Health Canada Santé Canada

Guidelines for Canadian Drinking Water Quality:

Supporting Documentation

# Protozoa: *Giardia* and *Cryptosporidium*

Prepared by the
Federal-Provincial-Territorial Committee on Drinking Water
of the
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Other supporting documents in the Canadian Guidelines for Drinking Water Quality can be found on the Water Quality and Health Bureau web page at http://www.hc-sc.gc.ca/hecs-sesc/water/dwgsup.htm.

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## 1. Guideline

*Although* Giardia *and* Cryptosporidium *can be responsible for severe and, in some cases, fatal gastrointestinal illness, it is not possible to establish maximum acceptable concentrations (MACs) for these protozoa in drinking water at this time. Routine methods available for the detection of cysts and oocysts suffer from low recovery rates and do not provide any information on their viability or human infectivity. Nevertheless, until better monitoring data and information on the viability and infectivity of cysts and oocysts present in drinking water are available, measures should be implemented to reduce the risk of illness as much as possible. If the presence of viable, human-infectious cysts or oocysts is known or suspected in source waters, or if* Giardia *or* Cryptosporidium *has been responsible for past waterborne outbreaks in a community, a treatment and distribution regime and a watershed or wellhead protection plan (where feasible) or other measures known to reduce the risk of illness should be implemented. Treatment technologies in place should achieve at least a 3-log reduction in and/or inactivation of cysts and oocysts, unless source water quality requires a greater log reduction and/or inactivation.*

## 2. Executive Summary for the Microbiological Quality of Drinking Water

### 2.1 Introduction

The information contained in this Executive Summary applies to the microbiological quality of drinking water as a whole. It contains background information on microorganisms, their health effects, sources of exposure, and treatment. Information specific to protozoa is included as a separate paragraph. It is recommended that this document be read in conjunction with other documents on the microbiological quality of drinking water, including the supporting document on Turbidity.

### 2.2 Background

There are three main types of microorganisms that can be found in drinking water: bacteria, viruses, and protozoa. These can exist naturally or can occur as a result of contamination from human or animal waste. Surface water sources, such as lakes, rivers, and reservoirs, are more likely to contain microorganisms than groundwater sources, unless the groundwater sources are under the influence of surface water.

The main goal of drinking water treatment is to remove or kill these organisms to reduce the risk of illness. Although it is impossible to completely eliminate the risk of waterborne disease, adopting a multi-barrier, source-to-tap approach to safe drinking water will reduce the numbers of microorganisms in drinking water. This approach includes the protection of source water (where possible), the use of appropriate and effective treatment methods, well-maintained

distribution systems, and routine verification of drinking water safety. All drinking water supplies should be disinfected, unless specifically exempted. In addition, surface water sources and groundwater sources under the influence of surface water should be filtered.

The performance of the drinking water filtration system is usually assessed by monitoring the levels of turbidity, a measure of the relative clarity of water. Turbidity is caused by matter, such as clay, silt, fine organic and inorganic matter, plankton, and other microscopic organisms, that is suspended within the water. Suspended matter can protect pathogenic microorganisms from chemical and ultraviolet light disinfection.

Currently available detection methods do not allow for the routine analysis of all microorganisms that could be present in inadequately treated drinking water. Instead, microbiological quality is determined by testing drinking water for *Escherichia coli*, a bacterium that is always present in the intestines of humans and animals and that would indicate faecal contamination of the water. The maximum acceptable concentration of *E. coli* in drinking water is none detectable per 100 mL.

## 2.3 *Giardia* and *Cryptosporidium*

Protozoa such as *Giardia* and *Cryptosporidium* are relatively large pathogenic microorganisms that multiply only in the gastrointestinal tract of humans and other animals. They cannot multiply in the environment, but they can survive longer in water than intestinal bacteria and are more infectious and resistant to disinfection than most other microorganisms. Routine methods detect only a fraction of the total number present and do not provide any information on the viability of these organisms or their ability to infect humans. As a result, it is not currently possible to establish maximum acceptable concentrations for *Giardia* and *Cryptosporidium* in drinking water. Instead, the use of a multi-barrier approach to safeguard drinking water supplies and reduce exposures to *Giardia* and *Cryptosporidium* in drinking water is recommended. Routine water quality monitoring for *E. coli* is also important, as the presence of *E. coli* is an indication that *Giardia* and *Cryptosporidium* could also be present. However, because *Giardia* and *Cryptosporidium* are more resistant to disinfection, the absence of *E. coli* does not necessarily mean that they are also absent.

## 2.4 Health Effects

The health effects of exposure to disease-causing bacteria, viruses, and protozoa in drinking water are varied. The most common manifestation of waterborne illness is gastrointestinal upset (nausea, vomiting, and diarrhoea), and this is usually of short duration. However, in susceptible individuals such as infants, the elderly, and immunocompromised individuals, the effects may be more severe, chronic (e.g., kidney damage), or even fatal. Bacteria (such as *Shigella* and *Campylobacter*), viruses (such as Norovirus and Hepatitis A), and protozoa (such as *Giardia* and *Cryptosporidium*) can be responsible for severe gastrointestinal illness. Other pathogens may infect the lungs, skin, eyes, central nervous system, or liver.

If the safety of drinking water is in question to the extent that it may be a threat to public health, authorities in charge of the affected water supply should have a protocol in place for issuing, and cancelling, advice to the public about boiling their water. Surveillance for possible waterborne diseases should also be carried out. If a disease outbreak is linked to a water supply, the authorities should have a plan to quickly and effectively contain the illness.

### 2.5 Exposure

Drinking water contaminated with human or animal faecal wastes is just one route of exposure to disease-causing microorganisms. Outbreaks caused by contaminated drinking water have occurred, but they are relatively rare compared with outbreaks caused by contaminated food. Other significant routes of exposure include contaminated recreational waters (e.g., bathing beaches and swimming pools) and objects (e.g., doorknobs) or direct contact with infected humans or domestic animals (pets or livestock). Although surface waters and groundwater under the influence of surface waters may contain quantities of microorganisms capable of causing illness, effective drinking water treatment can produce water that is virtually free of disease-causing microorganisms.

### 2.6 Treatment

The multi-barrier approach is an effective way to reduce the risk of illness from pathogens in drinking water. If possible, water supply protection programmes should be the first line of defence. Microbiological water quality guidelines based on indicator organisms (e.g., *E. coli*) and treatment technologies are also part of this approach. Treatment to remove or inactivate pathogens is the best way to reduce the number of microorganisms in drinking water and should include effective filtration and disinfection and an adequate disinfection residual. Filtration systems should be designed and operated to reduce turbidity levels as low as possible without major fluctuations.

It is important to note that all chemical disinfectants (e.g., chlorine, ozone) used in drinking water can be expected to form disinfection by-products that may affect human health. Current scientific data show that the benefits of disinfecting drinking water (reduced rates of infectious illness) are much greater than any health risks from disinfection by-products. While every effort should be made to reduce concentrations of disinfection by-products to as low a level as possible, any method of control used must not compromise the effectiveness of water disinfection.

## 3. Description

### 3.1 *Giardia*

*Giardia* is a small, flagellated protozoan (small single-cell organism lacking cell walls) parasite (Phylum Protozoa, Subphylum Sarcomastigophora, Superclass Mastigophora, Class Zoomastigophora, Order Diplomonadida, Family Hexamitidae) that inhabits the small intestines

of humans and other animals. The trophozoite, or feeding stage, lives mainly in the duodenum but is often found in the jejunum and ileum of the small intestine. Trophozoites (9–21 µm long, 5–15 µm wide, and 2–4 µm thick) have a pear-shaped body with a broadly rounded anterior end, two nuclei, two slender median rods, eight flagella in four pairs, a pair of darkly staining median bodies, and a large ventral sucking disc (cytostome). Trophozoites are normally attached to the surface of the intestinal villi, where they are believed to feed primarily upon mucosal secretions. After detachment, the binucleate trophozoites form cysts (encyst) and divide within the cyst, so that four nuclei become visible. Cysts are ovoid, 8–14 µm long by 7–10 µm wide, with two or four nuclei and remnants of organelles visible. Environmentally stable cysts are passed out in the faeces, often in large numbers. A complete life cycle description and diagram can be found in a review paper by Meyer and Jarroll (1980).

*Giardia lamblia* cysts can survive up to 77 days in tap water at 8°C (Bingham *et al*., 1979), but survival decreases with increasing temperature (54 days at 21°C and 4 days at 37°C). *Giardia muris* cysts remain viable for up to 2.8 months in river water when the temperature is <10°C and for approximately 1 month at 15–20°C in lake water (deRegnier *et al*., 1989). Cysts have no external features and are recognized by shape and visible internal morphology, as described above. Upon ingestion of the cysts by a suitable host, excystation is triggered by acid and enzymes in the stomach; by the time the parasite reaches the duodenum, a quadranucleate mass of protoplasm emerges, which rapidly divides into two trophozoites from each cyst (Meyer, 1994). Colonization of the small intestine then occurs by asexual reproduction.

The taxonomy of the genus *Giardia* is based on the species definition proposed by Filice (1952), who defined three species: *G. duodenalis* (syn. *G. intestinalis*, *G. lamblia*), *G. muris*, and *G. agilis*, based on the shape of the median body, an organelle composed of microtubules that is most easily observed in the trophozoite. Three species, *G. ardea*, *G. psittaci*, and *G. microti*, have subsequently been described on the basis of cyst morphology and small-subunit rRNA sequence analysis (Adam, 2001). These six species have been reported from mammals, birds, rodents, and amphibians and are not easily distinguished. Their host preferences have been widely debated — except for *agilis*, which is morphologically different, has been reported only from amphibians, and is not regarded as infective to humans. The name *Giardia lamblia* is commonly applied to isolates from humans.

Giardiasis is believed to be a zoonotic disease, although most of the evidence is circumstantial or compromised by inadequate controls. It is known that beaver, dogs, and muskrat can become infected with human-source *G. duodenalis* (Davies and Hibler, 1979; Hewlett *et al*., 1982; Erlandsen and Bemrick, 1988; Erlandsen *et al*., 1988), but the pathogenicity to humans for *Giardia* reported from birds, cattle, bears, cats, and other hosts is uncertain.

*Giardia duodenalis* can adapt to a variety of hosts, and, being asexual, its population genetics are best described in terms of clonal expansion (Tibayrenc, 1993) of virulent individuals within heterogeneous populations. *Giardia duodenalis* is capable of high rates of chromosomal rearrangement (Lymbery and Tibayrenc, 1994) and may indeed be able to adapt to new hosts (and even to *in vitro* cultivation) more readily than other parasites. *Giardia muris* from voles and

mice is generally regarded not to be infective to humans, although mice can be used as an animal model for human *Giardia* isolates (Byrd *et al*., 1994). The environmental resistance and prolonged viability of *Giardia* cysts in water at low temperature, the endemic nature of *Giardia* infections in humans and animals, and cross-species transmission, together with the low infectious dose needed to establish colonization within a new host, all point towards the potential for waterborne spread of this disease (Erlandsen, 1994). Recent research has focussed on distinguishing human-infective *Giardia* from other strains or species; however, the applicability of these methods to analysis of *Giardia* within water has been limited. Thus, at present, it is necessary to consider that any *Giardia* cysts found in water are potentially infectious to humans (Erlandsen, 1994). Molecular methods, such as the polymerase chain reaction (PCR), have been successfully used to differentiate *Giardia* species (Mahbubani *et al.*, 1992; Ionas *et al.*, 1997); however, further research is required to validate these methods.

### 3.2 *Cryptosporidium*

*Cryptosporidium parvum* is a protozoan parasite (Phylum Apicomplexa, Class Sporozoasida, Subclass Coccodiasina, Order Eucoccidiorida, Suborder Eimeriorina, Family Cryptosporidiidae) that was first recognized as a potential human pathogen in 1976 in a previously healthy 3-year-old child. A second case occurred 2 months later in an individual who was immunosuppressed as a result of drug therapy (Ungar, 1990). Subsequently, the disease became best known in immunosuppressed individuals exhibiting the symptoms now referred to as acquired immune deficiency syndrome, or AIDS (Meisel *et al*., 1976). The recognition of *C. parvum* as a human pathogen led to increased research into the life cycle of the parasite and an investigation of the possible vectors of transmission. *Cryptosporidium* has a multi-stage life cycle, typical of enteric coccidia, that takes place in a single host and evolves in six major stages: excystation, where sporozoites are released from an excysting oocyst; merogony, where asexual reproduction takes place; gametogeny, the stage at which gametes are formed; fertilization of the gamete by a microgamete to form a zygote; oocyst wall formation; and sporogony, sporozoite formation within the oocyst (Current, 1986).A complete life cycle description and diagram can be found in a review paper by Smith and Rose (1990).

As a waterborne pathogen, the most important stage in the life cycle is the round, thick-walled, environmentally stable oocyst, 4–6 µm in diameter. There is sometimes a visible external suture line, and the nuclei of sporozoites can be stained with fluorogenic dyes such as 4',6-diamidino-2-phenylindole (DAPI). *Cryptosporidium* oocysts have been shown to survive in cold waters (4°C) in the laboratory for up to 18 months (AWWA, 1988). Robertson *et al.* (1992) reported that *C. parvum* oocysts could withstand a variety of environmental stresses, including freezing (viability greatly reduced) and exposure to seawater. Upon ingestion by humans, the parasite completes its life cycle in the digestive tract. Ingestion initiates excystation of the oocyst and releases four sporozoites, which adhere to the epithelial surface of the gastrointestinal tract. Conflicting evidence from electron microscopic studies has led to some disagreement as to whether the parasite is intracellular (invasive) or extracellular (Anderson, 1982; Anderson *et al*.,

1982; Tzipori, 1983). All stages possess a feeding organelle that is protected along with the parasite body itself by an outer membrane. It is not certain whether the outer membrane is derived from the host cell (intracellular) or of parasitic origin (extracellular). The sporozoite undergoes asexual reproduction at the site and develops into a gamete. Some of these gametes release microgametes, which fertilize other macrogametes to form zygotes. A small number of zygotes retain a thin cell wall, which ruptures after the development of the sporozoites to aid in maintaining the infection within the host. The majority of the zygotes develop a thick cell wall and four sporozoites to become oocysts, which are then passed in the faeces.

The first description of *Cryptosporidium* was made by Tyzzer (1907), when he isolated the organism, which he named *Cryptosporidium muris*, from the gastric glands of mice. Tyzzer (1912) found a second isolate, which he named *C. parvum*, in the intestine of the same species of mice. This isolate was considered to be structurally and developmentally distinct by Upton and Current (1985). Although numerous species names have been proposed based on the identity of the host, most isolates of *Cryptosporidium* from mammals, including humans, are similar to *C. parvum* as described by Tyzzer (1907, 1912). Ten valid species have been recognized (Fayer *et al.*, 2000): *C. parvum*, *C. muris*, *C. andersoni*, *C. felis*, and *C. wrairi*, which infect mammals; *C. baileyi* and *C. meleagridis*, which infect birds; *C. serpentis* and *C. saurophilum*, which infect reptiles; and *C. nasorum*, which infects fish (Smith, 1990). *Cryptosporidium muris* has been reported from cattle in North America and Europe as well as from camels, deer, and other animals. Experimental infections of dogs, cats, and rabbits with *C. muris* have been described by Iseki *et al.* (1989). *Cryptosporidium parvum* is generally regarded as the major species responsible for clinical disease in humans and domestic animals, and zoonotic transmission is possible, especially from lambs, calves, and adult cattle. Symptomatic cryptosporidiosis has been reported from humans, cattle (common), lambs, goats, birds, horses, and monkeys. Human-source *Cryptosporidium* has been shown to be infective to cattle and lambs (Tzipori, 1983; Upton and Current, 1985). Other species of *Cryptosporidium*, such as *C. felis*, *C. canis*, *C. muris*, and *C. meleagridis*, have also been implicated in human infections (Katsumata *et al.*, 2000; Guyot *et al.*, 2001; Pedraza-Diaz *et al.*, 2001a,b; Yagita *et al.*, 2001; Tiangtip and Jongwutiwes, 2002).

As many as 18 distinct genotypes of *C. parvum* have been proposed, including human, bovine I, bovine II, rabbit, pig, mouse, deer, deer mouse, ferret, marsupial, opossum I, opossum II, skunk, bear, fox, muskrat, monkey, and sheep genotypes (Chalmers *et al.*, 2002; Xiao and Lal, 2002). The molecular analysis of *C. parvum* human and bovine isolates, linked to human cryptosporidiosis outbreaks, indicates the existence of two predominantly distinct genotypes in humans (Morgan *et al*., 1997; Peng *et al*., 1997; Spano *et al*., 1998; Sulaiman *et al*., 1998; Widmer *et al*., 1998; Awad-El-Kariem, 1999; Ong *et al*., 1999; Caccio *et al*., 2000; McLauchlin *et al.*, 2000; Xiao *et al*., 2001).Genotype 1 (currently referred to as *C. hominis*) isolates have been reported only in humans, while genotype 2 isolates have been reported in calves and humans exposed to infected cattle and in materials contaminated with cattle faeces. Genotype 2 isolates are able to infect mice and calves, whereas *C. hominis* isolates are not. Recent studies have

identified novel *C. parvum* genotypes in humans. Pieniazek *et al*. (1999) identified two novel *Cryptosporidium* genotypes in persons infected with human immunodeficiency virus (HIV): a dog and a cat genotype. Ong *et al.* (2002) also identified two new *C. parvum* genotypes in humans, one cervine (deer) and a not-yet-identified genotype (i.e., not been previously identified in humans or other animals). These findings have important implications for communities whose source water may be impacted by faeces from wildlife.

# 4. Health Effects

## 4.1 *Giardia*

The prepatent period (time between ingestion of cysts and excretion of new cysts) for giardiasis is 6–16 days (Rendtorff, 1978; Stachan and Kunstýr, 1983; Nash *et al*., 1987), and the minimal infective dose can be as low as 1–10 cysts (Rendtorff, 1978; Stachan and Kunstýr, 1983), although there are large differences between isolates of the parasite in terms of their virulence and antigenic diversity (Nash, 1994). The $ID_{50}$ (number of cysts ingested resulting in 50% of the test subjects becoming infected) was found to be 19 cysts by Rendtorff (1978) (calculated from his data) using human-source cysts in humans, but it can be as high as 543 for human-source *Giardia* in gerbils. *Giardia* strains that are well adapted to their hosts (e.g., by serial passage) can frequently infect with 50 cysts or less (Hibler *et al*., 1987). Research with animal models has shown that smaller inocula result in longer prepatent periods but do not influence the resulting parasite burden (Belosevic and Faubert, 1983).

Exposure to the parasite resulted in partial or total immunity for periods of up to 21 weeks in mice (Roberts-Thomson *et al*., 1976; Belosevic and Faubert, 1983). Olson *et al.* (1994) observed lower cyst output and greater weight gain in kittens immunized subcutaneously. Humoral immune response is revealed by increased levels of circulating IgG and IgM antibodies and secretion of IgA in milk, saliva, and possibly intestinal mucus. These immune products are active in eliminating disease (Heyworth, 1988), but lasting immunity has not been demonstrated. Very little is known about cellular immunity, but spontaneous killing of trophozoites by human peripheral blood monocytes has been described (denHollander *et al*., 1988). The host–parasite relationship is complex, and *Giardia* has been shown to be versatile in the expression of antigens (Nash, 1994), so universal lasting immunity is improbable. Olson *et al.* (1994) showed that potential for a vaccine exists, but infections and symptoms are only attenuated, and prevention of infection is not feasible at this time. Symptoms include nausea, anorexia, an uneasiness in the upper intestine, malaise, and perhaps low-grade fever or chills. The onset of diarrhoea is usually sudden and explosive, with watery and foul-smelling stools (Wolfe, 1984). The acute phase of the infection commonly resolves spontaneously, and organisms may disappear from the faeces. Some patients become asymptomatic cyst passers for a period and have no further clinical manifestations. Other patients, particularly children, suffer recurring bouts of the disease that may persist for months (Lengerich *et al*., 1994). Giardiasis can be treated using a number of drugs, including metronidazole, quinacrine, furazolidone, tinidazole, ornidazole, and nimorazole.

Lengerich *et al.* (1994) evaluated hospitalization rates for severe giardiasis in the United States. An estimated 4600 persons were hospitalized annually, a rate similar to that of shigellosis. The median length of hospital stay was 4 days.

### 4.2 *Cryptosporidium*

Although a complete pathogenesis for *C. parvum* in humans has yet to be determined, more information is becoming available through the study of both immunocompetent individuals and AIDS patients. DuPont *et al.* (1995) found that 18 of 29 healthy volunteers became infected after administration of doses of from 30 to 1 000 000 oocysts, 39% of whom were asymptomatic. The $ID_{50}$ was 132 oocysts, and 61% of the infected subjects experienced enteric symptoms. A follow-up experiment conducted 1 year after the primary exposure demonstrated that an initial exposure is inadequate to protect against future bouts of cryptosporidiosis (Okhuysen *et al*., 1998). Although the rates of diarrhoea were similar after each of the exposures, the severity of diarrhoea was lower after re-exposure. The $ID_{50}$ of 132 oocysts compares well with an $ID_{50}$ of 79 oocysts reported for a bovine strain of *C. parvum* in CD-1 mice (Finch *et al*., 1993b), although the minimum infective dose of oocysts required to produce infection in animals ranges in published research data from 10 to 100 oocysts (Miller *et al*., 1986; Ernest *et al*., 1987; Blewett *et al*., 1993).

*Cryptosporidium parvum* genotypes appear to have unique virulence and infectious dose properties. The TAMU strain of *C. parvum* (originally isolated from a horse) was shown to have an $ID_{50}$ of 9 oocysts and an illness attack rate of 86%, compared with the UCP strain of *C. parvum* (isolated from a cow), which had an $ID_{50}$ of 1042 oocysts and an illness attack rate of 59% (Okhuysen *et al.*, 1999; Messner *et al.*, 2001).Virulence genes responsible for this phenomenon are unknown (Okhuysen and Chappell, 2002). DuPont *et al*. (1995) found that the prepatent period ranged from 2 to 25 days (although most occurred within 3–11 days), with the shortest time to cyst excretion occurring with an inoculum of 1 000 000 oocysts. All individuals recovered spontaneously. Similarly, an investigation of a *Cryptosporidium* infection in travellers returning from the Caribbean indicated a prepatent period of 4–9 days (Ma *et al*., 1985). The usual symptom associated with the disease is diarrhoea, characterized by very watery, non-bloody stools. The volume of diarrhoea can be extreme, with 3 L/day being common and with reports of up to 17 L/day (Navin and Juranek, 1984). This symptom can be accompanied by nausea, vomiting (particularly in children), low-grade fever (below 39°C), anorexia, and dehydration. The symptoms reported from a waterborne outbreak are diarrhoea (100%), abdominal cramps (76%), nausea (45%), vomiting (19%), fever (14%), headache (29%), and muscle aches (13%) (D'Antonio *et al*., 1985). Some protective immunity appears to develop in infected populations. The primary mechanism of host defence appears to be cellular immunity, although humoral immunity is also known to be involved (Janoff and Reller, 1987).

The duration of infection is dependent on the condition of the immune system (Juranek, 1995) and can be broken down into three categories: (1) immunocompetent individuals who clear the infection in 7–14 days, (2) AIDS patients or others with severely weakened immune

systems (i.e., individuals with CD4 cell counts <180 cells/mm$^3$) who in most reported cases never completely clear the infection (it may develop into an infection with long bouts of remission followed by mild symptoms), and (3) individuals who are immunosuppressed following chemotherapy, short-term depression or illness (e.g., chicken pox), or malnutrition. In cases where the immunosuppression is not AIDS related, the infection usually clears (no cyst excretion, and symptoms disappear) within 10–15 days of the time the immune system returns to normal, although there have been reported cases involving children in which the infection has persisted for up to 30 days. The sensitivity of diagnosis of cryptosporidiosis by stool examination is low — so low that oocyst excreters may be counted as negative prematurely. Immunocompetent individuals usually carry the infection for a maximum of 30 days. With the exception of AIDS cases, individuals may continue to pass oocysts for up to 24 days. In an outbreak in a day care facility, children shed oocysts for up to 5 weeks (Stehr-Green *et al*., 1987). The reported rate of asymptomatic infection is believed to be low, but a report on an outbreak at a day care facility in Philadelphia, Pennsylvania, concluded that up to 11% of the children were asymptomatic (Alpert *et al*., 1986), and Ungar (1994) discussed three separate studies in day care centres where the asymptomatic infection rate ranged from 67 to 100%. It has been suggested that many of these asymptomatic cases were mild cases that were incorrectly diagnosed (Navin and Juranek, 1984). In AIDS patients, Juranek (1995) showed that only 13% (5/39) of patients with CD4 cell counts of <180 cells/mm$^3$ had self-limiting disease, but 100% (8/8) of those with counts >180 cells/mm$^3$ had infections that cleared.

Infections of *Cryptosporidium* spp. in the human intestine are known to cause damage to the mucosa, including villous atrophy and lengthening of the crypt (Tzipori, 1983). Most of the pathological data available have come from AIDS patients, and the presence of other opportunistic pathogens has made assessment of damage attributable to *Cryptosporidium* spp. difficult. There is some suggestion in the literature that outbreaks of cryptosporidiosis are of seasonal duration. This has yet to be addressed by investigation, but the seasons reported, by country, are as follows: February–April (Great Britain), April–July (Bangladesh), May–July (United States), and May–October (Italy) (D'Antonio *et al*., 1985; Baxby and Hart, 1986; Nigar *et al*., 1987; Caprioli *et al*., 1989).

No effective antimicrobial treatment for cryptosporidiosis in adults has been approved, although more than 120 drugs have been tested (Tzipori, 1983; O'Donoghue, 1995). The U.S. Food and Drug Administration (FDA) recently approved Alinia™ (nitazoxanide) for treatment of cryptosporidiosis and giardiasis in (1- to 11-year-old) children (U.S. FDA, 2002). Some progress has been reported with furazolidone in reducing the symptoms of immunocompetent patients. Spiramycin has apparently been used with some success in Chile and the United States, but at this time it is not licensed for general use by the FDA (Janoff and Reller, 1987). A functional immune system will usually eliminate symptoms and organisms spontaneously, but the immunocompromised individual may suffer from chronic infection.

During the Milwaukee, Wisconsin, cryptosporidiosis outbreak, investigators surveyed 285 patients with laboratory-confirmed infection. Of these, 130 were hospitalized, including 48 immunocompromised patients (MacKenzie *et al*., 1994).

## 5. Emerging Pathogenic Waterborne Protozoans

*Acanthamoeba* are free-living amoebae found in a variety of environments, including soil, air, and water. *Acanthamoeba* have been detected in a number of aquatic environments, including chlorinated swimming pools and drinking water (Rivera *et al.*, 1993; Vesaluoma *et al.*, 1995; Michel *et al.*, 1998; Rohr *et al.*, 1998). Although this organism is common in the environment and is capable of infecting mammals, including humans, infections in humans are rare. Most human infections have been associated with the use of "homemade" contact lens (saline) solutions, which resulted in keratitis (inflammation of the cornea) (Buck *et al.*, 2000; Seal, 2000; Yeung *et al.*, 2002). Since *Acanthamoeba* are relatively large, water filtration processes should be efficient for their removal; however, their cyst form is resistant to typical levels of chlorine (King *et al.*, 1988). Water treatment processes applied for the removal/inactivation of *Giardia* and *Cryptosporidium* should be effective against this organism. Additional concern over *Acanthamoeba* stems from the fact that it may harbour opportunistic pathogens (e.g., *Legionella pneumophila*, *Mycobacterium avium*) (Henke and Seidel, 1986; King *et al.*, 1988; Steinert *et al.*, 1998; Newsome *et al.*, 2001). Thus, if *Acanthamoeba* cysts are able to pass through the water treatment process into potable water, these pathogenic bacterial symbionts could cause human illness.

Microsporidia are spore-forming intracellular, obligate parasites that are widely distributed in the environment. Microsporidia are opportunistic pathogens, primarily affecting persons infected with HIV (Fournier *et al.*, 2000; Svedhem *et al.*, 2002). However, immunocompetent individuals can become infected as well. Microsporidia capable of causing human illness have not been detected in surface or potable water. This is likely due to the limited sensitivity of existing detection methods. Since microsporidia are similar in size to large bacteria, they should be removed by conventional coagulation/sedimentation and filtration processes; however, no information is available regarding the efficacy of these processes in removing microsporidia. Very little is known about the susceptibility of microsporidia to disinfectants. However, recent research suggests that ultraviolet (UV) light disinfection is highly effective for inactivating microsporidia (Huffman *et al.*, 2002).

*Toxoplasma gondii* is an obligate, intracellular parasite that affects almost all warm-blooded animals, including humans. Cats shed the oocyst form of this organism in their faeces. Oocysts are extremely resistant to environmental conditions and appear to retain their infectivity for several months (at temperatures of -5°C) (Dubey, 1998). Although this organism tends to cause mild flu-like symptoms, it can be life-threatening for immunocompromised individuals and pregnant women. Little is known about the distribution of this organism in water sources; however, oocysts have been reported to survive for up to 17 months in tap water. In 1995, a toxoplasmosis outbreak was reported in British Columbia, involving 110 acute cases, including

42 pregnant women and 11 neonates (Bowie *et al.*, 1997). This outbreak was thought to be due to contamination of a water reservoir by (domestic and wild) cat faeces (Isaac-Renton *et al.*, 1998; Aramini *et al.*, 1999). Limited information is available on the efficacy of water treatment processes in removing or inactivating *Toxoplasma gondii*. However, because of its size, it should be readily removed by conventional coagulation/sedimentation and filtration processes. In effect, water treatment processes applied for the removal/inactivation of *Giardia* and *Cryptosporidium* should be effective against this organism.

## 6. Sources and Exposure

### 6.1 *Giardia*

*Giardia* is the most commonly reported intestinal parasite in North America and the world (Farthing, 1989; Adam, 1991). Giardiasis has been shown to be endemic in humans and in over 40 species of animals, with prevalence rates ranging from 1 to 90+%. The prevalence rate among humans in Canada is typically 5–10%, but accurate rates are difficult to estimate because of the large number of asymptomatic cases (Keystone *et al*., 1978) and the inadequacy of reporting. Over 5000 confirmed cases of giardiasis were reported in 1999 in Canada. This represents a significant decline from the 9000 cases that were reported in 1987. Incidence rates have similarly declined over this period (34.44–17.7 cases per 100 000 persons) (Health Canada, 2003).

Most waterborne outbreaks have been associated with zoonotic transmission, particularly beaver (Kirner *et al*., 1978; Lopez *et al*., 1980; Lippy, 1981; Isaac-Renton *et al*., 1993). It is now clear, however, that other mammals, including dogs, muskrat, cattle, and sheep, can also be responsible for the introduction of cysts to surface water used as sources of drinking water. As population pressures increase and as more human-related activity occurs in catchment areas, the potential for faecal contamination becomes greater, and the possibility of contamination with human sewage must always be considered. Erlandsen and Bemrick (1988) concluded that *Giardia* cysts in water may be derived from multiple sources and that epidemiological studies that focus on beavers may be missing important sources of cyst contamination. Some waterborne outbreaks have been traced back to human sewage contamination (Wallis *et al*., 1998). Ongerth *et al.* (1995) showed that there is a statistically significant relationship between increased human use of water for domestic and recreational purposes and the prevalence of *Giardia* in animals and surface water. It is known that beaver and muskrat can be infected with human-source *Giardia* (Erlandsen *et al*., 1988), and these animals are frequently exposed to raw or partially treated sewage in Canada. Thus, it is likely that mammals act as a reservoir of human-infective *Giardia* from sewage-contaminated water and in turn amplify concentrations of *Giardia* cysts in water. If infected mammals live in close proximity to drinking water treatment plant intakes, then they could play an important role in the waterborne transmission of *Giardia*. Watershed management to control both sewage inputs and the populations of aquatic mammals in the vicinity of water intakes is just as important to disease prevention as adequate water treatment. It should also be

remembered that, in addition to water, giardiasis can be transmitted person to person via poor hygiene, food handling, and sexual practices.

In Canada, *Giardia* cysts are commonly found in sewage and surface waters and occasionally in drinking water. In a cross-Canada survey, Wallis *et al*. (1995) found that 56.2% of 162 raw sewage samples contained *Giardia* cysts, ranging in concentration from 1 to 88 000 cysts/L, and 10% of 1215 raw and treated drinking water samples contained 0.001–2 cysts/L. In samples at three sites on two rivers in the Montreal, Quebec, area, average *Giardia* cyst concentrations ranged from 0.07 to 14 cysts/L (Payment and Franco, 1993). The average cyst level in treated water prepared from the river waters was <0.002 cysts/L. Additional data from Quebec collected by the Ministry of the Environment and Wildlife showed that 45% of polluted and 34% of pristine water sources in the province were contaminated with *Giardia* cysts (total number of samples was 71), most of which were from rivers (Barthe and Brassard, 1994). *Giardia* cysts have been monitored in raw and treated drinking water in Ottawa, Ontario (Chauret *et al*., 1995). Cysts were not detected in treated water but were present in 83.3% and 66.6% of samples collected at the intakes of the two treatment plants. Concentrations ranged from <1 to 25 cysts/100 L (arithmetic means 6.0 and 5.8/100 L). Samples from the wastewater treatment plant were also examined. The arithmetic mean concentration for *Giardia* in treated wastewater was 73 cysts/100 L (representing a 99.3% reduction). Between 1990 and 1996, the annual geometric mean concentration of *Giardia* cysts in raw water at two treatment plants in Edmonton, Alberta, ranged from 8 to 193 cysts/100 L (Goatcher and Fok, 2000). No cysts were detected in almost all of the 1000-L treated water samples collected. On three occasions, one or two cysts were detected. However, in 1997, heavy spring runoff produced record levels of 2500 cysts/100 L of raw water. Cyst levels in treated water peaked at 34 cysts/1000 L, prompting municipal health officials to issue a precautionary boil water advisory to immunocompromised individuals. No increase in the number of cases of giardiasis in the Edmonton area was detected during this period. Isaac-Renton and colleagues (Isaac-Renton *et al*., 1987, 1993, 1994, 1996; Ong *et al*., 1996) have reported *Giardia* cyst contamination from a number of sites in British Columbia, some of which experienced waterborne outbreaks of giardiasis. In a province-wide survey, cysts were detected in 68% of raw water samples and 59% of disinfected samples. Mean cyst concentrations in raw water samples (2.9/100 L) were greater than those from treated water samples (2.1/100 L). The proportion of viable cysts in the raw and treated water samples was not determined. Infectivity in Mongolian gerbils was more frequent following inoculation with raw water concentrates than following inoculation with treated water concentrates (Isaac-Renton *et al*., 1996). In another study, levels in two adjacent watersheds in the interior of the province were determined (Ong *et al*., 1996). At the drinking water intake in one watershed, the geometric mean concentration of *Giardia* during a 21-month period was 173 cysts/100 L (range 4.6–2215 cysts/100 L). At the intake in the other watershed, *Giardia* was detected in samples collected over a 17-month period, with a geometric mean concentration of 26 cysts/100 L (range 2–114 cysts/100 L). In the Yukon, 7 of 42 drinking water samples collected over a period of 1 year at Whitehorse contained *Giardia*, with concentrations ranging from 0.2 to 1.4 cysts/100 L (Roach

*et al*., 1993). None of the 10 samples collected from Dawson's drinking water contained *Giardia*. In eight municipal drinking water supplies in the Atlantic region, 44% of 152 samples collected between 1991 and 1993 contained *Giardia* (Wallis *et al*., 1995). Although most positive samples contained less than 2.5 cysts/100 L, a few contained more than 150 cysts/100 L. Very few published data are available from Ontario, but an outbreak of giardiasis at Temagami in the spring of 1994 was characterized by an attack rate of 30% and cyst concentrations up to 2/L in treated water (Wallis *et al*., 1998). LeChevallier *et al.* (1991a) found *Giardia* cysts in 81% of 83 raw water samples and in 17% of 83 filtered water samples from the northeastern United States. Concentrations in raw water ranged from 0.05 to 242 cysts/L. One raw water sample from Alberta was included in the survey, and a concentration of 4.94 cysts/L was reported. Concentrations in finished drinking water samples ranged from 0.29 to 64 cysts/100 L.

LeChevallier *et al.* (1991a) also found significant positive correlations between cyst concentration and other raw water quality parameters, such as turbidity and total and faecal coliform densities; however, data from previous studies do not support these associations, possibly because of early reports of waterborne giardiasis from places like Colorado, which normally experience very low turbidities in raw water (Karlin and Hopkins, 1983). LeChevallier *et al.* (1991a) concluded that cyst contamination could be modelled in terms of watershed characteristics and that water reuse and sewage contamination were important factors in predicting cyst concentrations.

The viability of *Giardia* cysts found in water is commonly assumed to be high, but monitoring experience suggests otherwise. Cysts found in surface waters are often dead, as shown by propidium iodide (PI) dye exclusion (approximately 50% viability was observed using this technique during the Temagami outbreak), and water and sewage isolates infected only 9.4% of gerbils inoculated in the Canadian survey reported by Wallis *et al.* (1995). It is possible that not all of the *Giardia* isolates tested were actually infective to gerbils, but it is common to observe cysts that are non-refractile under phase microscopy and that have obviously damaged cyst walls. The immunofluorescent technique commonly used for detection is very sensitive and frequently reveals the presence of empty cysts ("ghosts"), particularly in sewage. Observations by LeChevallier *et al.* (1991b) also suggest that most of the cysts present in water are non-viable; 40 of 46 cysts isolated from drinking water exhibited "non-viable-type" morphologies (i.e., distorted or shrunken cytoplasm).

Several outbreaks associated with public drinking water systems have occurred in British Columbia (Penticton, 100 Mile House, Creston, Kimberley, Kelowna, Kitimat, Fernie, West Trail/Rossland, Barriere) (Health Canada, 1975–1995; Isaac-Renton *et al*., 1994), Alberta (Canmore, Banff, Morley, Exshaw, Edmonton) (Health Canada, 1975–1995), Saskatchewan (Brightsand, Flaxcombe) (Health Canada, 1975–1995), Manitoba (Dauphin) (Federal–Provincial Drinking Water Subcommittee, 1998), Ontario(Temagami, Muskoka/Perry Sound) (Wallis *et al*., 1998), Quebec(Shenley, St. Ferreol-les-Neiges, St. Perpetue, Les Escoumins) (Health Canada, 1975–1995; Federal–Provincial Drinking Water Subcommittee, 1998), New Brunswick (St. Quentin, Plaster Rock) (Federal–Provincial Drinking Water Subcommittee, 1998), and

Newfoundland (Botwood, Corner Brook, Roberts Arm, Springdale, Deer Lake, Bird Cove, St. Anthony Bight, Harbour Grace) (Health Canada, 1975–1995; Federal–Provincial Drinking Water Subcommittee, 1998). Outbreaks associated with semi-public drinking water supplies have also been reported in Ontario (Kingston, Peterborough, Guelph, Camp Tawingo), Quebec (Outaouais), and Saskatchewan (Swift Current) (Health Canada, 1975–1995). In the United States, outbreaks have been reported from 24 states (Jakubowski, 1994), especially Colorado and New England. During the period from 1965 to 1992, 115 outbreaks were reported that resulted in 26 530 known cases of giardiasis in the United States (Moore *et al.*, 1993; Jakubowski, 1994). Craun (1979), in an earlier study, identified reliance on surface water, minimal treatment (usually only chlorination), and inadequate treatment facilities as common causes of waterborne giardiasis. Small water treatment systems that used otherwise good-quality surface water of low turbidity seemed to be most commonly affected. A useful review of some select U.S. outbreaks has been compiled by Lin (1985), who concluded that these and other outbreaks had been caused by lack of filtration, improper filter operations, inadequate chlorination, cross-connections to sewers, and drinking contaminated surface waters.

### 6.2 *Cryptosporidium*

As with giardiasis, cryptosporidiosis may be waterborne, foodborne, or transmitted sexually or by the faecal–oral route. Reported prevalence rates of human cryptosporidiosis range from 0.6 to 20% (Caprioli *et al*., 1989; Zu *et al*., 1992; Mølbak *et al*., 1993; Nimri and Batchoun, 1994), based on stool samples. A survey of 1346 Canadian patients revealed a prevalence rate of 1.25% in 1985 (Janoff and Reller, 1987), but data are limited because the disease is not universally reportable. Infection rates for patients with AIDS are reported to be 4% in the United States and 2.5% in Canada (Janoff and Reller, 1987; Soave and Johnson, 1988). The parasite in immunocompetent individuals does not seem to be more prevalent among any particular age group, although the probability of seroconversion increases with age (Kuhls *et al*., 1994). Infected human hosts can excrete up to $10^{10}$ oocysts/g faeces (Smith and Rose, 1990).

Although not conclusive, existing literature suggests that livestock may be a significant source of *C. parvum* in surface waters. Olson *et al.* (1997) reported that *Cryptosporidium* is common in Canadian farm animals. It was present in faecal samples from cattle (20%), sheep (24%), hogs (11%), and horses (17%). Oocysts were more prevalent in calves than in adult animals; conversely, they were more prevalent in mature pigs and horses than in young animals. Infected calves can excrete up to $10^{7}$ oocysts/g faeces (Smith and Rose, 1990). Other studies have also suggested that cattle may be a source of *Cryptosporidium* in surface waters. For example, a weekly examination of creek samples upstream and downstream of a cattle ranch in the B.C. interior during a 10-month period revealed that the downstream location had significantly higher levels (geometric mean 13.3 oocysts/100 L, range 1.4–300 oocysts/100 L) than the upstream location (geometric mean 5.6/100 L, range 0.5–34.4 oocysts/100 L) (Ong *et al*., 1996). A pronounced spike was observed in downstream samples following calving in late February. Although the sample size was limited, none of the faecal specimens collected during

the study was positive for oocysts. However, during a confirmed waterborne outbreak of cryptosporidiosis in British Columbia, oocysts were detected in 70% of the cattle faecal specimens collected in the watershed close to the reservoir intake (Ong *et al*., 1997). Waterfowl can also act as a source of *C. parvum*. Graczyk *et al*. (1998) demonstrated that *C. parvum* oocysts retain infectivity in mice following passage through ducks. Histological examination of the respiratory and digestive systems at 7 days post-inoculation revealed that the protozoa were unable to establish infection in the birds. In an earlier study (Graczyk *et al*., 1996), the authors found that faeces from migratory Canada geese collected from seven of nine sites on Chesapeake Bay contained *Cryptosporidium* oocysts. Oocysts from three of the sites were infectious to mice and were identified as *C. parvum*. Based on these studies, it appears that waterfowl can pick up infectious *C. parvum* oocysts from their habitat and can carry and deposit them in the environment, including drinking water supplies. Although the evidence collected to date is scarce, it appears that, unlike the case with *Giardia*, wild ungulates and rodents are not a significant source of human-infectious *Cryptosporidium* (Roach *et al*., 1993; Ong *et al*., 1996).

*Cryptosporidium* oocysts have been reported in wastewater (3.3–20 000/L), surface waters receiving agricultural or wastewater discharges (0.006–2.5/L), pristine surface water (0.02–0.08/L), drinking water (0.006–4.8/L), and recreational water (0.66–500/L) in various studies, as summarized by Smith (1990). Similar results were reported by Madore *et al*. (1987) in the United States. Rose *et al*. (1991) found oocysts in 55% of 257 surface water samples at an average concentration of 43 oocysts/L and in 17% of 36 drinking water samples at concentrations ranging from 0.5 to 1.7 oocysts/L. In a multi-year Canada-wide survey, Wallis *et al*. (1995) found that 11.1% of 162 raw sewage samples contained *Cryptosporidium* oocysts ranging in concentration from 1 to 120/L, and 6.4% of 1215 raw and treated drinking water samples contained 0.001–0.005 oocysts/L. In samples from three sites on two rivers in the Montreal area, average *Cryptosporidium* oocyst concentrations ranged from <0.02 to 7/L (Payment and Franco, 1993). *Cryptosporidium* oocysts have been monitored in raw and treated drinking water in Ottawa (Chauret *et al*., 1995). Oocysts were not detected in treated water but were present in 50% and 100% of samples collected at the intake of the two treatment plants. Concentrations ranged from <1 to 95 oocysts/100 L (arithmetic means 4.0 and 22.3 oocysts/100 L). Samples from the wastewater treatment plant were also examined. The arithmetic mean concentration of *Cryptosporidium* in treated wastewater was 56.0 oocysts/100 L (representing a 96.8% reduction). Between 1990 and 1996, the annual geometric mean concentration of *Cryptosporidium* oocysts in raw water at two treatment plants in Edmonton ranged from 6 to 83 oocysts/100 L (Goatcher and Fok, 2000). Oocysts were not detected in 1000 L of treated water collected at either plant. In 1997, heavy spring runoff produced record levels of 10 300 oocysts/100 L of raw water and 80 oocysts/1000 L of treated water. As a result, municipal health officials issued a precautionary boil water advisory to immunocompromised individuals. No increase in the number of cases of cryptosporidiosis in the Edmonton area was detected during this period. In British Columbia, Isaac-Renton and colleagues conducted a number of studies on *Cryptosporidium* in drinking water supplies. In one study, levels in two adjacent watersheds in the interior of the province

were determined (Ong *et al*., 1996). At the drinking water intake in one watershed, the geometric mean concentration of *Cryptosporidium* during a 9-month period was 3.5 oocysts/100 L, with a range of 1.7–44.3 oocysts/100 L. At the intake in the other watershed, oocysts were detected in samples collected over a 6-month period, with a geometric mean concentration of 9.2 oocysts/100 L (range 4.8–51.4 oocysts/100 L). In another study, *Cryptosporidium* was monitored in the drinking water of three communities (groundwater supply, unprotected surface supply, and protected surface supply) for 1 year (Isaac-Renton *et al*., 2000). Although *Cryptosporidium* was not found in the groundwater, intermittent detectable levels were found in the drinking water taken from surface supplies. In the drinking water from the unprotected supply, 71% of samples were positive, compared with 34% of the samples from the protected supply. LeChevallier *et al*. (1991a,b) reported that 87% of 83 raw surface water samples and 27% of 83 filtered water samples taken from the northeastern United States contained *Cryptosporidium* oocysts. Concentrations in raw water ranged from 0.07 to 484 oocysts/L. One raw water sample from Alberta contained 0.34 oocysts/L. As with *Giardia* cysts, LeChevallier *et al.* (1991a,b) concluded that water reuse and sewage contamination were important predictors of *Cryptosporidium* concentrations in water.

All *Cryptosporidium* oocysts found in water are frequently assumed to be viable, a view supported by the knowledge that the oocyst is highly resistant to environmental stress, but this is probably incorrect. Viability is probably less than 100%, as shown by Smith *et al.* (1993), who found that oocyst viability in surface waters is often very low. This point of view is supported directly by LeChevallier *et al.* (1991b), who found that 21 of 23 oocysts in filtered waters had "non-viable-type" morphology (i.e., absence of sporozoites and distorted or shrunken cytoplasm). No outbreaks of cryptosporidiosis occurred in any of the municipalities included in the LeChevallier *et al.* (1991b) survey. Similarly, Sorvillo *et al.* (1994) concluded that municipal drinking water was not an important risk factor for cryptosporidiosis in AIDS patients residing in Los Angeles County, California, on the basis of epidemiological data collected before and after the introduction of filtration in a major water supply for the area. However, it should be emphasized that although low concentrations of viable oocysts are routinely found in raw water, they may not represent an immediate public health risk; rather, it is the sudden and rapid influx of parasites into source waters that is likely responsible for the increased risk of infection associated with transmission through drinking water. Environmental events such as flooding or high precipitation can facilitate the rapid rise in oocyst concentration within a defined area of a watershed.

Outbreaks associated with public drinking water systems have been reported in Ontario (Kitchener–Waterloo and Collingwood) (Welker *et al*., 1996; Federal–Provincial Drinking Water Subcommittee, 1998) and British Columbia (Cranbrook, Kelowna, and Chilliwack) (Welker *et al*., 1996; Ong *et al*., 1997). Outbreaks associated with a semi-public drinking water supply and swimming pool were reported in British Columbia (West Bank) (Health Canada, 1975–1995; Bell *et al*., 1993; Meeds, 1993). Nineteen outbreaks have been reported in the United Kingdom; seven outbreaks have been reported in the United States (Craun *et al*., 1998). Attack rates were

typically high, ranging from 26 to 40%, and many thousands of people were affected. In addition, there have been several outbreaks associated with swimming pools, wave pools, and lakes.

# 7. Analytical Methods

## 7.1 Detection

Existing methodologies for routine monitoring of *Giardia* and *Cryptosporidium* are only semi-quantitative and do not provide any information on viability or human infectivity. The U.S. Environmental Protection Agency's (EPA) Method 1623, for example, is one of the most widely used methods for the simultaneous detection of *Cryptosporidium* and *Giardia* in water; however, this method does not determine (oo)cyst viability or infectivity (U.S. EPA, 2001). Most water samples contain few (oo)cysts, and concentration techniques are required to obtain even a small number of (oo)cysts. Moreover, neither organism can be reliably cultured from a water sample, although excystation and culture procedures have been established for both.

The routine analysis of protozoan parasites in water samples relies upon direct microscopic detection after concentration of particulate matter by filtration or centrifugation. Sample concentration is generally accomplished by filtration through a 1-µm nominal porosity wound filter (Jakubowski and Ericksen, 1978; APHA, 1995), 2-µm absolute porosity membrane filter (Spaulding *et al*., 1983; Wallis and Buchanan-Mappin, 1984; Ongerth, 1989), or 1-µm absolute porosity polysulphone filters (Fricker and Clancy, 1998). Recovery efficiencies ranging from 70 to 80% have been reported for the latter. Three filtration options have been validated for use with Method 1623, including the Envirochek™ capsule filter, CrypTest™ capsule filter, and Filta-Max™ foam filter (U.S. EPA, 2001).

If a wound or membrane filter is used for raw or treated drinking water, approximately 1000 L are pumped through the filter, and particulate matter is recovered by backflushing, rinsing, hand washing, or machine processing (using a stomacher bag) (LeChevallier *et al*., 1991a,b) and concentrated into a pellet. The background material in the pellet is then reduced by discontinuous density gradient centrifugation using zinc sulphate, 1.0 M sucrose, or a mixture of Percoll (Pharmacia Biotech) and sucrose. Centrifugation will cause denser particles to pass through the density medium and form a pellet at the bottom of the tube. Theoretically, *Giardia* cysts and *Cryptosporidium* oocysts will float on the surface of the density medium and may be recovered by pipette. Practically, the use of density media introduces significant errors. Dead *Cryptosporidium* oocysts tend to penetrate the density flotation medium and accumulate in the pellet. Sucrose and zinc sulphate density media selectively concentrate viable oocysts (Bukhari and Smith, 1995), whereas Percoll–sucrose concentrates empty (ghost) oocysts (LeChevallier *et al*., 1995). Material recovered from the surface of the density medium is then (re)centrifuged, and the final pellet is examined microscopically. Membrane filters offer higher recovery efficiencies, but the amount of water that can pass through without filter clogging is small, often only 10–20 L. Membrane filters are useful, however, because they retain more material and may be dissolved to recover cysts and oocysts (Aldom and Chagla, 1995).

Immunomagnetic separation (IMS)/immunocapture represents an alternative to the use of density-gradient flotation procedures and is increasingly being applied (McCuin *et al*., 2001; Moss and Arrowood, 2001; Rimhanen-Finne *et al*., 2001, 2002; Sturbaum *et al*., 2002; Ward *et al*., 2002). Typically, 10-L samples are processed, and the collected material is eluted with a detergent and concentrated by centrifugation. The pellet is resuspended in buffer and mixed with specific monoclonal antibodies attached to magnetized particles, also referred to as immunomagnetic beads. The (oo)cysts are then separated from the debris in a magnetic field. (Oo)cysts seeded into low-turbidity waters can be recovered with efficiencies of >90% (Fricker and Clancy, 1998). Although IMS aids in reducing false positives by reducing the level of debris on slide preparations for microscopic analysis, it is a relatively expensive procedure, with few manufacturers supplying the immunomagnetic beads (e.g., Dynal Inc., Aureon Biosystems, ImmuCell Inc., Miltenyi Biotech). Moreover, it has been reported that high levels of iron may inhibit immunomagnetic separation (Yakub and Stadterman-Knauer, 2000).

The use of antibodies specific for *Giardia* cysts and *Cryptosporidium* oocysts has greatly enhanced the probability of finding these organisms against a cluttered background and making a positive identification. Immunofluorescent staining is usually performed by trapping a portion of the pellet on a small membrane and rinsing antibodies through, but it can also be carried out in centrifuge tubes or on microscope slides (Sauch, 1985; LeChevallier *et al*., 1991a,b; Wallis, 1994). Unfortunately, there are some algae that are very close in size and staining characteristics to cysts and oocysts, and final identification often requires light, phase, and differential interference microscopy in addition to immunofluorescence. Murine monoclonal antibodies are commercially available from several manufacturers (Meridian Diagnostics Ltd., Cellabs Pty. Ltd., Waterborne Inc.) in both direct and indirect fluorescence kits. The intercalation of the vital dye DAPI highlights nuclei and aids identification (Grimason *et al*., 1994).

Detection of *Giardia* cysts and *Cryptosporidium* oocysts by immunofluorescence requires specialized equipment and a high level of technical skill. The analysis is tedious, expensive, and only semi-quantitative, but it has been used to confirm waterborne transmission of both parasites in many outbreaks and is being continuously improved. Clancy *et al.* (1994) conducted a blind survey (analysis of spiked wound filter samples) of 16 commercial labs in the United States and found that recovery of *Giardia* cysts ranged from 0.8 to 22.3% (average 9.3%) and that recovery of *Cryptosporidium* oocysts ranged from 1.3 to 5.5% (average 2.8%). In 1995, Health Canada commissioned a similar study of commercial, government, and research laboratories in Canada, and *Giardia* cyst recovery ranged from 0 to 90% (average 21%) for eight laboratories analysing 10 unknown samples. *Cryptosporidium* oocyst recovery ranged from 0 to 43% (average 5.3%) for the same samples (Clancy Environmental Consultants, Inc., 1996). LeChevallier *et al.* (1995) conducted a critical analysis of the immunofluorescence method and concluded that losses of *Cryptosporidium* oocysts typically exceed losses of *Giardia* cysts and that major losses occur during centrifugation and clarification.

Alternative techniques for detecting (oo)cysts following concentration and recovery have been proposed. For example, flow cytometry with fluorescence activated cell sorting (FACS) has

been used more and more as an alternative separation and enumeration technique (Vesey *et al.*, 1997; Bennett *et al.*, 1999; Reynolds *et al.*, 1999; Delaunay *et al.*, 2000; Lindquist *et al.*, 2001). However, the specificity and sensitivity of the FACS procedure are impaired by the presence of autofluorescent algae and cross-reaction of other organisms and particles with the monoclonal antibodies. Recent work by Ferrari *et al.* (2000) has led to the development of a two-colour FACS assay, which has addressed a number of these problems, leading to much improved specificity. Automated cell sorting devices (e.g., ChemScan RDI) have also been used to detect (oo)cysts and are relatively easy to operate (Rushton *et al.*, 2000; De Roubin *et al.*, 2002).

A number of molecular approaches have also been used in the detection of *Giardia* and *Cryptosporidium* (oo)cysts. PCR, especially in association with IMS (i.e., IMS-PCR), has been used by many groups (Deng *et al.*, 1997, 2000; Bukhari *et al.*, 1998; Di Giovanni *et al.*, 1999; Kostrzynska *et al.*, 1999; Rochelle *et al.*, 1999; Hallier-Soulier and Guillot, 2000; Hsu and Huang, 2001; McCuin *et al.*, 2001; Moss and Arrowood, 2001; Rimhanen-Finne *et al.*, 2001, 2002; Sturbaum *et al.*, 2002; Ward *et al.*, 2002). PCR is highly sensitive and specific and, when combined with other molecular biology techniques, such as restriction fragment length polymorphism (RFLP), can be used to discriminate between species and genotypes of *Cryptosporidium*. Genotype information can be used to help identify the potential host sources of *Cryptosporidium* responsible for an outbreak (Morgan *et al.*, 1997; Widmer, 1998; Lowery *et al.*, 2000, 2001a,b). PCR is highly sensitive (i.e., level of a single (oo)cyst), is amenable to automation, and may permit discrimination of viable and non-viable (oo)cysts. However, several PCR inhibitors are frequently found in water, including divalent cations and humic and fulvic acids (Sluter *et al.*, 1997). Despite the potential for inhibition of amplification, many PCR assays have been developed for detection of waterborne (oo)cysts. Some of these include primers directed at the 18S rRNA coding region (Lowery *et al.*, 2000; Ong *et al.*, 2002; Sturbaum *et al.*, 2002; Ward *et al.*, 2002) or mRNA coding for heat shock proteins (i.e., reverse transcriptase-PCR assay, or RT-PCR) (Stinear *et al.*, 1996; Kaucner and Stinear, 1998; Griffin *et al.*, 1999; Gobet and Toze, 2001; Karasudani *et al.*, 2001). Fluorescence *in situ* hybridization (FISH) has also been used in the detection of *Giardia* and *Cryptosporidium* (oo)cysts. However, because of relatively weak signals, there have been difficulties in microscopic interpretation, resulting in the limited use of this method (Deere *et al.*, 1998; Vesey *et al.*, 1998; Dorsch and Veal, 2001).

### 7.2 Viability and Infectivity

As indicated above, routine detection methods provide no indication of (oo)cyst viability or infectivity. Viability (but not infectivity) can be estimated by excystation. *Giardia* can be excysted using acid and enzymes such as trypsin and grown in TYI-S-33 medium (Diamond *et al.*, 1978; Rice and Schaefer, 1981), but the excystation rate for *G. duodenalis* is often low. *Cryptosporidium parvum* oocysts can also be excysted as a measure of viability (Black *et al.*, 1996). However, excystation methods have been shown to be relatively poor indicators of C*ryptosporidium* oocyst viability. Neumann *et al.* (2000b) observed that non-excysted oocysts recovered after commonly used excystation procedures are still infectious to neonatal mice.

Oocyst viability can also be determined using mouse infectivity assays. Both parasites can be used to infect experimental animals such as the gerbil (for *Giardia*) (Belosevic *et al*., 1983) or neonatal CD-1 mice (for *Cryptosporidium*) (Finch *et al*., 1993b), and this technique is useful for pilot plant studies or isolate collection; however, most analytical laboratories do not maintain animal colonies, and the expense is high. Culturing and animal infection are therefore more useful for research purposes, such as disinfection effectiveness, than for routine monitoring (Delaunay *et al.*, 2000; Korich *et al.*, 2000; Matsue *et al.*, 2001; Noordeen *et al.*, 2002; Okhuysen *et al.*, 2002; Rochelle *et al.*, 2002).

Various staining methods have been developed to assess (oo)cyst viability (Robertson *et al.*, 1998; Freire-Santos *et al.*, 2000; Neumann *et al.*, 2000b; Gold *et al.*, 2001; Iturriaga *et al.*, 2001). Of these, the fluorogenic dyes DAPI and PI and nucleic acid stains have received the most attention. In general, DAPI/PI give good correlation with *in vitro* excystation (Campbell *et al*., 1992). Three classes of (oo)cysts can be identified: (1) viable (permeable to DAPI, impermeable to PI), (2) non-viable (permeable to both DAPI and PI), and (3) quiescent or dormant (impermeable to both DAPI and PI, but potentially viable). Neumann *et al.* (2000a) demonstrated a strong correlation between SYTO-9® and SYTO-59® staining intensity with animal infectivity of freshly isolated *C. parvum* oocysts. Nucleic acid dyes have also proved useful for determining the viability and infectivity of (oo)cysts in environmental samples. Stains like SYTO-59 have been used successfully in conjunction with FITC-labelled antibodies to determine the viability and infectivity of (oo)cysts in water samples, because their fluorescence spectra do not overlap with that of FITC (Belosevic *et al.*, 1997; Bukhari *et al.*, 2000; Neumann *et al.*, 2000b). However, dye permeability and excystation procedures overestimate viability and potential infectivity of treated or disinfected oocysts (Jenkins *et al*., 1997).

Recent advances have facilitated the use of *in vitro* tissue culture assays to estimate infectivity of oocysts in water (Di Giovanni *et al.*, 1999; Hijjawi *et al.*, 2001;Weir *et al.*, 2001; Rochelle *et al.*, 2002). Concentrated water samples are disinfected and typically inoculated on human illeocaecal adenocarcinoma (HCT-8) cell monolayers. After a 24- to 48-hour incubation, the monolayer is examined for the presence of specific reproductive stages using either an indirect antigen–antibody assay (Slifko *et al*., 1997) or RT-PCR (Rochelle *et al.*, 1997). In a comparison experiment, average percent viabilities of *C. parvum* oocysts less than 2 months old were 42, 40, and 78% for tissue culture infectivity, excystation, and DAPI/PI assays, respectively (Slifko, 1998). There are several advantages to the cell culture assay, including its high sensitivity (i.e., single oocyst), applicability to analysis of raw and treated water samples, ease of performance, and rapid turnaround time for results. The disadvantage of this method is that it requires the maintenance of a cell line and is often subject to poor reproducibility among similar samples for quantitative assessments. RT-PCR has also been applied to the direct detection of viable *Giardia* and *C. parvum* in water concentrates (Kaucner and Stinear, 1998). When compared with the immunofluorescence assay (IFA) DAPI/PI method, the frequency of detection of viable *Giardia* increased from 24% with IFA to 69% with RT-PCR. In contrast, viable *C. parvum* were detected in only 3% of samples with RT-PCR compared with 14% by IFA

DAPI/PI, suggesting that other *Cryptosporidium* species were present in the samples. RT-PCR possesses some disadvantages, including the need for small processed volumes, possible inhibition by environmental constituents, inefficient extraction of RNA from (oo)cysts, and its non-quantitative nature.

Alternative viability assays have been proposed, including FISH and nucleic acid probes to detect 18S rRNA in *Giardia* and *Cryptosporidium* (Fricker and Clancy, 1998). The 18S molecule is abundant in viable (oo)cysts but declines rapidly in non-viable (oo)cysts. This method is limited by its inability to assess (oo)cyst infectivity. Further research is required to improve the detection limit and validate the assay.

## 8. Treatment Technology

The removal and inactivation of *Giardia* cysts and *Cryptosporidium* oocysts from raw water are complicated by their small size and resistance to commonly used oxidants such as chlorine. *Cryptosporidium* oocysts are more difficult to eliminate but appear to be less common than *Giardia* cysts in Canadian surface waters. The detection procedure is less efficient for *Cryptosporidium* oocysts, however, and the national prevalence rate may be higher than suspected. Waterborne outbreaks of giardiasis and cryptosporidiosis have resulted both from inadequate treatment and from improper operating procedures (Lin, 1985). The multiple-barrier approach to treatment, including watershed or wellhead protection, optimized filtration and disinfection, a well-maintained distribution system, and monitoring the effectiveness of treatment (e.g., turbidity, disinfection residuals, etc.), is by far the best approach to reduce the risk of infection to acceptable or non-detectable levels. In communities where filtration is not economically feasible, an effective watershed protection plan, adequate disinfection, an intact distribution system, and, possibly, recognized point-of-entry or point-of-use treatment must be relied upon to reduce these risks. An exhaustive review of available treatment options is beyond the scope of this document. These methods have been reviewed in water treatment manuals prepared by UMA Engineering Ltd. *et al.* (1993), Health Canada (1993), and the U.S. EPA (1991).

Effective water treatment begins with watershed management to minimize the input of faecal contamination from human and other animal sources, by controlling aquatic mammalian populations and locating raw water intakes as far as possible from sewage outfalls (Crockett and Haas, 1997). The possible flooding of sewage collection and treatment systems cannot be overlooked, and sudden increases in indicator organisms can give advance warning of problems (e.g., the outbreak at Temagami, Ontario). Coagulation, flocculation, clarification, filtration (including direct filtration), and post-disinfection are all commonly used to good effect in municipal water treatment plants to remove or inactivate *Giardia* cysts, but problems can still occur with *Cryptosporidium* oocysts (because of their small size and resistance to oxidants). There are approximately 1000 communities in Canada, including some major cities, that use surface water supplies and rely solely upon chlorination with varying degrees of watershed management.

The degree of treatment required to remove and/or inactivate (oo)cysts is dependent upon their concentrations in source waters. For this reason, source waters should be periodically monitored for (oo)cysts, particularly at times when concentrations are expected to be highest — for example, following spring freshet or heavy rains — to determine appropriate levels of treatment. For example, the U.S. EPA has proposed that if average concentrations of oocysts in source waters are less than 0.075/L, then conventional filtration achieving 3-log reduction of oocysts should be adequate (U.S. EPA, 2002a). Where monitoring for oocysts is not feasible, *E. coli* can be used as an indicator of their presence and treatment requirements. It has been proposed by the U.S. EPA that treatment known to achieve 3-log reduction of oocysts is adequate, provided average concentrations of *E. coli* do not exceed 10 colony-forming units (cfu)/100 mL in lakes or 50 cfu/100 mL in flowing streams.

## 8.1 Municipal-scale Technologies

Barring system-specific exemptions, all public (municipal) supplies should be disinfected. A disinfectant residual should be maintained throughout the distribution system at all times. In addition to disinfection, minimum treatment of all supplies derived from surface water sources and groundwater impacted by surface waters should include coagulation, flocculation, clarification, and filtration, or equivalent technologies.

The efficacy of disinfection can be predicted based on a knowledge of the residual concentration of disinfectant, temperature, pH (for chlorine only), and contact time to first customer. This relationship is commonly referred to as the CT concept, where CT is the product of C (the residual concentration of disinfectant, measured in mg/L) and T (the disinfectant contact time, measured in minutes). CT values for chlorine, chlorine dioxide, chloramine, and ozone developed by the U.S. EPA to achieve various degrees of inactivation of *Giardia* are provided in Annex A to guide water purveyors on the conditions required to achieve adequate inactivation of *Giardia*. The degree of inactivation considered adequate will, of course, depend upon the levels of *Giardia* in the raw water and the level of acceptable risk of illness. It is possible to reduce the viability of *Giardia* cysts by as much as 99.9% using chlorination alone, but long contact times are required. Where source waters are of high quality, either naturally or because of an effective watershed protection programme, disinfection achieving less than a 99.9% inactivation may be sufficient. Ozone and chlorine dioxide are much better disinfectants, but both are expensive and result in the formation of unwanted by-products (particularly chlorite, in the case of chlorine dioxide, and bromate, in the case of ozone). Ozone is a better choice but is unreliable when turbidity is high or variable, because cysts are protected in flocculated particles. Chloramine should not be used as a primary disinfectant. A discussion of the effect of these variables can be found in the water treatment manuals mentioned above or in von Huben (1991) for chlorine, chlorine dioxide, chloramine, and ozone.

Chlorination alone does not appear practical for the inactivation of *Cryptosporidium* (Finch *et al.*, 1993a). Watershed protection followed by filtration and an intact distribution system are at present the best available means of reducing the risk of waterborne

cryptosporidiosis in treatment plants relying upon chlorination. Work carried out by Finch *et al.* (1997) has shown that ozonation may be effective when used properly. These authors also demonstrated that the use of the two disinfectants sequentially gave better results than were obtained when either was used by itself. Chlorination followed by chloramination is more effective than previously believed and can inactivate *Cryptosporidium* oocysts by up to 1.6 logs when viability is measured by infection of mice. Chlorine following ozone or chlorine dioxide was particularly effective. A discussion of the effects of ozonation and other water treatment processes may be found in Smith *et al.* (1995).

Filtration with the aid of coagulation/flocculation followed by disinfection is the most practical method to achieve high removal/inactivation rates of cysts and oocysts. Payment and Franco (1993) showed that 99.998% of *Giardia* cysts and *Cryptosporidium* oocysts were removed from heavily polluted water by full conventional treatment (flocculation, settling, pre- and post-disinfection with chlorine dioxide and chlorine, and filtration) at three Montreal water treatment plants. Slow sand and diatomaceous earth filtration can also be highly effective. Optimizing filtration is desirable to provide stable filter performance and minimize breakthrough of cysts and oocysts. The recycling of filter backwash water containing cysts or oocysts, without treatment, is not recommended. Pressure filters vary widely in their performance and are not as reliable as properly operated gravity filter operations. Water types vary, however, and the choice of the most appropriate system must be made by experienced engineers after suitable pilot testing. An effective system of operator training and process control is essential in areas of known contamination where the risk is high. Monitoring of raw water for cysts and oocysts is useful for establishing prevalence, and analysis of treated water provides an indication of risk if viability or infectivity assays are incorporated. This can be particularly important in the spring after heavy rains or snowmelt. Useful data for process control may also be obtained by monitoring for cysts and oocysts or for their appropriate surrogates in treated water. Abnormal turbidity measurements or particle counts can quickly indicate a malfunction in filter performance. LeChevallier and Norton (1992) observed that particles >5 µm and turbidity were useful predictors of *Giardia* and *Cryptosporidium*. A 1-log removal of particles and turbidity corresponded to a 0.66- and 0.89-log removal of cysts and oocysts, respectively.

Membrane filtration has become an (increasingly) important component of drinking water treatment systems. Microfiltration, ultrafiltration, nanofiltration, and reverse osmosis are the most commonly used membrane processes for microbial removal. Microfiltration membranes have the largest pore size (≥0.1 µm), while reverse osmosis membranes have the smallest pore size (≥0.0001 µm) (Taylor and Weisner, 1999). While all of these processes appear effective in removing protozoan (oo)cysts, microfiltration and ultrafiltration are most commonly applied/used because of their cost-effectiveness. Jacangelo *et al.* (1995) evaluated the removal of *G. muris* and *C. parvum* from three source waters of varying quality using a variety of microfiltration and ultrafiltration membranes. Microfiltration membranes of 0.1 µm and 0.2 µm and ultrafiltration membranes of 100, 300, and 500 kilodaltons were assessed. Both microfiltration and ultrafiltration resulted in log removals of >4.7–7.0 for *G. muris* and >4.4–7.0

for *C. parvum.* Karami *et al.* (1999) also evaluated the effectiveness of microfiltration membranes (0.2 µm) for removal of (oo)cysts. Average log removals of 3.3–4.4 were reported for *Giardia*-sized particles, and log removals of 2.3–3.5 were reported for *Cryptosporidium*-sized particles. More recently, States *et al.* (1999) reported absolute removal of *Cryptosporidium* and *Giardia* by microfiltration, and Parker *et al.* (1999) reported a 5.3-log removal of *C. parvum* using microfiltration membranes (0.2 µm). Although membrane filtration is highly effective for microbial removal, including removal of protozoan (oo)cysts, membrane fouling (caused by accumulation of particles, chemicals, and biological growth on membrane surfaces) and degradation (caused by hydrolysis and oxidation) must be considered. Because the physical characteristics of the membrane could vary during the manufacturing process by different manufacturers, the cyst and oocyst removal efficiency for a specific membrane must be demonstrated through challenge testing and verified by direct integrity testing (e.g., measuring pressure loss upstream or downstream of the membrane, or assessing removal of spiked particulates using a marker-based approach). More detailed information on filtration techniques can be found in Health Canada's Turbidity supporting document (http://www.hc-sc.gc.ca/hecs-sesc/water/publications/turbidity1/toc.htm).

UV light disinfection is an emerging (alternative) treatment approach that appears to be highly effective for inactivating *Giardia* and *Cryptosporidium*. Whereas earlier studies (i.e., those prior to 1998) (Rice and Hoff, 1981; Karanis *et al.*, 1992; Lorenzo-Lorenzo *et al.*, 1993; Campbell *et al.*, 1995) reported protozoan inactivation only at very high UV doses, recent studies have shown that low doses can achieve substantial inactivation (Clancy *et al.*, 1998; Bukhari *et al.*, 1999; Craik *et al.*, 2000, 2001; Belosevic *et al.*, 2001; Drescher *et al.*, 2001; Linden *et al.*, 2001, 2002; Shin *et al.*, 2001; Campbell and Wallis, 2002; Mofidi *et al.*, 2002; Rochelle *et al.*, 2002). These contrasting observations are the result of *in vitro* viability assays, used in earlier studies, which greatly overestimate the UV dose necessary for inactivation (Clancy *et al.*, 1998; Bukhari *et al.*, 1999; Craik *et al.*, 2000). Current studies rely on *in vivo* assays (e.g., neonatal mouse model) and cell culture techniques for assessing (oo)cyst inactivation. Based on these and other studies, the U.S. EPA developed a UV light dose table, also known as an "IT table," which was released in June 2003 as part of the draft "Ultraviolet Disinfection Guidance Manual" (U.S. EPA, 2003). According to this dose table, a (low-pressure UV light) dose of 12 mJ/cm$^2$ is required for a 3-log inactivation of *Cryptosporidium* (refer to Table B.1). In contrast, a dose greater than 140 mJ/cm$^2$ is required to achieve the same level of inactivation for certain viruses. Although UV light disinfection appears to be highly effective for inactivating protozoans, the possibility of reactivation after UV light treatment must be considered. The ability of microorganisms to repair their UV-damaged DNA (reactivate) has been reported. Belosevic *et al.* (2001) observed reactivation of *G. muris* after treatment with relatively low doses (<25 mJ/cm$^2$) of medium-pressure UV light. However, *C. parvum* and *G. muris* (oo)cysts exposed to medium-pressure UV doses of ≥60 mJ/cm$^2$ did not exhibit reactivation after treatment. Linden *et al.* (2002) did not observe any reactivation of *G. lamblia* after exposure to UV light doses of 16 and 40 mJ/cm$^2$.

Even the most sophisticated municipal treatment system cannot provide water that is absolutely free of disease-causing microorganisms all the time. The real goal of treatment is to reduce the presence of disease-causing organisms and associated health risks to an acceptable or safe level. The risk of illness can be minimized by maximizing the number and efficacy of treatment barriers present. This level of acceptability or safety may vary from community to community and depends on many site-specific environmental, human health, and economic conditions. For example, determinations of safety must consider the various types and, where possible, concentrations of infectious organisms in the raw water, the seriousness of the illnesses they cause, the degree of resistance to the illnesses in the exposed population, the extent of disease surveillance in the community, and the available financial resources that must be shared among drinking water treatment and other common services in a community. Nevertheless, minimum treatment of all supplies derived from surface water sources and groundwater under the influence of surface water should include coagulation, flocculation, clarification, and filtration, or equivalent technologies, in addition to disinfection. Because *Giardia* and *Cryptosporidium* are ubiquitous in surface waters in Canada and are more resistant to disinfection than most other infectious organisms, it is desirable that treatment known to achieve at least a 99.9% reduction of *Giardia* and *Cryptosporidium* be in place.

In the United States, the EPA has promulgated the Surface Water Treatment Rule (SWTR) to control the presence of *Giardia* and viruses in public drinking water systems using either surface water or groundwater under the influence of surface water (U.S. EPA, 1989). All systems using filtered or unfiltered surface water must achieve at least a 99.9% (3-log) removal and/or inactivation of *G. lamblia* cysts. This level of removal/inactivation is believed to reduce the risk of waterborne giardiasis to less than $10^{-4}$ (i.e., <1 in 10 000 people infected) per year. Under the rule, a public water system using surface water must use filtration unless it meets certain water quality, operational, and public health standards. It is assumed that filtration removes 99% (2-log) of *Giardia* cysts and that disinfection need only provide a further 90% (1-log) inactivation (see Annex A). Systems using conventional treatment that are able to achieve turbidity levels of less than 0.5 nephelometric turbidity units (NTU) in the filtered water in 95% of samples are assumed to achieve 2.5-log removal of *Giardia* cysts, providing that coagulation and flocculation conditions are optimized for turbidity removal. Disinfection in these systems need only inactivate 68.4% (0.5-log) of *Giardia* cysts (see Annex A).

Recognizing that systems with very poor source water may not be adequately protected by a 3-log reduction in *Giardia* cysts and that the requirements for *Giardia* reduction may not be applicable to *Cryptosporidium*, the U.S. EPA has promulgated an Interim Enhanced Surface Water Treatment Rule (IESWTR) (U.S. EPA, 1998). One component of this rule establishes that systems required to filter under the SWTR must achieve a 2-log removal of *Cryptosporidium*. Conventional or direct filtration plants producing water with turbidities of 0.3 NTU or less in 95% of monthly samples and in which the turbidity never exceeds 1 NTU are deemed to meet this requirement. The Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR), promulgated in January 2002, builds upon the requirements of the SWTR and the IESWTR and

is designed to strengthen microbial controls for small systems (i.e., those serving <10 000 people) that use surface water and groundwater under the influence of surface water (U.S. EPA, 2002b). The U.S. EPA recently proposed the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR), which will apply to all public water systems that use surface water or groundwater under the influence of surface water. This rule defines system-specific treatment requirements and assigns systems into different categories (known as bins) based on the results of source water *Cryptosporidium* monitoring. For example, systems serving at least 10 000 people with a *Cryptosporidium* source water concentration of >0.075/L but <1.0/L (as determined by at least 1 year of monitoring) are classified into Bin 2. Systems within this bin category are required to achieve a total *Cryptosporidium* removal/inactivation of at least 4 logs (U.S. EPA, 2002a). The LT2ESWTR will also establish a "toolbox" of various disinfection technologies, such as ozone and UV light, that can be used to obtain *Cryptosporidium* disinfection credits. Included in the toolbox will be specific disinfection criteria, such as ozone CT products and UV IT dosages required for certain levels of *Cryptosporidium* inactivation.

Drinking water technologies meeting the turbidity limits prescribed in the *Guidelines for Canadian Drinking Water Quality* (Health Canada, 2004) can apply the estimated potential removal credits for *Giardia*, *Cryptosporidium*, and enteric viruses given in Table 1. These log reduction credits are based on the mean or median removals established by the U.S. EPA as part of the LT2ESWTR (U.S. EPA, 2002a). Facilities that do not meet the requirements or facilities that believe they can achieve a higher log credit than is automatically given can be granted a log reduction credit based on a demonstration of performance.

## 8.2 Residential-scale Treatment Options

Minimum treatment of all semi-public and private supplies derived from surface water sources or groundwater under the influence of surface water should include adequate filtration (or equivalent technologies) and disinfection. Semi-public and private supplies are considered to be residential-scale for the purposes of this document.

An array of options is available for treating source waters to provide high-quality pathogen-free drinking water. For public systems, these include various filtration methods, disinfection with chlorine-based compounds, or alternative technologies, such as UV light or ozonation. Semi-public and private systems can employ many of the same technologies but on a smaller scale, along with others, such as distillation, not used by public systems. These technologies have been incorporated into point-of-entry devices that treat all water entering the system or point-of-use devices that treat water only at a single location — for example, at the kitchen tap. The commonly used drinking water disinfectants are chlorine, chloramine, chlorine dioxide, ozone, and UV light. All of the above disinfectants are used in public systems; however, semi-public and private systems using disinfection are more apt to rely on chlorine or UV light.

**Table 1: *Giardia*, *Cryptosporidium*, and virus potential removal credits for various technologies meeting the turbidity limits prescribed in the *Guidelines for Canadian Drinking Water Quality***

| Technology | Cyst/oocyst credit[c] | Virus credit |
|---|---|---|
| Conventional filtration[a] | 3.0 log | 2.0 log |
| Direct filtration[a] | 2.5 log | 1.0 log |
| Slow sand or diatomaceous earth filtration[a] | 3.0 log | 2.0 log |
| Micro- and ultrafiltration, nanofiltration, and reverse osmosis[b] | Removal efficiency demonstrated through challenge testing and verified by direct integrity testing | No credit for micro- and ultrafiltration; for nanofiltration and reverse osmosis, removal efficiency demonstrated through challenge testing and verified by direct integrity testing |

[a] Conventional/direct/slow sand/diatomaceous earth filtration should be followed by free chlorination to obtain additional virus credit.
[b] Micro- and ultrafiltration should be followed by free chlorination for the inactivation of viruses.
[c] Depending on (oo)cyst levels in source water, additional treatment is required using UV light, ozone, chlorine, or chlorine dioxide.

Health Canada does not recommend specific brands of drinking water treatment devices, but it strongly recommends that consumers look for a mark or label indicating that the device has been certified by an accredited certification body as meeting the appropriate NSF International/ American National Standards Institute (ANSI) standard. These standards have been designed to safeguard drinking water by helping to ensure the material safety and performance of products that come into contact with drinking water. Certification organizations provide assurance that a product or service conforms to applicable standards. In Canada, the following organizations have been accredited by the Standards Council of Canada (http://www.scc.ca) to certify drinking water devices and materials as meeting the appropriate NSF/ANSI standards:

- Canadian Standards Association International (http://www.csa-international.org);
- NSF International (http://www.nsf.org);
- Underwriters Laboratories Inc. (http://www.ul.com);
- Quality Auditing Institute (http://www.qai.org); and
- International Association of Plumbing & Mechanical Officials (http://www.iapmo.org).

## 9. Assessment

*Giardia* and *Cryptosporidium* are infectious protozoans that can be transmitted by water, poor hygiene, sexual activities, and food. Both these organisms are enteric pathogens that cause serious illness in immunocompetent and immunocompromised individuals. Cryptosporidiosis is the more serious of the two because (1) most cases are symptomatic, (2) there is no effective

drug treatment for adults, and (3) the illness is capable of causing death. *Cryptosporidium parvum* may be fatal in immunocompromised individuals, particularly those suffering from AIDS.

The risk of becoming infected by protozoan cysts or oocysts in drinking water depends upon (1) the number of viable cysts or oocysts ingested (dose), (2) the virulence or infectivity of the ingested cysts or oocysts, and (3) the susceptibility of the host population to infection. The dose can be estimated based upon measurements of cyst or oocyst concentrations in drinking water and the amount of water consumed over the period of exposure. The viability of cysts or oocysts can be estimated using dye exclusion or excystation, but, as noted above, these methods probably provide an overestimate of true viability. Infectivity can be determined from animal or human infection assays. Human tissue culture assays can also be used to determine *Cryptosporidium* infectivity. The susceptibility of individuals in the host population varies by age, immunological status, history of previous exposures, and other genetic and environmental factors. These factors vary widely between individuals, so it is only meaningful to estimate the risk to populations in terms of the number of individuals who may become infected rather than the number of individuals who may become ill.

The application of a mathematical risk model to waterborne giardiasis and cryptosporidiosis has been proposed by Regli *et al.* (1991), Rose *et al.* (1991), Haas and Rose (1994), and Teunis *et al*. (1997), based on work by Haas (1983), Rendtorff (1978), and DuPont *et al*. (1995). Using this model, the probability ($P_i$) of an infection resulting from the ingestion of a single volume of liquid (V) containing μ organisms per litre can be described by a simple exponential probability density function:

$$P_i = 1 - e^{-r\mu V} \quad (1)$$

where r is the fraction of ingested organisms that survive to initiate infection. The probability that less than 1 person in 10 000 per year will become infected after exposure to pathogens in drinking water has been proposed as an acceptable level of risk (Regli *et al.*, 1991). This corresponds to an acceptable daily risk of $2.75 \times 10^{-7}$ or, rearranging equation 1, to an acceptable daily intake of N organisms ($N = \mu V$), equivalent to:

$$N = -(1/r) \ln (1 - P_i) \quad (2)$$

The proposed exponential model makes several assumptions that introduce uncertainty into the assessment. First, it assumes that the distribution of cysts and oocysts in water is random (Poisson). However, it is likely that the (oo)cysts are not randomly distributed but rather occur in clusters, either loosely associated with each other or tightly bound to or within particles (Gale, 1996). Such clustering means that most consumers will not be exposed to any pathogens, but a small portion will be exposed to infectious doses. Models that do not account for clustering will therefore underestimate the probability of exposure and infection. Second, as there are no

practical procedures to determine the viability and infectivity of the small number of cysts and oocysts recovered from drinking water, it is assumed that one cyst or oocyst is capable of causing an infection. This assumption will therefore lead to an overestimation of the risk. Until routine practical methods to identify human-infectious strains of *Giardia* and *Cryptosporidium* are available, it is desirable, from a health protection perspective, to assume that all viable (oo)cysts recovered from drinking water are infectious to humans unless evidence to the contrary exists. It must also be remembered that the current risk assessment model assumes that errors caused by overestimating viability are at least partially counterbalanced by poor (oo)cyst recoveries during the detection method. Third, the model assumes a daily tap water consumption of 1.5 L per person; for many segments of the population, however, some or most of this quantity is boiled and therefore of no microbiological significance. For example, a study in the United Kingdom has indicated that, on average, 89% of the tap water consumed is boiled (Gale, 1996). Assessments that do not consider boiled tap water consumption will yield a conservative estimate of risk of infection. Fourth, dose–response experiments use single laboratory strains; therefore, it is unknown whether organism age, prior environmental exposures, or strain type influences infectivity. Furthermore, the experiments are conducted on healthy humans or other animals and do not consider the immune status of the exposed population, and they are therefore likely to underestimate the risk. Finally, all cysts and oocysts ingested are considered to be viable and pathogenic to humans, which is probably not true, but it is assumed that errors caused by inefficiency in cyst and oocyst recovery during the analytical procedure are counterbalanced by overestimates in viability and pathogenicity.

### 9.1 *Giardia*

The risk model has been applied to Canadian *Giardia* data by Wallis *et al*. (1995). Based on a review of human and gerbil infection studies, they proposed that a value of $r = 0.0105$ be used in the model. The use of gerbil infection data may be questioned, but these data show that different isolates have markedly different $ID_{50}$ values within genetically similar hosts. Until more human dose–response data become available, it is proposed that this value be used as the most comprehensive estimate of the infectivity of *Giardia* in humans, recognizing that both host and parasite are variable in their response.

Using equation (2), the theoretical acceptable daily intake of human-infectious *Giardia* cysts can be calculated to be $2.6 \times 10^{-5}$. If it is assumed that each person consumes 1.5 L of tap water per day, then a theoretical maximum acceptable concentration (MAC) for *Giardia* would be $1.7 \times 10^{-2}$ cysts/1000 L. This concentration is well below the detection limits of current methods and would require filtration of at least 60 000 L of water to detect a single cyst. A more practical approach would be to periodically monitor the source water for cysts, especially during times when highest cyst concentrations would be expected, and to determine the adequacy of treatment by comparing existing treatment with published treatment guidelines (U.S. EPA, 1991; Health Canada, 1993; UMA Engineering Ltd. *et al.*, 1993). For example, water treatment plants

maintaining a 3-log reduction could accept raw water levels of 1.7 cysts/100 L or less and continue to maintain an annual risk of infection of less than $1 \times 10^{-4}$.

Monitoring data by Wallis *et al*. (1995) have shown that cyst concentrations averaging 3 cysts/1000 L in finished water, which produce a theoretical daily risk of $4.75 \times 10^{-5}$ and a theoretical annual risk of 0.0172 (17 cases in 1000), do not cause detectable outbreaks of waterborne giardiasis. Monitoring data reported by others in the absence of outbreaks present similar levels of *Giardia* cysts in treated drinking water (LeChevallier *et al.*, 1991b; Payment and Franco, 1993; Goatcher and Fok, 2000). Although an annual rate of 17 cases per 1000 people may not cause noticeable levels of giardiasis, drinking water treatment officials in large centres are understandably reluctant to be responsible for outbreaks that could potentially affect tens of thousands of people annually. The theoretical infection rate predicted by the model is reduced to the much lower reported illness rate from official data by several factors. These could include (1) variable viability and infectivity of cysts, (2) variable susceptibility of the host population to infection, (3) asymptomatic cases, (4) use of effective treatment devices in the home or other sources of drinking water, and (5) incomplete reporting. Based on known waterborne outbreaks of giardiasis in Canada, Wallis *et al*. (1996) proposed an action level of 3–5 cysts/100 L in treated drinking water.

### 9.2 *Cryptosporidium*

The risk model described for *Giardia* can also be applied to *Cryptosporidium*, but there are fewer data available from dose–response experiments. An excellent data set was published by DuPont *et al*. (1995), which permits calculation of an r value of 0.0047 (Haas *et al.*, 1996). This value is close to the value for *Giardia* (r = 0.0105), and the resulting calculations are almost the same after rounding. Using the dose–response data of DuPont *et al*. (1995), Haas *et al*. (1996) determined that the theoretical acceptable daily intake of oocysts is $6.54 \times 10^{-5}$. Assuming a daily tap water consumption of 1.5 L/person, the theoretical MAC would be $4.4 \times 10^{-2}$ oocysts/1000 L. This concentration is well below the detection limits of current methods and would require filtration of at least 23 000 L of water to detect a single oocyst. A more practical approach would be to monitor the source water for oocysts, particularly during periods when levels would be expected to be high, and to determine the adequacy of treatment by comparing existing treatment with published treatment guidelines. Drinking water treatment plants using direct or conventional filtration optimized to achieve a filtered water turbidity of <0.3 NTU can be expected to achieve at least a 2-log removal of *Cryptosporidium* oocysts (Patania *et al.*, 1995). Thus, in theory, filtration plants could accept raw water containing 0.44 oocysts/100 L or less and maintain an annual risk of infection of less than $1 \times 10^{-4}$. Using the model, Haas and Rose (1994) estimated that the mean concentration of *Cryptosporidium* in finished water during the outbreak in Milwaukee was 1.2 oocysts/L. Perz *et al.* (1998) used a risk assessment model to determine the potential role of New York City tap water in the transmission of endemic cryptosporidiosis. Assuming a reasonable baseline concentration of 1 oocyst/1000 L, they estimated that tap water is responsible for more than 6000 infections annually, with 99% occurring in the non-AIDS

subgroup. The authors concluded that low-level transmission via tap water may represent an important exposure route for endemic cryptosporidiosis. Based upon outbreak and routine monitoring data from the United States and the United Kingdom, Haas and Rose (1995) proposed a finished water action level of 10–30 oocysts/100 L.

### 9.3 Balancing Risks

Havelaar *et al.* (2000) recently described a quantitative risk assessment approach for comparing the risks associated with disinfection by-products with those associated with *C. parvum* infection. This group evaluated the use of disability-adjusted life-years (DALYs) as a measure of disease burden. DALYs take into account the loss of healthy life due to mortality and morbidity and are expressed as:

$$DALY = LYL + YLD$$

where LYL represents the number of life-years lost due to mortality and YLD represents the number of years lived with a disability, weighted with a factor between 0 and 1 for severity of the disability. LYL represents the product of deaths due to a particular illness/disease (d) and the standard life expectancy at the age of death due to that illness/disease (e*), whereas YLD represents the product of the number of persons affected by a non-lethal illness/disease (N), the duration of the illness/disease (L), and its severity (W). Overall, for a given agent, the population health burden is calculated as:

$$DALY = \sum_i d_i e^*_i + \sum_i N_i L_i W_i$$

Using this approach, Havelaar *et al.* (2000) compared the risks associated with ozonation of water with those associated with *C. parvum* infection and determined that ozonation led to an approximate 7-fold reduction in the median risk of *C. parvum* infection. This decrease was associated with an increase in bromate concentration, along with an increase in the health burden of renal cancer. This group concluded that the health benefits of preventing gastroenteritis in the general population and premature death in immunocompromised patients (associated with *C. parvum* infection) outweigh the health losses associated with premature death from renal cancer (due to exposure to increased concentrations of bromate) by a factor of greater than 10 (i.e., net benefit of 1 DALY/million person-years).

Although the DALY approach is being used by many groups, no consensus has been reached regarding the use of a common health metric. The U.S. EPA, for example, has been assessing quality-adjusted life-years (QALYs) as a measure of disease burden (Murphy *et al.*, 2000).

## 10. Rationale

It is not possible at this time to establish MACs for *Giardia* and *Cryptosporidium* in drinking water. Methods available for the detection of cysts and oocysts suffer from low recovery rates, do not provide any information on their viability or human infectivity, and provide temporally restricted information about potential parasite numbers. Nevertheless, until better monitoring data and information on the viability and infectivity of cysts and oocysts present in drinking water are available, measures should be implemented to reduce the risk of illness as much as possible. If the presence of viable, human-infectious cysts or oocysts is known or suspected in source waters, or if *Giardia* or *Cryptosporidium* has been responsible for past waterborne outbreaks in a community, a treatment and distribution regime and a watershed or wellhead protection plan (where feasible) or other measures known to reduce the risk of illness should be implemented. Periodic monitoring of source waters for changes in cyst and oocyst concentrations should be used to adjust treatment processes and to confirm cyst and oocyst concentrations and the adequacy of current treatment processes. This guideline is primarily intended to protect the health of the immunocompetent population. Immunocompromised people may be at increased risk of illness and should discuss their risks and the need for extra precautionary measures with their physicians.

Even the most sophisticated municipal treatment system cannot provide water that is absolutely free of disease-causing microorganisms all the time. The real goal of treatment is to reduce the presence of disease-causing organisms and associated health risks to an acceptable or safe level. This level of acceptability or safety may vary from community to community and depends on many site-specific environmental, human health, and economic conditions. Nevertheless, minimum treatment of all supplies derived from surface water sources and groundwater impacted by surface water should include coagulation, flocculation, clarification, and filtration, or equivalent technologies, in addition to disinfection. Because *Giardia* and *Cryptosporidium* are ubiquitous in surface waters in Canada and are more resistant to disinfection than most other infectious organisms, it is desirable that treatment known to achieve at least a 99.9% reduction of *Giardia* and *Cryptosporidium* be in place.

Many disciplines, including those responsible for source water protection, treatment plant operation, water quality monitoring, and disease surveillance, are involved in protecting the public from drinking water-related illnesses. Therefore, it is essential that these groups have cooperative strategies in place, not only to control waterborne outbreaks of giardiasis or cryptosporidiosis promptly, but also to manage effectively any incident where the microbiological safety of the water may have been compromised.

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**ANNEX A:**

**CT Tables for the Inactivation of *Giardia lamblia* Cysts by Chlorine, Chlorine Dioxide, Chloramine, and Ozone at Various Temperatures**

## *CHLORINE*

### *(a) 99.9% (3-log) inactivation*

**Table A.1. CT values (in mg·min/L) for 99.9% inactivation of *Giardia lamblia* cysts by free chlorine at 0.5°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 137 | 163 | 195 | 237 | 277 | 329 | 390 |
| 0.6 | 141 | 169 | 200 | 239 | 286 | 342 | 407 |
| 0.8 | 145 | 172 | 205 | 246 | 295 | 354 | 422 |
| 1.0 | 148 | 176 | 210 | 253 | 304 | 365 | 437 |
| 1.2 | 152 | 180 | 215 | 259 | 313 | 376 | 451 |
| 1.4 | 155 | 184 | 221 | 266 | 321 | 387 | 464 |
| 1.6 | 157 | 189 | 226 | 273 | 329 | 397 | 477 |
| 1.8 | 162 | 193 | 231 | 279 | 338 | 407 | 489 |
| 2.0 | 165 | 197 | 236 | 286 | 346 | 417 | 500 |
| 2.2 | 169 | 201 | 242 | 297 | 353 | 426 | 511 |
| 2.4 | 172 | 205 | 247 | 298 | 361 | 435 | 522 |
| 2.6 | 175 | 209 | 252 | 304 | 368 | 444 | 533 |
| 2.8 | 178 | 213 | 257 | 310 | 375 | 452 | 543 |
| 3.0 | 181 | 217 | 261 | 316 | 382 | 460 | 552 |

**Table A.2. CT values (in mg·min/L) for 99.9% inactivation of *Giardia lamblia* cysts by free chlorine at 5°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 97 | 117 | 139 | 166 | 198 | 236 | 279 |
| 0.6 | 100 | 120 | 143 | 171 | 204 | 244 | 291 |
| 0.8 | 103 | 122 | 146 | 175 | 210 | 252 | 301 |
| 1.0 | 105 | 125 | 149 | 179 | 216 | 260 | 312 |
| 1.2 | 107 | 127 | 152 | 183 | 221 | 267 | 320 |
| 1.4 | 109 | 130 | 155 | 187 | 227 | 274 | 329 |
| 1.6 | 111 | 132 | 158 | 192 | 232 | 281 | 337 |
| 1.8 | 114 | 135 | 162 | 196 | 238 | 287 | 345 |
| 2.0 | 116 | 138 | 165 | 200 | 243 | 294 | 353 |
| 2.2 | 118 | 140 | 169 | 204 | 248 | 300 | 361 |
| 2.4 | 120 | 143 | 172 | 209 | 253 | 306 | 368 |
| 2.6 | 122 | 146 | 175 | 213 | 258 | 312 | 375 |
| 2.8 | 124 | 148 | 178 | 217 | 263 | 318 | 382 |
| 3.0 | 126 | 151 | 182 | 221 | 268 | 324 | 389 |

**Table A.3. CT values (in mg·min/L) for 99.9% inactivation of *Giardia lamblia* cysts by free chlorine at 10°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 73 | 88 | 104 | 125 | 149 | 177 | 209 |
| 0.6 | 75 | 90 | 107 | 128 | 153 | 183 | 218 |
| 0.8 | 78 | 92 | 110 | 131 | 158 | 189 | 226 |
| 1.0 | 79 | 94 | 112 | 134 | 162 | 195 | 234 |
| 1.2 | 80 | 95 | 114 | 137 | 166 | 200 | 240 |
| 1.4 | 82 | 98 | 116 | 140 | 170 | 206 | 247 |
| 1.6 | 83 | 99 | 119 | 144 | 174 | 211 | 253 |
| 1.8 | 86 | 101 | 122 | 147 | 179 | 215 | 259 |
| 2.0 | 87 | 104 | 124 | 150 | 182 | 221 | 265 |
| 2.2 | 89 | 105 | 127 | 153 | 186 | 225 | 271 |
| 2.4 | 90 | 107 | 129 | 157 | 190 | 230 | 276 |
| 2.6 | 92 | 110 | 131 | 160 | 194 | 234 | 281 |
| 2.8 | 93 | 111 | 134 | 163 | 197 | 239 | 287 |
| 3.0 | 95 | 113 | 137 | 166 | 201 | 243 | 292 |

**Table A.4. CT values (in mg·min/L) for 99.9% inactivation of *Giardia lamblia* cysts by free chlorine at 15°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 49 | 59 | 70 | 83 | 99 | 118 | 140 |
| 0.6 | 50 | 60 | 72 | 86 | 102 | 122 | 146 |
| 0.8 | 52 | 61 | 73 | 88 | 105 | 126 | 151 |
| 1.0 | 53 | 63 | 75 | 90 | 108 | 130 | 156 |
| 1.2 | 54 | 64 | 76 | 92 | 111 | 134 | 160 |
| 1.4 | 55 | 65 | 78 | 94 | 114 | 137 | 165 |
| 1.6 | 56 | 66 | 79 | 96 | 116 | 141 | 169 |
| 1.8 | 57 | 68 | 81 | 98 | 119 | 144 | 173 |
| 2.0 | 58 | 69 | 83 | 100 | 122 | 147 | 177 |
| 2.2 | 59 | 70 | 85 | 102 | 124 | 150 | 181 |
| 2.4 | 60 | 72 | 86 | 105 | 127 | 153 | 184 |
| 2.6 | 61 | 73 | 88 | 107 | 129 | 156 | 188 |
| 2.8 | 62 | 74 | 89 | 109 | 132 | 159 | 191 |
| 3.0 | 63 | 76 | 91 | 111 | 134 | 162 | 195 |

**Table A.5. CT values (in mg·min/L) for 99.9% inactivation of *Giardia lamblia* cysts by free chlorine at 20°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 36 | 44 | 52 | 62 | 74 | 89 | 105 |
| 0.6 | 38 | 45 | 54 | 64 | 77 | 92 | 109 |
| 0.8 | 39 | 46 | 55 | 66 | 79 | 95 | 113 |
| 1.0 | 39 | 47 | 56 | 67 | 81 | 98 | 117 |
| 1.2 | 40 | 48 | 57 | 69 | 83 | 100 | 120 |
| 1.4 | 41 | 49 | 58 | 70 | 85 | 103 | 123 |
| 1.6 | 42 | 50 | 59 | 72 | 87 | 105 | 126 |
| 1.8 | 43 | 51 | 61 | 74 | 89 | 108 | 129 |
| 2.0 | 44 | 52 | 62 | 75 | 91 | 110 | 132 |
| 2.2 | 44 | 53 | 63 | 77 | 93 | 113 | 135 |
| 2.4 | 45 | 54 | 65 | 78 | 95 | 115 | 139 |
| 2.6 | 46 | 55 | 66 | 80 | 97 | 117 | 141 |
| 2.8 | 47 | 56 | 67 | 81 | 99 | 119 | 143 |
| 3.0 | 47 | 57 | 68 | 83 | 101 | 122 | 146 |

**Table A.6. CT values (in mg·min/L) for 99.9% inactivation of *Giardia lamblia* cysts by free chlorine at 25°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 24 | 29 | 35 | 42 | 50 | 59 | 70 |
| 0.6 | 25 | 30 | 36 | 43 | 51 | 61 | 73 |
| 0.8 | 26 | 31 | 37 | 44 | 53 | 63 | 75 |
| 1.0 | 26 | 31 | 37 | 45 | 54 | 65 | 78 |
| 1.2 | 27 | 32 | 38 | 46 | 55 | 67 | 80 |
| 1.4 | 27 | 33 | 39 | 47 | 57 | 69 | 82 |
| 1.6 | 28 | 33 | 40 | 48 | 58 | 70 | 84 |
| 1.8 | 29 | 34 | 41 | 49 | 60 | 72 | 86 |
| 2.0 | 29 | 35 | 41 | 50 | 61 | 74 | 89 |
| 2.2 | 30 | 35 | 42 | 51 | 62 | 75 | 90 |
| 2.4 | 30 | 36 | 43 | 52 | 63 | 77 | 92 |
| 2.6 | 31 | 37 | 44 | 53 | 65 | 78 | 94 |
| 2.8 | 31 | 37 | 45 | 54 | 66 | 80 | 96 |
| 3.0 | 32 | 38 | 46 | 55 | 67 | 81 | 97 |

### *(b) 90% (1-log) inactivation*

**Table A.7. CT values (in mg·min/L) for 90% inactivation of *Giardia lamblia* cysts by free chlorine at 0.5°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 46 | 54 | 65 | 79 | 92 | 110 | 130 |
| 0.6 | 47 | 56 | 67 | 80 | 95 | 114 | 136 |
| 0.8 | 48 | 57 | 68 | 82 | 98 | 113 | 141 |
| 1.0 | 49 | 59 | 70 | 84 | 101 | 122 | 146 |
| 1.2 | 51 | 60 | 72 | 86 | 104 | 125 | 150 |
| 1.4 | 52 | 61 | 74 | 89 | 107 | 129 | 155 |
| 1.6 | 52 | 63 | 75 | 91 | 110 | 132 | 159 |
| 1.8 | 54 | 64 | 77 | 93 | 113 | 136 | 163 |
| 2.0 | 55 | 66 | 79 | 95 | 115 | 139 | 167 |
| 2.2 | 56 | 67 | 81 | 99 | 118 | 142 | 170 |
| 2.4 | 57 | 68 | 82 | 99 | 120 | 145 | 174 |
| 2.6 | 58 | 70 | 84 | 101 | 123 | 148 | 178 |
| 2.8 | 59 | 71 | 86 | 103 | 125 | 151 | 181 |
| 3.0 | 60 | 72 | 87 | 105 | 127 | 153 | 184 |

**Table A.8. CT values (in mg·min/L) for 90% inactivation of *Giardia lamblia* cysts by free chlorine at 5°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 32 | 39 | 46 | 55 | 66 | 79 | 93 |
| 0.6 | 33 | 40 | 49 | 57 | 68 | 81 | 97 |
| 0.8 | 34 | 41 | 49 | 58 | 70 | 84 | 100 |
| 1.0 | 35 | 42 | 50 | 60 | 72 | 87 | 104 |
| 1.2 | 36 | 42 | 51 | 61 | 74 | 89 | 107 |
| 1.4 | 36 | 43 | 52 | 62 | 76 | 91 | 110 |
| 1.6 | 37 | 44 | 53 | 64 | 77 | 94 | 112 |
| 1.8 | 38 | 45 | 54 | 65 | 79 | 96 | 115 |
| 2.0 | 39 | 46 | 55 | 67 | 81 | 98 | 118 |
| 2.2 | 39 | 47 | 56 | 68 | 83 | 100 | 120 |
| 2.4 | 40 | 48 | 57 | 70 | 84 | 102 | 123 |
| 2.6 | 41 | 49 | 58 | 71 | 86 | 104 | 125 |
| 2.8 | 41 | 49 | 59 | 72 | 88 | 106 | 127 |
| 3.0 | 42 | 50 | 61 | 74 | 89 | 108 | 130 |

**Table A.9. CT values (in mg·min/L) for 90% inactivation of *Giardia lamblia* cysts by free chlorine at 10°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 24 | 29 | 35 | 42 | 50 | 59 | 70 |
| 0.6 | 25 | 30 | 36 | 43 | 51 | 61 | 73 |
| 0.8 | 26 | 31 | 37 | 44 | 53 | 63 | 75 |
| 1.0 | 26 | 31 | 37 | 45 | 54 | 65 | 78 |
| 1.2 | 27 | 32 | 38 | 46 | 55 | 67 | 80 |
| 1.4 | 27 | 33 | 39 | 47 | 57 | 69 | 82 |
| 1.6 | 28 | 33 | 40 | 48 | 58 | 70 | 84 |
| 1.8 | 29 | 34 | 41 | 49 | 60 | 72 | 86 |
| 2.0 | 29 | 35 | 41 | 50 | 61 | 74 | 88 |
| 2.2 | 30 | 35 | 42 | 51 | 62 | 75 | 90 |
| 2.4 | 30 | 36 | 43 | 52 | 63 | 77 | 92 |
| 2.6 | 31 | 37 | 44 | 53 | 65 | 78 | 94 |
| 2.8 | 31 | 37 | 45 | 54 | 66 | 80 | 96 |
| 3.0 | 32 | 38 | 46 | 55 | 67 | 81 | 97 |

**Table A.10. CT values (in mg·min/L) for 90% inactivation of *Giardia lamblia* cysts by free chlorine at 15°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 16 | 20 | 23 | 28 | 33 | 39 | 47 |
| 0.6 | 17 | 20 | 24 | 29 | 34 | 41 | 49 |
| 0.8 | 17 | 20 | 24 | 29 | 35 | 42 | 50 |
| 1.0 | 18 | 21 | 25 | 30 | 36 | 43 | 52 |
| 1.2 | 18 | 21 | 25 | 31 | 37 | 45 | 53 |
| 1.4 | 18 | 22 | 26 | 31 | 38 | 46 | 55 |
| 1.6 | 19 | 22 | 26 | 32 | 39 | 47 | 56 |
| 1.8 | 19 | 23 | 27 | 33 | 40 | 48 | 59 |
| 2.0 | 19 | 23 | 28 | 33 | 41 | 49 | 59 |
| 2.2 | 20 | 23 | 28 | 34 | 41 | 50 | 60 |
| 2.4 | 20 | 24 | 29 | 35 | 42 | 51 | 61 |
| 2.6 | 20 | 24 | 29 | 36 | 43 | 52 | 63 |
| 2.8 | 21 | 25 | 30 | 36 | 44 | 53 | 64 |
| 3.0 | 21 | 25 | 30 | 37 | 45 | 54 | 65 |

**Table A.11. CT values (in mg·min/L) for 90% inactivation of *Giardia lamblia* cysts by free chlorine at 20°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 12 | 15 | 17 | 21 | 25 | 30 | 35 |
| 0.6 | 13 | 15 | 18 | 21 | 26 | 31 | 36 |
| 0.8 | 13 | 15 | 18 | 22 | 26 | 32 | 38 |
| 1.0 | 13 | 16 | 19 | 22 | 27 | 33 | 39 |
| 1.2 | 13 | 16 | 19 | 23 | 28 | 33 | 40 |
| 1.4 | 14 | 16 | 19 | 23 | 28 | 34 | 41 |
| 1.6 | 14 | 17 | 20 | 24 | 29 | 35 | 42 |
| 1.8 | 14 | 17 | 20 | 25 | 30 | 36 | 43 |
| 2.0 | 15 | 17 | 21 | 25 | 30 | 37 | 44 |
| 2.2 | 15 | 18 | 21 | 26 | 31 | 38 | 45 |
| 2.4 | 15 | 18 | 22 | 26 | 32 | 38 | 46 |
| 2.6 | 15 | 18 | 22 | 27 | 32 | 39 | 47 |
| 2.8 | 16 | 19 | 22 | 27 | 33 | 40 | 48 |
| 3.0 | 16 | 19 | 23 | 28 | 34 | 41 | 49 |

**Table A.12. CT values (in mg·min/L) for 90% inactivation of *Giardia lamblia* cysts by free chlorine at 25°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 8 | 10 | 12 | 14 | 17 | 20 | 23 |
| 0.6 | 8 | 10 | 12 | 14 | 17 | 20 | 24 |
| 0.8 | 9 | 10 | 12 | 15 | 18 | 21 | 25 |
| 1.0 | 9 | 10 | 12 | 15 | 19 | 22 | 26 |
| 1.2 | 9 | 11 | 13 | 15 | 18 | 22 | 27 |
| 1.4 | 9 | 11 | 13 | 16 | 19 | 22 | 27 |
| 1.6 | 9 | 11 | 13 | 16 | 19 | 23 | 28 |
| 1.8 | 10 | 11 | 14 | 16 | 20 | 23 | 29 |
| 2.0 | 10 | 12 | 14 | 17 | 20 | 24 | 29 |
| 2.2 | 10 | 12 | 14 | 17 | 21 | 25 | 30 |
| 2.4 | 10 | 12 | 14 | 17 | 21 | 25 | 31 |
| 2.6 | 10 | 12 | 15 | 18 | 22 | 26 | 31 |
| 2.8 | 10 | 12 | 15 | 18 | 22 | 26 | 32 |
| 3.0 | 11 | 13 | 15 | 18 | 22 | 27 | 32 |

### *(c) 68.4% (0.5-log) inactivation*

**Table A.13. CT values (in mg·min/L) for 68.4% inactivation of *Giardia lamblia* cysts by free chlorine at 0.5°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 23 | 27 | 33 | 40 | 46 | 55 | 65 |
| 0.6 | 24 | 28 | 33 | 40 | 48 | 57 | 68 |
| 0.8 | 24 | 29 | 34 | 41 | 49 | 59 | 70 |
| 1.0 | 25 | 29 | 35 | 42 | 51 | 61 | 73 |
| 1.2 | 25 | 30 | 36 | 43 | 52 | 63 | 75 |
| 1.4 | 26 | 31 | 37 | 44 | 54 | 65 | 77 |
| 1.6 | 26 | 32 | 38 | 46 | 55 | 66 | 80 |
| 1.8 | 27 | 32 | 39 | 47 | 56 | 68 | 82 |
| 2.0 | 28 | 33 | 39 | 48 | 55 | 70 | 83 |
| 2.2 | 28 | 34 | 40 | 50 | 59 | 71 | 85 |
| 2.4 | 29 | 34 | 41 | 50 | 60 | 73 | 87 |
| 2.6 | 29 | 35 | 42 | 51 | 61 | 74 | 89 |
| 2.8 | 30 | 36 | 43 | 52 | 63 | 75 | 91 |
| 3.0 | 30 | 36 | 44 | 53 | 64 | 77 | 92 |

**Table A.14. CT values (in mg·min/L) for 68.4% inactivation of *Giardia lamblia* cysts by free chlorine at 5°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 16 | 20 | 23 | 28 | 33 | 39 | 47 |
| 0.6 | 17 | 20 | 24 | 29 | 34 | 41 | 49 |
| 0.8 | 17 | 20 | 24 | 29 | 35 | 42 | 50 |
| 1.0 | 18 | 21 | 25 | 30 | 36 | 43 | 52 |
| 1.2 | 18 | 21 | 25 | 31 | 37 | 45 | 53 |
| 1.4 | 18 | 22 | 26 | 31 | 38 | 46 | 55 |
| 1.6 | 19 | 22 | 26 | 32 | 39 | 47 | 56 |
| 1.8 | 19 | 23 | 27 | 33 | 40 | 48 | 58 |
| 2.0 | 19 | 23 | 28 | 33 | 41 | 49 | 59 |
| 2.2 | 20 | 23 | 28 | 34 | 41 | 50 | 60 |
| 2.4 | 20 | 24 | 29 | 35 | 42 | 51 | 61 |
| 2.6 | 20 | 24 | 29 | 36 | 43 | 52 | 63 |
| 2.8 | 21 | 25 | 30 | 36 | 44 | 53 | 64 |
| 3.0 | 21 | 25 | 30 | 37 | 45 | 54 | 65 |

**Table A.15. CT values (in mg·min/L) for 68.4% inactivation of *Giardia lamblia* cysts by free chlorine at 10°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 12 | 15 | 17 | 21 | 25 | 30 | 35 |
| 0.6 | 13 | 15 | 18 | 21 | 26 | 31 | 36 |
| 0.8 | 13 | 15 | 18 | 22 | 26 | 32 | 38 |
| 1.0 | 13 | 16 | 19 | 22 | 27 | 33 | 39 |
| 1.2 | 13 | 16 | 19 | 23 | 28 | 33 | 40 |
| 1.4 | 14 | 16 | 19 | 23 | 28 | 34 | 41 |
| 1.6 | 14 | 17 | 20 | 24 | 29 | 35 | 42 |
| 1.8 | 14 | 17 | 20 | 25 | 30 | 36 | 43 |
| 2.0 | 15 | 17 | 21 | 25 | 30 | 37 | 44 |
| 2.2 | 15 | 18 | 21 | 26 | 31 | 38 | 45 |
| 2.4 | 15 | 18 | 22 | 26 | 32 | 38 | 46 |
| 2.6 | 15 | 18 | 22 | 27 | 32 | 39 | 47 |
| 2.8 | 16 | 19 | 22 | 27 | 33 | 40 | 48 |
| 3.0 | 16 | 19 | 23 | 28 | 34 | 41 | 49 |

**Table A.16. CT values (in mg·min/L) for 68.4% inactivation of *Giardia lamblia* cysts by free chlorine at 15°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 8 | 10 | 12 | 14 | 17 | 20 | 23 |
| 0.6 | 8 | 10 | 12 | 14 | 17 | 20 | 24 |
| 0.8 | 9 | 10 | 12 | 15 | 18 | 21 | 25 |
| 1.0 | 9 | 11 | 13 | 15 | 18 | 22 | 26 |
| 1.2 | 9 | 11 | 13 | 15 | 19 | 22 | 27 |
| 1.4 | 9 | 11 | 13 | 16 | 19 | 23 | 28 |
| 1.6 | 9 | 11 | 13 | 16 | 19 | 24 | 28 |
| 1.8 | 10 | 11 | 14 | 16 | 20 | 24 | 29 |
| 2.0 | 10 | 12 | 14 | 17 | 20 | 25 | 30 |
| 2.2 | 10 | 12 | 14 | 17 | 21 | 25 | 30 |
| 2.4 | 10 | 12 | 14 | 18 | 21 | 26 | 31 |
| 2.6 | 10 | 12 | 15 | 18 | 22 | 26 | 31 |
| 2.8 | 10 | 12 | 15 | 18 | 22 | 27 | 32 |
| 3.0 | 11 | 13 | 15 | 19 | 22 | 27 | 33 |

**Table A.17. CT values (in mg·min/L) for 68.4% inactivation of *Giardia lamblia* cysts by free chlorine at 20°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 6 | 7 | 9 | 10 | 12 | 15 | 19 |
| 0.6 | 6 | 8 | 9 | 11 | 13 | 15 | 18 |
| 0.8 | 7 | 8 | 9 | 11 | 13 | 16 | 19 |
| 1.0 | 7 | 8 | 9 | 11 | 14 | 16 | 20 |
| 1.2 | 7 | 8 | 10 | 12 | 14 | 17 | 20 |
| 1.4 | 7 | 8 | 10 | 12 | 14 | 17 | 21 |
| 1.6 | 7 | 8 | 10 | 12 | 15 | 18 | 21 |
| 1.8 | 7 | 9 | 10 | 12 | 15 | 18 | 22 |
| 2.0 | 7 | 9 | 10 | 13 | 15 | 18 | 22 |
| 2.2 | 7 | 9 | 11 | 13 | 16 | 19 | 23 |
| 2.4 | 8 | 9 | 11 | 13 | 16 | 19 | 23 |
| 2.6 | 8 | 9 | 11 | 13 | 16 | 20 | 24 |
| 2.8 | 8 | 9 | 11 | 14 | 17 | 20 | 24 |
| 3.0 | 9 | 10 | 11 | 14 | 17 | 20 | 24 |

**Table A.18. CT values (in mg·min/L) for 68.4% inactivation of *Giardia lamblia* cysts by free chlorine at 25°C**

| Residual (mg/L) | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| <0.4 | 4 | 5 | 6 | 7 | 8 | 10 | 12 |
| 0.6 | 4 | 5 | 6 | 7 | 9 | 10 | 12 |
| 0.8 | 4 | 5 | 6 | 7 | 9 | 11 | 13 |
| 1.0 | 4 | 5 | 6 | 8 | 9 | 11 | 13 |
| 1.2 | 5 | 5 | 6 | 8 | 9 | 11 | 13 |
| 1.4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
| 1.6 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
| 1.8 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
| 2.0 | 5 | 6 | 7 | 8 | 10 | 12 | 15 |
| 2.2 | 5 | 6 | 7 | 9 | 10 | 13 | 15 |
| 2.4 | 5 | 6 | 7 | 9 | 11 | 13 | 15 |
| 2.6 | 5 | 6 | 7 | 9 | 11 | 13 | 16 |
| 2.8 | 5 | 6 | 8 | 9 | 11 | 13 | 16 |
| 3.0 | 5 | 6 | 8 | 9 | 11 | 14 | 16 |

## *CHLORINE DIOXIDE*

**Table A.19. CT values (in mg·min/L) for inactivation of *Giardia*, pH 6.0–9.0**

| Log inactivation | Water temperature (°C) | | | | | |
|---|---|---|---|---|---|---|
| | ≤1 | 5 | 10 | 15 | 20 | 25 |
| 0.5 | 10 | 4.3 | 4.0 | 3.2 | 2.5 | 2.0 |
| 1.0 | 21 | 8.7 | 7.7 | 6.3 | 5.0 | 3.7 |
| 1.5 | 32 | 13 | 12 | 10 | 7.5 | 5.5 |
| 2.0 | 42 | 17 | 15 | 13 | 10 | 7.3 |
| 2.5 | 52 | 22 | 19 | 16 | 13 | 9 |
| 3.0 | 63 | 26 | 23 | 19 | 15 | 11 |

## *CHLORAMINE*

**Table A.20. CT values (in mg·min/L) for inactivation of *Giardia*, pH 6.0–9.0**

| Log inactivation | Water temperature (°C) | | | | | |
|---|---|---|---|---|---|---|
| | ≤1 | 5 | 10 | 15 | 20 | 25 |
| 0.5 | 635 | 365 | 310 | 250 | 185 | 125 |
| 1.0 | 1270 | 735 | 615 | 500 | 370 | 250 |
| 1.5 | 1900 | 1100 | 930 | 750 | 550 | 375 |
| 2.0 | 2535 | 1470 | 1230 | 1000 | 735 | 500 |
| 2.5 | 3170 | 1830 | 1540 | 1250 | 915 | 625 |
| 3.0 | 3800 | 2200 | 1850 | 1500 | 1100 | 750 |

## *OZONE*

**Table A.21. CT values (in mg·min/L) for inactivation of *Giardia***

| Log inactivation | Water temperature (°C) | | | | | |
|---|---|---|---|---|---|---|
| | ≤1 | 5 | 10 | 15 | 20 | 25 |
| 0.5 | 0.48 | 0.32 | 0.23 | 0.16 | 0.12 | 0.08 |
| 1.0 | 0.97 | 0.63 | 0.48 | 0.32 | 0.24 | 0.16 |
| 1.5 | 1.5 | 0.95 | 0.72 | 0.48 | 0.36 | 0.24 |
| 2.0 | 1.9 | 1.3 | 0.95 | 0.63 | 0.48 | 0.32 |
| 2.5 | 2.4 | 1.6 | 1.2 | 0.79 | 0.60 | 0.40 |
| 3.0 | 2.9 | 1.9 | 1.43 | 0.95 | 0.72 | 0.48 |

**ANNEX B:**

**UV Dose (IT) Table for the Inactivation of *Giardia* and *Cryptosporidium***

**Table B.1. UV dose (mJ/cm$^2$) requirements for up to 3-log (99.9%) inactivation of *Cryptosporidium* and *Giardia* (U.S. EPA, 2003)**

| | Log inactivation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Microorganism | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | 3.5 | 4 |
| *Cryptosporidium* | 1.6 | 2.5 | 3.9 | 5.8 | 8.5 | 12 | – | – |
| *Giardia* | 1.5 | 2.1 | 3.0 | 5.2 | 7.7 | 11 | – | – |