Guidance on
Waterborne Bacterial Pathogens

Document for Public Comment

Prepared by the Federal-Provincial-Territorial
Committee on Drinking Water

Consultation period ends
September 21, 2012

Canada

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Document for Public Consultation
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Purpose of consultation

The Federal-Provincial-Territorial Committee on Drinking Water (CDW) has assessed the available information on waterborne bacterial pathogens with the intent of establishing a drinking water guidance document. The purpose of this consultation is to solicit comments on this guidance document.

The CDW has requested that this document be made available to the public and open for comment. Comments are appreciated, with accompanying rationale, where required. Comments can be sent to the CDW Secretariat via email at water_eau@hc-sc.gc.ca. If this is not feasible, comments may be sent by mail to the CDW Secretariat, Water, Air and Climate Change Bureau, Health Canada, 3rd Floor, 269 Laurier Avenue West, A.L. 4903D, Ottawa, Ontario K1A 0K9. All comments must be received before September 21, 2012.

It should be noted that this Guidance Document on Waterborne Bacterial pathogens will be revised following evaluation of comments received, and a final guidance document will be posted. This document should be considered as a draft for comment only.

Guidance on waterborne bacterial pathogens

Background on guidance documents

The main role of the Federal-Provincial-Territorial Committee on Drinking Water is the development of the Guidelines for Canadian Drinking Water Quality. This role has evolved over the years, and new methodologies and approaches have led the Committee to develop a new type of document, Guidance documents, to provide advice and guidance on issues related to drinking water quality for parameters that do not require a formal Guideline for Canadian Drinking Water Quality.

There are two instances when the Federal-Provincial-Territorial Committee on Drinking Water may choose to develop guidance documents. The first would be to provide operational or management guidance related to specific drinking water related issues (such as boil water advisories), in which case the documents would provide only limited scientific information or health risk assessment. The second instance would be to make risk assessment information available when a guideline is not deemed necessary.

The Federal-Provincial-Territorial Committee on Drinking Water establishes the Guidelines for Canadian Drinking Water Quality specifically for contaminants that meet all of the following criteria:
1. exposure to the contaminant could lead to adverse health effects;
2. the contaminant is frequently detected or could be expected to be found in a large number of drinking water supplies throughout Canada; and
3. the contaminant is detected, or could be expected to be detected, at a level that is of possible health significance.

If a contaminant of interest does not meet all these criteria, the Federal-Provincial-Territorial Committee on Drinking Water may choose not to establish a numerical guideline or develop a Guideline Technical Document. In that case, a guidance document may be developed.

Guidance documents undergo a similar process as Guideline Technical Documents, including public consultations through the Health Canada web site. They are offered as information for drinking water authorities, and in some cases to help provide guidance in spill or other emergency situations.

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Part A. Guidance on waterborne bacterial pathogens

Throughout history, consumption of drinking water supplies of poor sanitary quality has been linked to illnesses in human populations. These illnesses most commonly present as gastrointestinal-related symptoms, such as diarrhoea and nausea. The organisms identified within this document as enteric waterborne bacterial pathogens are those that have been well-established as having a history of being responsible for waterborne outbreaks of gastrointestinal illness. Although there are methods capable of detecting and measuring pathogenic bacteria in drinking water, routine monitoring for these organisms still remains difficult and impractical. This is because there are a number of types of bacterial pathogens that can be present in human and/or animal wastes that can vary significantly in their distribution depending on the sources of contamination impacting the water supply; and conducting detection and identification procedures for each possible type can be difficult, requiring significant resources. As a result, monitoring for a broad indicator of fecal contamination such as *E. coli* is useful in verifying the microbiological water quality and safety of the drinking water supply. Detection of elevated numbers of *E. coli* is indicative of fecal contamination and thus indicates that enteric bacterial pathogens may possibly be present.

In recent decades, there has been an increasing amount of interest in waterborne bacterial pathogens that occur naturally in the water environment and thus have the potential to be transmitted through water. These other waterborne bacterial pathogens discussed within this document can cause human infection resulting in gastrointestinal and non-gastrointestinal illnesses (particularly respiratory illnesses). Routine monitoring for these pathogens remains impractical as well. As these organisms occupy different environmental niches and have primary sources other than human or animal faeces, there are presently no satisfactory microbiological indicators for their presence. To date, none of these organisms have been associated with outbreaks of illness as a result of exposure to drinking water supplies in Canada. However, as they do have the potential to be spread through drinking water, it is important to ensure that the treatment and disinfection strategies in place are capable of providing adequate control of these organisms.

Health Canada maintains that the best means of safeguarding against the presence of waterborne pathogens (including non-faecal pathogens) in drinking water is the application of the multi-barrier approach that includes adequate treatment, a well-maintained distribution system, and, in the case of enteric bacteria - source protection. Treatment and disinfection requirements in the provision of microbiologically safe drinking water are based on health-based treatment goals for the removal and inactivation of the enteric protozoa *Giardia* and *Cryptosporidium* and enteric viruses. These organisms present a significant challenge to water treatment and disinfection technologies because of their difficulty of removal, high infectivity and high disinfectant resistance. As a result, current drinking water treatment and disinfection practices applied to meet the treatment goals for viruses and protozoa are expected to be similarly capable of controlling waterborne bacterial pathogens in drinking water. This approach can reduce both faecal and non-faecal pathogens to non-detectable levels or to levels that have not been associated with human illness.

Consequently, it remains unnecessary and impractical to establish maximum acceptable concentrations for the waterborne bacterial pathogens described in this document. The

monitoring of indicators such as *E. coli* continues to be used in the verification of the microbiological quality of drinking water.

Under the multi-barrier approach to safe drinking water, numerous process controls are required to function alongside bacteriological analysis in order to reliably produce drinking water of an acceptable quality. Important individual elements under this approach include:

- source water protection (where possible);
- optimized treatment performance (e.g., for turbidity reduction and particle removal);
- proper application of disinfection technologies;
- a well-designed and maintained distribution system; and
- maintenance of a disinfection residual.

The potential for introduction of waterborne bacterial pathogens into the distribution system and the capability for survival and regrowth in biofilms are of concern in drinking water treatment. Additional considerations specific for aiding in the control of biofilms in the distribution system can include:

- use of proper construction materials;
- control measures to reduce levels of natural organic matter, scaling and corrosion;
- measures to prevent low flow rates or water stagnation and to control temperatures (where possible); and
- maintenance activities such as flushing and cleaning.

Contamination problems involving waterborne bacterial pathogens can occur in water systems outside of water treatment plants' distribution network, such as plumbing systems, or heating, ventilation and air conditioning systems. Specific information pertaining to guidance and requirements for these systems can be found by consulting the proper regulatory authority.

Part B. Supporting information

B.1   Enteric waterborne pathogens

B.1.1   Pathogenic *Escherichia coli*

     Escherichia coli are bacteria found naturally in the digestive tract of warm-blooded animals, including humans. As such, it is used in the drinking water industry as the definitive indicator of recent faecal contamination of water. While most strains of *E. coli* are non-pathogenic, some possess virulence traits that can enable them to cause serious diarrhoeal infections in humans. These pathogenic *E. coli* are divided into groups based on the mechanisms with which they interact with human intestinal tract and cause symptoms (e.g., produce specific types of toxin; or invade, bind to, or cause structural alterations of intestinal cells) (Percival et al., 2004). The six groups are: enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enteroaggregative (EAEC), and diffuse adherent (DAEC) *E. coli* (Percival et al., 2004; AWWA, 2006). The EHEC group has emerged as a group that is of particular significance to the water industry (AWWA, 2006). This group is a broad group which contains many different serotypes that have been implicated as causes of human illness (Muniesa et al., 2006). One member of the EHEC group *E. coli* O157:H7, has been most commonly associated with pathogenic *E. coli* outbreaks worldwide (Muniesa et al., 2006) and has been implicated in a few waterborne outbreaks (Bruce-Grey-Owen Sound Health Unit, 2000; Schuster et al., 2005; Craun et al., 2006; Clark et al., 2010). In Canada, the Walkerton outbreak of 2000 was the first documented outbreak of *Escherichia coli* O157:H7 infection associated with a Canadian municipal water supply and the largest multiwaterborne bacterial outbreak in the country to date (Bruce-Grey-Owen Sound Health Unit, 2000). Surveillance reports published for other countries have indicated that over the period from 1990 to the early 2000's, *E. coli* O157:H7 has been identified as the causative agent of approximately 6% of the reported drinking water outbreaks in England and Wales (Smith et al., 2006) and roughly 7% of those reported in the United States (Craun et al., 2006).

     Cattle and human sewage are the primary and secondary sources, respectively, of EHEC (Jackson et al., 1998; Percival et al., 2004). Human sewage is the major source of the other pathogenic *E. coli* groups (Percival et al., 2004; AWWA, 2006). Transmission of pathogenic *E. coli* occurs through the fecal-oral route, and the primary routes of exposure are from contaminated food, water or from person to person (Percival et al., 2004, AWWA, 2006). Pathogenic *E. coli* are not usually a concern in treated drinking water, however outbreaks of *E. coli* O157:H7 involving consumption of drinking water contaminated with human sewage or cattle faeces have been documented (Swerdlow et al., 1992; Bruce-Grey-Owen Sound Health Unit, 2000).

     The probability of becoming ill depends on the number of organisms ingested, the health status of the person, and the resistance of the person to the organism or toxin (AWWA, 1999).

     With the exception of EHEC, most pathogenic *E. coli* require a high number of bacteria to be ingested in order to produce illness. Infectious dose estimates for non-EHEC strains range from 10^5 to 10^{10} organisms (Percival et al., 2004). EHEC strains on the other hand have a very low infectious dose. It has been suggested that ingestion of fewer than 100 cells may be sufficient to cause infection (Percival et al., 2004, Pond, 2005). The onset and duration of pathogenic *E. coli* related illness will be strain dependent, but symptoms can begin in as little as 8-12 hours and last from a few days to up to a few weeks (Percival et al., 2004).

Pathogenic *E. coli* can cause diarrhoea that ranges in severity from mild and self-limiting to severe and life-threatening (Percival et al., 2004; AWWA, 2006). Most non-EHEC illness is marked by a watery diarrhoea that can be accompanied by vomiting, abdominal pain, fever and muscle pain depending on the group or strain involved.

EHEC illness can begin with watery and bloody diarrhoea in combination with vomiting, but in some cases can progress to the more serious and potentially life-threatening symptoms of hemorrhagic colitis (grossly bloody diarrhoea) and haemolytic uremic syndrome (kidney failure). These symptoms are caused by shiga-like toxins, potent toxins that are related to *Shigella dysenteriae* toxins (Percival et al., 2004). It has been proposed that up to 10% of *E. coli* O157:H7 infections can progress to haemolytic uremic syndrome (Moe, 1997; Sherman et al., 2010). Children and the elderly are most susceptible to the complications that arise from EHEC infections (Percival et al., 2004). One area of recent study interest has been the examination of possible long-term health effects in adults as a result of contracting haemolytic uremic syndrome from *E. coli* O157:H7, as these to date have been largely unknown (Clark et al., 2010). Clark et al. (2010) reported on the results of a health study among persons exposed and unexposed to gastrointestinal illness from *E. coli* O157:H7 and Campylobacter during the Walkerton outbreak in May 2000. The author's conclusions were that the data provided evidence of increases in the incidence of hypertension, cardiovascular disease and indicators of kidney impairment in persons who experienced acute gastroenteritis during the outbreak. Further study in this area is required.

B.1.1.1 Treatment technology

In the majority of treatment and disinfection studies involving pathogenic *E. coli* it is the EHEC strain O157:H7 that has been selected as the model organism due to its health significance and prominence in foodborne and waterborne outbreaks. Regardless, review of the evidence generated to date suggests that the proper application of water treatment and disinfection technologies will be capable of controlling strains of pathogenic and non-pathogenic *E. coli* in drinking water (Percival et al., 2004; AWWA, 2006).

In terms of chlorine and monochloramine effectiveness, laboratory studies have demonstrated *E. coli* O157:H7 log inactivation capabilities of up to 4 log at concentrations and contact times that would be encountered in municipal drinking water treatment (Rice et al., 1999; Wojcicka et al., 2007; Chauret et al., 2008).

For UV disinfection, Zimmer-Thomas et al. (2007) observed log inactivations of 4.5 log or greater for *E. coli* O157:H7 at all tested doses of low pressure (LP) and medium pressure (MP) UV. These included UV doses commonly used in water disinfection (20 and 40 mJ/cm^2, LP), as well as low doses intended to be representative of compromised UV dose delivery (5 and 8 mJ/cm^2, LP and MP). In UV inactivation experiments, Sommer et al. (2000) observed considerable divergence in sensitivity of different pathogenic (including enterohemorrhagic) strains of *E. coli*. The authors further demonstrated that a UV dose of 125 J/m^2 (equivalent to 12.5 mJ/cm^2) was sufficient to produce a 6-log inactivation of all of the strains under study. Further information on treatment technology for *E. coli* can be found in the *Escherichia coli* guideline technical document (Health Canada, 2012).

B.1.1.2 Assessment

Studies have shown that the survival and susceptibility to disinfection of pathogenic *E. coli* strains approximates that of typical *E. coli* (AWWA, 1999; Rice, 1999). Also, although routine examination methods for generic *E. coli* are not designed to distinguish pathogenic *E. coli* strains, the former will always occur in greater concentration in faeces, even during outbreaks.

Pathogenic *E. coli* will not occur in the absence of generic *E. coli*. As a result, the presence of *E. coli* can be used as an indicator of the presence of pathogenic *E. coli*.

B.1.2 *Salmonella* **and** *Shigella*

Salmonella and *Shigella* are agents of gastrointestinal illness that belong to the same microbiological family as *E. coli*.

Salmonella is a complex taxonomic genus consisting of over 2000 different varieties or serological types that can cause infections in animals and humans (AWWA, 2006). According to experts, the genus is officially made up of only two species, *Salmonella enterica* and *Salmonella bongori* (Percival et al., 2004, AWWA, 2006). *Salmonella enterica* is the species of most relevance for human infections and it can be further broken down into 6 subspecies, of which one, *Salmonella enterica* subspecies *enterica* contains the majority of serotypes that are associated with cases of human gastroenteritis (Percival et al., 2004). By convention, when referring to *Salmonella* serotypes, the serotype is adopted as the species name (for example: *Salmonella enterica* subsp. *enterica* serovar *enteritidis* becomes *Salmonella enteritidis*).

The vast majority of *Salmonella* serotypes encountered in developed countries are zoonotic pathogens. Reservoirs for these organisms include poultry, pigs, birds, cattle, cats, dogs, rodents and turtles (AWWA, 2006). Infected humans and as a result, sewage, are also source of *Salmonella*. Transmission of *Salmonella* occurs through the fecal-oral route, predominantly through food. Drinking water is not often implicated as a source of *Salmonella* infection (Percival et al., 2004). As *Salmonella* is a zoonotic pathogen, runoff from agricultural lands can provide a mechanism for the transfer of animal fecal wastes to source waters.

The taxonomy of *Shigella* is much simpler than that of *Salmonella*. The genus is categorized into four major serological groups. *Shigella sonnei* and *Shigella flexneri* are the two species of importance as causes of gastrointestinal illness in developed countries (Percival et al., 2004). Infected humans are the only significant reservoir (AWWA, 2006). Transmission is fecal-oral, through drinking water or food that has been contaminated with human fecal wastes. Person-person is also a significant route of exposure for *Shigella*, particularly among children. *Shigella* is a human-specific pathogen and is not expected to be found in the environment (AWWA, 2006). Thus contamination of water supplies is suggestive of a source of human fecal contamination such as from sewage or on-site wastewater disposal systems.

Numerous outbreaks linked to contaminated drinking water have been reported worldwide (Boring et al., 1971; White and Pedersen, 1976; Auger et al., 1981; CDC, 1996; Angulo et al., 1997; Alamanos et al., 2000; Taylor et al., 2000; Chen et al., 2001). Schuster et al. (2005) reported that *Shigella* and *Salmonella* were identified as the causative agent in 9 and 16 confirmed proposed or suspected drinking water outbreaks in Canada respectively over the years 1974-2001. In the United States, *Salmonella* and *Shigella* accounted for approximately 2% and 5% of drinking water outbreak reported from 1991-2002 according to U.S. Surveillance data (Craun et al., 2006). Common causes of waterborne outbreaks by these organisms are poor source water, inadequate treatment or post-treatment contamination (e.g., by cross-connections) (AWWA, 2006).

Both organisms give rise to acute, self-limiting gastrointestinal illness with symptoms of diarrhoea, vomiting and abdominal pain. *Shigella*-associated illness is more dysenteric in nature, marked by a more watery diarrhoea containing blood and mucus (AWWA, 2006). Once infected recovering individuals may continue to shed either of these organisms in their faeces for days up to several weeks or months. Published reports regarding the median infective doses for these two organisms have suggested they may be as low as 10^3-10^5 organisms for *Salmonella* serotypes and

10^2-10^3 organisms for *Shigella flexneri* and *Shigella sonnei* (Hunter et al., 1997; Kothary and Babu, 2001). The factors that contribute to the virulence of these organisms are still under investigation. Both possess mechanisms that enable the bacteria to invade, survive, replicate and disrupt the function of the human intestinal lining (Percival et al., 2004). In addition, *Shigella* sonnei and *Shigella flexneri* are known to produce an exotoxin that affects intestinal water absorption and retention (Percival et al., 2004).

B.1.2.1 Treatment technology
        Salmonella and *Shigella* survival characteristics in water and their susceptibility to disinfection have been demonstrated to be similar to those of coliform bacteria, including *E. coli* (McFeters et al., 1974; Mitchell and Starzyk, 1975; Chang et al., 1985; Koivunen and Heinonen-Tanski, 2005). It is generally recognized that well-operated disinfection will be sufficient in controlling *Salmonella* and *Shigella* in treated drinking water (AWWA, 2006).

B.1.2.2 Assessment
        The absence of *E. coli* during routine verification should be an adequate indication of the sufficient removal and inactivation of *Salmonella* and *Shigella*.

B.1.3   *Campylobacter* and *Yersinia*
        Campylobacters are pathogenic bacteria found primarily in the intestinal tract of domestic and wild animals, especially birds. Poultry, cattle, sheep and pigs are considered significant reservoirs for these organisms (Percival et al., 2004; AWWA, 2006). *Yersinia* can be found in the faeces of wild animals as well as domestic livestock such as cattle, pigs and sheep (Percival, 2004). It is the *Campylobacter* species *C. jejuni*, *C. coli* and *C. upsaliensis*, and the *Yersinia* species *Y. enterocolitica* that are most important to the water industry (AWWA, 2006). Human sewage also contains large number of both of these organisms.
        Both *Campylobacter* and *Yersinia enterocolitica* are transmitted through the fecal-oral route, mostly through contaminated food, and sometimes through water (Percival et al., 2004). Person to person transmission of *Campylobacter* or *Yersinia enterocolitica* is uncommon (Percival et al., 2004; AWWA, 2006).
        Waterborne outbreaks of gastroenteritis involving *Campylobacter jejuni* and *Yersinia enterocolitica* have been recorded on numerous occasions, with improper treatment, post-treatment contamination or consumption of untreated water supplies being the most frequent causes (Eden et al., 1977; McNeil et al., 1981; Mentzing, 1981; Vogt et al., 1982; Taylor et al., 1983; Lafrance et al., 1986; Sacks et al., 1986; Thompson and Gravel, 1986). In a review of Canadian data on waterborne outbreaks for the period spanning from 1974-2001, Schuster et al. (2005), reported that *Campylobacter* was implicated in 24 outbreaks and was second only to *Giardia* (51 outbreaks) in outbreaks where a causative agent was identified. The most notable Canadian waterborne outbreak was the May 2000 Walkerton outbreak involving *Campylobacter*, and *E. coli* O157:H7 where faecally contaminated well water was not properly treated before consumption (Clark et al., 2003). No outbreaks of *Yersinia* gastroenteritis have been reported for municipal drinking water supplies in North America over the past two decades (Schuster et al., 2005; Craun et al., 2006)
        Campylobacter enteritis typically presents as flu-like symptoms and/or abdominal pain, followed by a profuse watery diarrhoea caused by the presence of an enterotoxin similar to cholera toxin (AWWA, 2006). A known, but rare complication of *Campylobacter* illness is Guillian-Barré syndrome — a nervous system disorder causing rapidly progressing weakness of

the muscles and nerves (Percival, 2004; AWWA, 2006). *Yersinia* enterocolitica-associated gastroenteritis is associated with symptoms of fever, diarrhoea, abdominal cramps and occasionally vomiting (AWWA, 2006). The gastrointestinal illnesses caused by both organisms are considered to be self-limiting (Percival et al., 2004).

B.1.3.1 *Treatment technology*

Studies have demonstrated the susceptibility of *Campylobacter* species and *Yersinia enterocolitica* to disinfectants commonly used in water treatment (Wang et al., 1982; Blaser et al., 1986; Sobsey, 1989; Lund, 1996; Rose et al., 2007). It is generally recognized that treatment technologies effective in the removal and inactivation of *E. coli* will be effective against these pathogenic bacteria (AWWA, 2006).

B.1.3.2 *Assessment*

Studies have a lack of a correlation between indicator organisms (e.g., *E. coli*, total coliforms) and the presence of *Campylobacter* and *Yersinia* in raw surface water supplies (Carter et al., 1987; Lund, 1996; Hörman et al., 2004). Thus, *E. coli* may not be an adequate indicator of the presence of both *C. jejuni* and *Y. enterocolitica* in source waters at all times. However, as it is expected that properly operated treatment and disinfection technologies are effective in controlling these organisms in treated drinking water, it is expected that the *E. coli* guideline is sufficiently protective against their potential presence.

B.2 Other bacterial pathogens

B.2.1 *Legionella*

Legionellae are recognized human pathogens; they are a cause of respiratory illness which can be serious for persons with weakened immune systems. They are free-living aquatic bacteria that occur widely in water environments. The presence of *Legionella* is more of a concern for water systems outside of municipal water treatment systems, such as cooling towers, hospital and residential plumbing systems. However, the organisms are also capable of colonizing drinking water distribution system biofilms. *Legionella* species exhibit a number of survival properties that make them quite resistant to the effects of chlorination and elevated water temperatures.

The bacteria themselves are weakly Gram-negative, small, motile, rods that have precise nutritional requirements and as a result do not grow well on culture media. At least 50 different *Legionella* species have been identified, and approximately half of these species have been associated with disease. *L. pneumophila* (serogroup 1) is the agent responsible for most cases of illness in humans. Other than *L. pneumophila*, species causing far fewer infections but still considered to be clinically relevant include: *L. micdadei, L. bozemanii, L. longbeachae,* and *L. dumoffi* (Rheingold et al., 1984; Doyle and Heuzenroeder, 2002; Roig et al., 2003).

B.2.1.1 *Sources and exposure*

Legionella species are naturally present in a wide range of freshwater environments, including surface water (Fliermans et al., 1981; Palmer et al., 1993) and groundwater (Brooks et al., 2004; Costa et al., 2005). The bacteria are not considered to be enteric pathogens and are not transmitted via the fecal-oral route. However, *Legionella* can occasionally be detected in human fecal samples, as diarrhoea is a symptom of illness in a percentage of cases (Rowbotham, 1998).

Similarly, animals are not reservoirs for *Legionella* (U.S. EPA, 1999).

Legionella can be isolated from human-made systems (e.g., cooling towers, hot water tanks, shower heads, aerators). The presence of *Legionella* in these systems is almost exclusively associated with biofilms (Lau and Ashbolt, 2009). In general, the amount of legionellae in source waters is low compared with the concentrations that can be reached in human-made systems (Mathys et al., 2008). In an investigation of biofilm formation and *Legionella* colonization on various plumbing materials (Rogers et al., 1994), the numbers of biofilm-bound *Legionella* spanned from 1 to 80 times those detected in free water. Biofilms are important for the survival of the fastidious legionellae: they provide protection to *Legionella,* which are also able to utilize nutrients supplied by other organisms in this nutrient-rich environment (Borella et al., 2005; Temmerman et al., 2006; Lau and Ashbolt, 2009).

Some naturally occurring waterborne protozoa, such as *Acanthamoeba*, *Hartmanella*, *Naegleria*, *Valkampfia* and *Echinamoeba*, can also harbour *Legionella* organisms (Rowbotham, 1986;Kilvington and Price, 1990; Kramer and Ford, 1994; Fields, 1996). *Legionella* can infect and remain within the protozoan cyst form, where they are protected from disinfectants (Kilvington and Price, 1990; Thomas et al., 2004; Declerk et al., 2007). They are also able to multiply within these protozoa, which has been proposed as likely to be the only way that *Legionella* replicate within aquatic systems (Abu-Kwaik et al., 1998; Thomas et al., 2004). Thus, as well as offering protection, this association suggests a mechanism for the increase and transport of *L. pneumophila* in human-made systems (Declerk et al., 2009).

Temperature is an additional factor that influences *Legionella* colonization of water systems. Temperatures between 20°C and 50°C are hospitable for colonization, although legionellae typically only grow to high concentrations at temperatures below 42°C (Percival et al., 2004).

Plumbing systems outside of public water supply systems (e.g., in residential buildings, hotels, institutional settings) are most commonly implicated in *L. pneumophila* infections (Yoder et al., 2008). Since *Legionella* is a respiratory pathogen, systems that generate aerosols, such as cooling towers, whirlpool baths and shower heads, are the more commonly implicated sources of infection. The hot water supply system is commonly pinpointed as the origin of the contamination (Hershey et al., 1997; McEvoy et al., 2000; Borella et al., 2004; Oliver et al., 2005; Burnsed et al., 2007; Yoder et al., 2008). However, the cold water supply, when held within the range of *Legionella* multiplication (25°C), has also been implicated (Hoebe et al., 1998; Cowgill et al., 2005). *Legionella* infection can occur when people breathe in contaminated aerosols. The bacteria have not been found to be transmitted from person to person (U.S. EPA, 1999).

Legionella contamination is particularly troublesome in hospitals, where susceptible human populations are present and can be exposed to aerosols containing hazardous concentrations of *L. pneumophila*. Within the community, large buildings such as hotels, community centres, industrial buildings, and apartment buildings are most often implicated as sources of outbreaks (Riemer et al., 2010). Studies have shown that contamination of domestic hot water systems with *Legionella* can occur in single-family homes (Joly et al., 1985; Alary and Joly, 1991; Stout et al., 1991; Marrie et al., 1994; Dufresne et al., 2011). In a study of hot water plumbing systems in homes in the Quebec City area, Alary and Joly (1991) reported that *Legionella* was detected in 39% (69/178) of hot water tanks with electric heaters, and in 0% (0/33) of tanks with oil or gas-fired heaters. The authors further observed that in a proportion of those homes whose hot water tanks tested positive for *Legionella*, the organism could also be detected at distal locations such as faucets (12%) and showerheads (15%). The position of the

heat source in the design of electrically-heated hot water tanks observed at the time of study was cited as the reason for the difference in contamination between the two water heater types (Alary and Joly, 1991). In the electric tanks, the heating elements were located above the bottom of the heater, which could allow bottom sediments to remain at lower temperatures (< 50-60°C) permissible for *Legionella* growth.

Similarly, evidence has been provided that sporadic cases of Legionnaires' disease can plausibly be acquired from aerosols in residential plumbing systems (Stout et al., 1992; Straus et al., 1996; Lück et al., 2008). In a study conducted in the province of Quebec, Dufresne et al. (2011) observed that among 36 legionellosis-confirmed patients residing in homes with domestic hot water tanks, residential and clinical isolates of *Legionella* were microbiologically-related by pulse-field gel electrophoresis in 14% (5/36) of the cases. Similar studies conducted in Pittsburgh (Stout et al., 1992), and the state of Ohio (Straus et al., 1996) showed comparable results.

Persons thought to be at the highest risk of contracting Legionnaires' disease are those with lung conditions or compromised immune systems (e.g., persons receiving transplants or chemotherapy, persons with diabetes or kidney disease). The risk of infection is higher among persons 40-70 years of age, and the disease is seen more frequently in males than in females (Percival et al., 2004). Other risk factors include smoking and excessive use of alcohol. Legionnaires' disease is considered a very rare cause of pneumonia in children. In contrast, age, gender and smoking do not seem to be risk factors for Pontiac fever (Diederen, 2008).

The concentration of *Legionella* required to cause infection is not well understood (Armstrong and Haas, 2008). It has been suggested that amoebae harbouring *Legionella* may increase the potential for infectivity by providing a mechanism to expose humans to hundreds of *Legionella* cells if inhaled or aspirated in an aerosol (Rowbotham, 1986; Greub and Raoult, 2004).

B.2.1.2 Health effects

There are two distinct illnesses caused by *Legionella*: Legionnaires' disease and Pontiac fever. Collectively, these illnesses are referred to as legionellosis. Legionnaires' disease is a serious respiratory illness involving pneumonia. Other features include: fever, cough and headache; chest and muscle pain; and a general feeling of unwellness (malaise) (Fields et al., 2002; CDC, 2008b). The time from the point of infection to the onset of symptoms is about 2–10 days, and the disease period can last up to several months. One problem in diagnosing Legionnaires' disease is a lack of any specific symptom that distinguishes it from other bacterial pneumonias. Reported rates of Legionnaires' disease in Canada over the period 2000-2004 (the latest year for which data has been published) ranged from 0.13 to 0.20 cases per 100,000 population (PHAC, 2011). The mortality rate of Legionnaires' disease in the United States as of 1998 was reported at roughly 10% and 14% for community-acquired and hospital-acquired cases, respectively (Benin et al., 2002). Once in the lungs, *Legionella* is able to cause disease and avoid human immune defenses by infecting macrophages – immune system cells which ordinarily capture foreign bodies and present them to other cells for digestion (Fields et al., 2002). *Legionella* replicates within these macrophages and then causes their death, which results in the release of new organisms to continue the infection. Early diagnosis and antibiotic therapy are keys to successfully treating Legionnaires' disease.

Pontiac fever is a less serious respiratory illness that does not involve pneumonia and is more flu-like in nature. The time to the onset of symptoms is 24–48 hours (AWWA, 2006; CDC, 2008a). Disease is self-limiting, and typically resolves without complications in 2–5 days (CDC, 2008b). No known fatalities have been reported with this illness. Pontiac fever is difficult to

distinguish from other respiratory diseases because of a lack of specific clinical features. Experts have speculated that disease may be caused by exposure to a mixture of live and dead *Legionella* cells and non-*Legionella* endotoxin (Diederen, 2008). Antibiotic treatment is typically not prescribed because of the short, self-limiting nature of the disease (CDC, 2008b).

B.2.1.3 Treatment technology

Successful control of *Legionella* in water supplies requires focused attention not only on the organisms themselves, but also on the control of free-living amoebae and biofilms that support their persistence.

Physical removal mechanisms used during drinking water treatment, such as coagulation, flocculation, sedimentation, and filtration, will reduce the number of *Legionella* present in finished water. Disinfectants shown to be effective in reducing the number of *Legionella* present include: chlorine, monochloramine, chlorine dioxide, ozone and UV. In comparison with *E. coli, Legionella* cells have been shown to be more resistant to chlorination (Delaedt et al., 2008; Wang et al., 2010). Survival strategies exhibited by the organisms (colonization of biofilms and residence within free-living amoebae) also further protect *Legionella* from the action of disinfectants. In the distribution system, currently recommended disinfectant residuals are sufficient to keep the concentration of non-biofilm-associated *Legionella* at levels that have not been associated with disease (Storey et al., 2004; Delaedt et al., 2008). Hyperchlorination has been employed as a control strategy; however, studies have demonstrated that *Legionella* residing in biofilms or in cysts of *Acanthamoeba polyphaga* can survive following exposure to 50 mg/L free chlorine (Kilvington and Price, 1990; Cooper and Hanlon, 2010). There is also the concern of an increased potential for corrosion of plumbing systems with continued high concentrations of chlorine (Kool et al., 1999).

Various alternative disinfection methods have been examined for their potential to control *Legionella* colonization in the distribution system. Monochloramine has been shown to be more effective than chlorine as a residual disinfectant against legionellae. Weintraub et al. (2008) observed that converting from chlorine to monochloramine for residual disinfection in a municipal distribution system resulted in a significant reduction in the number of distribution samples, point-of-use sites and water heaters positive for *Legionella* colonization. Further, Kool et al. (1999) reported that hospitals using monochloramine for secondary disinfection were less likely to have reported outbreaks of Legionnaires' disease than those using free chlorine. Monochloramine is considered better able to penetrate into biofilms (LeChevallier, 1981), and is more stable, therefore able to maintain its concentration over greater distances in the distribution system (Kool et al., 1999).

Chlorine dioxide has demonstrated similar advantages over chlorine as a residual disinfectant for *Legionella* control. Sidari et al. (2004) observed significantly decreased *Legionella* concentrations at hot and cold point of use sites of a hospital's plumbing system upon switching to chlorine dioxide as the residual disinfectant. However, Health Canada (2008) has determined that chlorine dioxide is not effective to maintain a disinfectant residual in the distribution system.

It has been suggested that ozone may be a more effective disinfectant against *Legionella* than chlorine, but its main drawback is that it does not stay in water sufficiently long to provide a disinfectant residual (Kim et al., 2002; Blanc et al., 2005). Loret et al. (2005) observed that ozone at a concentration of 0.5 mg/L was effective in reducing Legionella, protozoa and biofilms in a model distribution system, but was not as effective as chorine (2 mg/L) or chlorine dioxide (0.5 mg/L), which both showed longer residual concentrations in the system. Ozone at a

concentration of 0.1-0.3 µg/mL was shown to be as effective as 0.4 mg/L free chlorine in inactivating *Legionella* suspensions, producing a 2-log reduction of the organism within five minutes in laboratory experiments (Dominique et al. 1988).

      Copper-silver ionization systems have also received much study and have shown effectiveness in controlling *Legionella* in drinking water supplies (Stout et al., 1998; Kusnetsov et al., 2001; Stout et al., 2003; Cachafeiro et al., 2007). Stout et al. (1998) observed that copper-silver ionization (mean hot water tank concentrations of 0.29 mg/L and 0.054 mg/L, respectively) was more effective than a superheat-and-flush method in reducing the recovery of *Legionella* from a hospital distribution system. In a survey of the experiences of hospital systems using copper-silver ionization, Stout et al. (2003) reported that following installation of the disinfection systems, the percentage of hospitals reporting cases of (1) Legionnaires' disease, and (2) positive Legionella samples at more than 30% of the sites measured, had both been reduced to 0% from 100% and 47%, respectively, before the systems had been installed.

      Additional measures recommended for distribution systems include temperature control; control of water system design and construction to prevent the accumulation of biofilms, sediments or deposits; and nutrient control strategies (Bartram et al., 2007; Bentham et al., 2007).

      In plumbing systems in hospitals and large buildings, thermal disinfection (elevating hot water to temperatures above 70°C, and flushing use points such as taps and showerheads) has been routinely employed either on its own or in conjunction with chemical disinfection. Typically this is recognized as a temporary control strategy as recolonization can occur within a few months of treatment (Storey et al., 2004).

      General recommendations regarding the control of *Legionella* in domestic plumbing systems involve maintaining proper water temperatures. The National Plumbing Code of Canada includes requirements of a minimum of 60°C for water temperature in hot water storage tanks, to address the growth of *Legionella* (NRCC, 2010). Where increased hot water temperatures create an increased risk of scalding for vulnerable groups (e.g., children, the elderly), appropriate safety measures should be applied to limit the temperature to 49°C. Thermostatic or pressure-balanced mixing valves can be installed to control the water temperature at the tap to reduce the risk of scalding (Bartram et al., 2007; Bentham et al., 2007).

      Control of *Legionella* in water systems outside of plumbing systems also requires controlling its growth in biofilms. The heating, ventilation and air conditioning industry has guidelines for reducing *Legionella* growth in cooling systems (ASHRAE, 2000) Hotel and lodging industry requirements for the operation and maintenance of plumbing facilities, including procedures for the proper disinfection of plumbing equipment in their facilities, are generally specified under various public health regulations and/or legislation. Consulting with the appropriate Provincial or Territorial Ministry of Health is advisable for obtaining information on these requirements.

B.2.1.4 Assessment

      The increasing importance of *Legionella* as a cause of human infection can be in part linked to continued human development and the resulting dependence on human made plumbing systems (Fields et al., 2002). Despite being ubiquitous in source waters, *Legionella* pneumophila and other *Legionella* species have only been recovered in low concentrations from Canadian drinking water supplies (Dutka et al., 1984; Tobin et al., 1986), and no illnesses have been linked to these low concentrations. For these reasons, the presence of the organism is not sufficient evidence to warrant remedial action in the absence of disease cases (Dufour and Jakubowski, 1982; Tobin et al., 1986).

Due to the existence of *Legionella* outside of fecal sources in nature, *E. coli* is not expected to be a reliable indicator of the presence of these bacteria. No suitable indicators have been identified to signal increasing concentrations of *Legionella* in a building's plumbing system. There is some evidence that increasing *Legionella* concentrations are accompanied, or preceded, by elevated HPC measurements (WHO, 2002). However, the correlation between HPC and *Legionella* is not consistent.

Legionella have also been included on the U.S. EPA's Candidate Contaminant List (CCL) as one of the priority contaminants for regulatory decision making and information collection (U.S. EPA, 2009). Guidelines or regulations that have been developed for *Legionella* in Canada, the United States and other countries worldwide relate to control of the organism in water environments outside of municipal water distribution networks (e.g., piped water systems, cooling towers, health care facilities) (Cunliffe, 2007).

B.2.2 *Mycobacterium avium* complex

The *Mycobacterium avium* complex (Mac) is a grouping of environmental mycobacteria that can cause illness in humans. The group consists of *Mycobacterium avium* (includes subspecies: *avium*, *sylvaticum* and *paratuberculosis*); and *Mycobacterium intracellulare* (Cangelosi et al., 2004). Mac organisms are considered ubiquitous in natural waters. Transmission is primarily through contact with contaminated waters via either ingestion or through inhalation (AWWA, 2006). Mac disease comes mainly in the form of lung infections, and occurs largely in persons who have suppressed immune systems (Percival et al., 2004).

Mycobacteria themselves are motile rod to coccoid-shaped bacteria that have characteristically high levels of waxy lipids in their cell wall. They are Gram negative, but are more commonly considered to be "acid-fast" due to the way their cell walls respond to diagnostic staining procedures (AWWA, 2006). Mac organisms are also referred to as "non-tuberculous" or "atypical" mycobacteria. This is to distinguish them from the more well-known mycobacteria species that are responsible for tuberculosis and leprosy that are not a concern for drinking water (Nichols et al., 2004). Other environmental mycobacteria are known that have been linked to skin infections through waterborne contact; but these are also of lesser importance to drinking water supplies (Nichols et al., 2004).

B.2.2.1 Sources and exposure

Mac organisms are natural inhabitants of water and soil environments (Falkinham, 2004). Water is considered the main reservoir (Percival et al., 2004; Vaerewijck et al., 2005), and the organisms can be encountered in natural aquatic systems worldwide including marine waters and fresh water lakes, streams, ponds and springs (Falkinham, 2004; Percival et al., 2004). Mac organisms can be encountered in drinking water supplies, but generally in low numbers and at low frequency (Peters et al., 1995; Covert et al., 1999; Falkinham et al., 2001; Hillborn et al., 2006). However, Mac bacteria can survive in distribution system biofilms, and grow there to reach significant populations (Falkinham et al., 2001). Following an 18-month survey of Mycobacteria in drinking water of 8 water treatment plants, Falkinham et al. (2001) noted that mycobacteria numbers were on average, 25,000-fold higher in distribution samples than samples taken upstream in the treatment plant. Counts of *M. intracellulare* in biofilms were observed to reach 600 CFU/cm^2, on average (Falkinham et al., 2001). Feazel et al. (2009) observed that Mycobacteria were enriched in plumbing system (showerhead) biofilms, reaching counts 100 times above those in water samples. In another study, Tsintzou et al. (2000) observed a statistically significant decrease in the presence of environmental mycobacteria in drinking water

samples after the replacement of the city's water distribution network. The authors attributed the reduction to the absence of distribution system biofilms (Tsintzou et al., 2000). Surveys of water samples from water treatment plants and residential dwellings have reported Mac isolation rates of 2-60% (von Reyn et al., 1993; Glover et al., 1994; Peters et al., 1995; Covert et al., 1999; Hillborn et al., 2006). Hillborn et al. (2006) recovered *M. avium* from roughly 50-60% of point-of-use (POU) samples (cold-water taps) served by two water treatment plants. Concentrations ranged from 200 to greater than 300 CFU/500 mL (Hillborn et al., 2006). Von Reyn et al. (1993) isolated Mac organisms from 17-25% of water supply samples, collected from hot water taps at patient care facilities (hospitals and clinics).

Other studies have reported a failure to isolate Mac organisms from water systems, instead detecting only other non-Mac mycobacteria (von Reyn et al., 1993; Le Dantec et al., 2002a; September et al., 2004; Sebakova et al., 2008). It has been suggested that the likelihood of exposure to Mac bacteria in water is diverse in various areas of the world but in general, may be less in developing countries (von Reyn et al., 1993, September et al., 2004). Mac organisms and biofilms have also been found in other human-made systems such as: cooling towers (Pagnier et al., 2009); ice-machines (LaBombardi et al., 2002); nebulizer reservoirs and toilets and sinks (AWWA, 2006); and water meters (Falkinham et al., 2001). Studies have reported the isolation of non-tuberculous mycobacteria from ground water, though *M. avium* has not been frequently detected (Falkinham et al., 2001; Vaerewijck et al., 2005)

Similar to *Legionella*, the growth and survival of Mac organisms can be enhanced by their ability to invade and survive in free-living amoebae such as *Acanthamoeba polyphaga* or *A. castellanii* (Cirillo et al., 1997; Steinert et al., 1998). A key difference between Mac and *Legionella* however, is that Mac organisms are able to replicate outside of amoebae, in biofilms (Steinert et al., 1998; Vaerewijck et al., 2005).

The ubiquitous nature of Mac organisms results from their ability to survive and grow under varied conditions. Mycobacteria can survive in water with little nutrients. Archuleta et al. (2002) observed that *M. intracellulare* was capable of surviving for over a year in reverse osmosis deionized water. Mac organisms have also been shown to grow in natural waters over wide ranges of pH (5-7.5), salinity (0-2%) and temperature (10-51°C) (Sniadack et al., 1992; Falkinham et al., 2001). Water conditions that have been identified as being more favourable for the growth of Mac organisms include high levels of humic and fulvic acids, high zinc concentrations, low pH and low dissolved oxygen levels (Kirschner et al., 1992; Kirschner et al., 1999; Vaerewijck et al., 2005)

Infection through contact with *M. avium* and *M. intracellulare* has been well documented (Wendt et al., 1980; Grange, 1991; Glover et al., 1994; Montecalvo et al., 1994; von Reyn et al., 1994; Kahana et al., 1997; Aronson et al., 1999; Mangione et al., 2001). Inhalation of contaminated aerosols, during contact with contaminated hot tubs, spa pools or similar facilities is most frequently cited as the route and source of infection (Kahana et al., 1997; Mangione et al., 2001; Rickman et al., 2002; Cappulluti et al., 2003; Lumb et al., 2004; Sood et al., 2007). Person-person transmission of the organisms is thought to be uncommon (Nichols et al., 2004; Falkinham, 1996). Evidence of the link between water supplies, particularly hot water supplies and Mac infection has also been provided (von Reyn et al., 1994; Tobin-D'Angelo et al., 2004; Marras et al., 2005). Von Reyn et al. (1994) reported the detection of the same strain of *M. avium* in patients and hospital potable water supplies to which they had been exposed; but not in water supplies collected from patient's homes. Marras et al. (2005) documented a case of Mac-associated hypersensitivity pneumonitis where the patient strain was recovered from the shower and bathtub from the patient's home, but not the hot tub. Despite these links, it has been

suggested that hospital and domestic drinking water-related cases represent a small proportion of Mac illness (von Reyn et al., 1994, Phillips et al., 2001). The infectious dose of Mac has not been well established. Rusin et al. (1997) proposed an oral infectious dose for mice of 10^4 to 10^7 organisms. True estimations of the inhaled infectious dose would be dependent upon (among other factors) the virulence of the organism and the immune status of the host.

B.2.2.2 *Health effects*

Mac organisms largely cause opportunistic infections in humans. The vast majority of people exposed to Mac do not develop disease (Field et al., 2004). Infections occur mostly in individuals who have weakened or suppressed immune systems (AIDS patients, the elderly, the very young) or persons with underlying conditions such as cystic fibrosis. Mac disease rarely occurs in healthy people (Field et al., 2004). Mac organisms have low pathogenicity, so individuals can become colonized with the organisms without any adverse health effects.

The main symptom of Mac lung infection is a chronic productive cough (cough with phlegm, saliva or mucus) (Field et al., 2004). Other symptoms can include fever, night sweats, fatigue and weight loss (Percival et al., 2004). However it has been suggested that the secondary symptoms are less common unless the individual has extensive lung disease (Crow et al., 1957; Field et al., 2004). In HIV/AIDS sufferers, Mac infection can spread to other parts of the body, including joints, skin, blood, liver and brain; and the disease can be debilitating and life-threatening for these patients (Percival et al., 2004). A possible role for Mac organisms in the development of Crohn's disease, an inflammatory bowel disease, has been suggested, but supporting data is presently inconclusive (Feller et al., 2007; Behr and Kapur, 2008). Epidemiological studies have shown evidence of a strong association between *Mycobacterium avium paratuberculosis* and the disease, but the causality of this association is unknown (Feller et al., 2007; Abubakar et al., 2008). Mac can also be a cause of lymphadenitis in children (lymph node infection - likely acquired through the oral route) and hypersensitivity pneumonitis in adults (inflammation of lung alveoli due to inhaled organic dusts) (Tortoli, 2009).

The true prevalence of Mac infections is not known, as it is not a reportable illness in Canada or the United States. Estimates of the rate of Mac-related pulmonary disease in the U.S. have been put from 1-2 cases to as high as 5 cases per 100,000 persons per year based on epidemiological studies conducted in various American cities (Reynolds et al., 2001; Marras and Daley, 2002). Marras et al. (2007) estimated the prevalence of pulmonary non-tuberculous mycobacteria in Ontario to range from 9-14 positive isolations per 100,000 population over the years from 1997-2003. The authors further reported that overall, Mac were isolated in roughly 60% of the cases (Marras et al., 2007).

Mac diseases are treatable, but the clearing of these infections can be difficult, and treatment can have a high rate of failure (Field et al., 2004). Mycobacteria have demonstrated strong resistance to antimicrobial agents (Daley and Griffith, 2010). Antibiotics are delivered at high doses and often require a long administration period (e.g., several months to over a year) (Percival et al., 2004; Daley and Griffith, 2010).

B.2.2.3 *Treatment technology*

Water treatment technologies commonly used, including chemical disinfection and physical removal methods, have been tested for their ability to inactivate or remove mycobacteria from water supplies. Of these technologies, the most effective has been physical removal using sand filtration and coagulation–sedimentation techniques. In one study, Falkinham et al. (2001) observed that water treatment plants treating surface water sources reduced mycobacteria

numbers by 2-4 log through filtration, and primary disinfection. A significant association between the frequency of detection of *M. avium* and high raw water turbidity was also reported. The authors were careful to note that reducing turbidity could represent one approach to reducing mycobacteria in drinking water, but that this procedure alone may not be completely sufficient to eliminate *M. avium* from the distribution system (Falkinham et al., 2001). Mac organisms are more resistant to the commonly used disinfectants. The highly hydrophobic quality of the organism's cell wall is thought to be largely responsible for this increased resistance (LeChevallier, 2004).

For chlorination, Le Dantec et al. (2002b) reported varying chlorine sensitivities among a collection of various mycobacteria isolated from the distribution system (note: Mac organisms were not isolated in this study). The authors calculated that a CT of 60 mg·min/L (e.g., 0.5 mg/L for 2 hours) would result in a log reduction for environmental mycobacteria ranging from 1.5 to 4 logs. Taylor et al. (2000) provided data on the susceptibility of environmental and patient isolates of *M. avium* to various disinfectants: chlorine, monochloramine ozone and chlorine dioxide. The mean $CT_{99.9}$ (mg·min/L) values for the individual disinfectants were: chlorine: (51-204), monochloramine (91-1710), ozone (0.10-0.17) and chlorine dioxide: (2-11). The authors did note that there was significant variation in the susceptibility of different strains (Taylor et al., 2000).

In another study using chlorine dioxide, Vicuna-Reyes et al. (2008) reported $CT_{99.9}$ values ranging from 3-36 mg·min/L (5-30°C), prompting the authors to conclude that the disinfectant can be effective in controlling mycobacteria. The CT values necessary for inactivation of Mac have been reported to range from near equivalency to a few times greater (monochloramine); to tens to hundreds of times greater (ozone, chlorine dioxide); to over two thousand times greater (chlorine) than that necessary to inactivate *E. coli* (Taylor et al., 2000). Data have been provided suggesting that mycobacteria are more sensitive than *Cryptosporidium* oocysts to chlorine, monochloramine chlorine dioxide and ozone, and is equal or more sensitive than *Giardia* to all of these with the exception of free chlorine (Jacangelo et al., 2002; LeChevallier, 2004).

In UV disinfection studies, Hayes et al. (2008) demonstrated that patient and environmental strains of *M. avium* and *M. intracellulare* exhibited greater than 4-log reduction at UV fluences less than 20 mJ/cm^2. The authors concluded that Mac organisms in free-suspension could be readily inactivated by UV doses commonly employed in drinking water treatment (Hayes et al., 2008). LeChevallier et al. (2004) reported that UV values required to inactivate mycobacteria are in the range of those required for other vegetative bacteria.

Even with good removal of organisms from the source water, the number of Mac organisms may increase in the distribution system (Falkinham et al., 2001). Residing within biofilms or free-living amoebae can further increase Mac organism resistance to inactivation. Steed et al. (2006) observed that *M. avium* and *M. intracellulare* cells in biofilms were up to 1.8-4 times more resistant than cells in free suspension when exposed to chlorine. Miller and Burmudez (2000) showed that *M. avium* growth within *Acanthamoeba* reduced the bacteria's susceptibility to antibiotics. As with *Legionella*, successful control of Mac organisms requires attention on the control of free-living amoebae and biofilms that support their persistence.

Mac organisms have also demonstrated resistance to elevated temperatures. Several authors have reported recovery of *M. avium* from hot water systems at temperatures between 50 and 57°C (DuMoulin et al., 1988; von Reyn et al., 1994; Covert et al., 1999; Norton et al., 2004). Additional factors thought to have a role in encouraging growth in the distribution system include high assimilable organic carbon levels, as well as distribution system materials and construction (e.g., pipe materials, gaskets, coatings, corroded pipes, dead ends, spaces, long storage times)

(Falkinham et al., 2001). Similarly to distribution system control strategies described for *Legionella* (Bartram et al., 2007; Bentham et al., 2007), temperature control; control of water system design and construction to prevent the accumulation of biofilms, sediments or deposits; and nutrient control strategies should also prove effective in the control of Mac organisms.

B.2.2.4 Assessment

No suitable indicators have been identified to signal increasing concentrations of Mac organisms in water systems. For example, studies have found no relationship between the numbers of non-tuberculous mycobacteria recovered from reservoir water and coliform counts, HPCs, and total and free chlorine levels (Glover et al., 1994; Aronson et al., 1999). There is some evidence that M. avium presence is associated with turbidity in raw waters (Falkinham et al., 2001), but further exploration of this issue is needed.

Currently, the presence of mycobacteria in water is not regulated by any country or international organization, including Canada. The U.S. EPA has identified *M. avium* and *M. intracellulare* as waterborne health-related microbes that need additional research on their health effects, their occurrence in water, and their susceptibility to treatment methods (Reynolds, 2001). These organisms have also been included in a list of candidate contaminants for possible regulation by the U.S. EPA (2009). At the present time, there is not sufficient information to warrant actions based on the presence of the organisms in the absence of disease.

B.2.3   *Aeromonas*

The genus *Aeromonas* has gained public health recognition as organisms that can cause opportunistic infections in humans. Species of *Aeromonas* have been associated with gastroenteritis, however understanding of the role the organisms play as a cause of diarrhoeal illness is presently incomplete. Skin, wound and soft tissue infections with *Aeromonas* species as a result of exposure to contaminated water in non-drinking water scenarios have been well documented. It is believed that drinking water has the potential to serve as a route of transmission, but direct evidence of *Aeromonas* as a cause of drinking water-acquired gastrointestinal illness is lacking.

Aeromonads are Gram-negative, short rod-shaped bacteria that share some similarities with *Vibrio* and *E. coli.* They are universally found, occurring naturally in virtually all water types. The genus *Aeromonas* contains more than 17 distinct genetic species. Three species: *A. hydrophila*, *A. veronii* biovar *sobria* (syn. *A. sobria*) and *A. caviae*, account for roughly 85% of human infections, and are therefore considered to be the species of most importance for drinking water systems (Janda and Abbott, 1998; 2010).

B.2.3.1 Sources and exposure

Aeromonas species can be found in virtually all surface water types (freshwater, marine, and estuarine) in all but the most extreme conditions of pH, salinity and temperature (Percival et al., 2004; AWWA, 2006). They are less frequently detected in groundwater, with their presence in these systems typically indicating well contamination (Havelaar et al., 1990; Massa et al., 2001; Borchardt et al., 2003).

Aeromonads are recognized animal pathogens (Percival et al., 2004; AWWA, 2006). The organisms have been isolated from the gastrointestinal tract and infected tissues of a number of cold-blooded and warm-blooded animals, most notably fish, birds, reptiles and domestic livestock (U.S. EPA, 2006; Janda and Abbott, 2010). They also have been recovered from retail food items such as meat, poultry and dairy products (Janda and Abbott, 2010). It has been

suggested that animals may be an environmental reservoir for *Aeromonas* (Janda and Abbott, 2010).

The organisms are not considered to be natural fecal pathogens (U.S. EPA, 2006). *Aeromonas* species are not normally found in human faeces in high numbers (Janda and Abbott, 2010); however a small percentage of the population can carry the bacteria in their intestinal tract without showing symptoms of disease (von Gravenitz, 2007). Estimates of the prevalence of *Aeromonas* in human fecal samples worldwide have been roughly put at 0-4% for asymptomatic persons and as high as 11% from persons with diarrhoeal illness (Burke et al., 1983; U.S. EPA, 2006, von Gravenitz, 2007; Khafanchi et al., 2010). Individual studies have observed rates that have been as high as 27.5% and 52.4% for asymptomatic persons and diarrhoeal illness cases respectively (Pazzaglia et al., 1990; 1991). Numbers of *Aeromonas* are much higher in sewage, with concentrations of $>10^8$ CFU/mL having been reported (Percival, 2004).

Levels of *Aeromonas* in clean rivers, lakes, and storage reservoirs, have generally been reported to be in the range of $1\text{-}10^2$ CFU/mL (Holmes et al., 1996). Surface waters receiving sewage contamination and nutrient-rich waters in the warmer summer months may reach concentrations of 10^3 - 10^5 CFU/mL (Holmes et al., 1996; U.S. EPA, 2006). Groundwaters generally contain less, with less than 1 CFU/mL (Holmes et al., 1996). Drinking water immediately leaving the treatment plant is typically in the range of <1 to 10^2 CFU/mL (Holmes et al., 1996; U. S. EPA, 2006; Pablos et al., 2009; Janda and Abbott, 2010) , with potentially higher concentrations in drinking water distribution systems, (Payment et al., 1988; Chauret et al., 2001; U.S. EPA, 2006). Concentrations in individual environments can be expected to vary, however,

The organisms can survive over wide ranges of pH (5-10) and temperature (2-42°C) (Percival, 2004). Water temperature is particularly important to *Aeromonas* growth. In temperate climes during the warmer months of the year, the bacteria have been shown to be more readily detected from source waters and water distribution systems (Chauret et al., 2001; U.S. EPA, 2006; Janda and Abbott, 2010). Aeromonads are also very versatile nutritionally. They are capable of growing to elevated numbers in water with high organic content and can also survive in low nutrient waters (Kersters et al., 1996).

Similar to other bacteria, *Aeromonas* species can enter into a viable-non-culturable (VNC) state under stressful conditions in aquatic environments. There is some debate at to what effect this state has on a species' viability and pathogenicity. Maalej et al. (2004) reported that cells of a strain of *A. hydrophila* rendered non-culturable under marine stress conditions lost haemolytic and cytotoxic properties; but that these could be regained following recovery at warmer temperatures. In contrast, Mary et al. (2002) observed that VNC cells of *A. hydrophila* lost viability, and this could not be regained following a temperature upshift to 25°C. It has been suggested that survival properties may differ depending on the species and strain of *Aeromonas* (Brandi et al., 1999; Mary et al., 2002).

The organisms have been detected in the distribution systems of chlorinated drinking water supplies worldwide (Chauret et al, 2001; Emekdas et al., 2006; Langmark et al., 2007; September et al., 2007). As with other bacterial pathogens, the presence of biofilms and free-living amoebae have been identified as factors contributing to higher concentrations of *Aeromonas* encountered in drinking water distribution systems relative to finished water (Rahman et al., 2008; September et al., 2007). During an assessment conducted as part of their Unregulated Contaminant Monitoring Regulations, the U.S. EPA (2002) provided data indicating that *Aeromonas* could be detected in 11% of municipal systems serving greater than 10,000 persons and 14% of systems serving less than 10,000 persons. The concentrations of *Aeromonas* reported were less than 10 CFU/100 mL in 78% of the samples (U.S. EPA, 2002). Limited

studies have been conducted on *Aeromonas*-protozoa interactions within the municipal supplies. Rahman et al. (2008) observed that the bacteria may use the free-living amoebae *Acanthamoeba* as a reservoir to improve transmission and for protection from disinfectants.

Exposure to *Aeromonas* species through direct contact of wounds or skin follicles with contaminated waters has been reported for recreational-type water environments such as lakes, rivers, swimming pools and hot tubs (Gold and Salit, 1993; Manresa, 2009). Unusual water situations brought about by floods or disaster events can be expected to create similar opportunities for *Aeromonas* exposure. Wound infection with species of *Aeromonas* was a problem among victims of the Thailand tsunami as a result of exposure to contaminated floodwaters (Hiransuthikul et al., 2005). Exposure to *Aeromonas* in contaminated floodwaters was also expected amongst victims and rescue workers following Hurricane Katrina (Presley et al., 2006). Person-person transmission of *Aeromonas* resulting in infection is not expected to occur (U.S. EPA, 2006).

The evidence for acquiring *Aeromonas* infection through the ingestion of drinking water is not well established, and the subject is one of debate (von Gravenitz et al., 2007). The presence of *Aeromonas* in finished drinking water supplies and distribution samples has been well documented suggesting a possible route of transmission (LeChevallier et al., 1980; Payment et al., 1988; Borchardt et al., 2003; Emekdas et al., 2006; Kuhn et al., 2007; Scoaris et al., 2008). However, other findings have been cited which oppose this suggestion. Epidemiological investigations have demonstrated little evidence of direct connections between patient isolates of *A. hydrophila* with isolates recovered from their drinking water supplies. Borchardt et al. (2003) observed that *Aeromonas* isolates were infrequently found in stool samples of gastroenteritis patients and that those detected were not genetically related to isolates recovered from drinking water. Additionally, researchers have cited the virtual absence of reported waterborne outbreaks of diarrhoea against the near universal presence of *Aeromonas* in water environments as evidence supporting that transmission of these organisms occurs by a mechanism other than through drinking water (von Gravenitz, 2007; Janda and Abbott, 2010). Some researchers have speculated that for many fecal isolates of *Aeromonas*, colonization of the human gastrointestinal tract may only be fleeting (Janda and Abbott, 2010).

The ingested dose of *Aeromonas* necessary to cause gastrointestinal infections is uncertain. Limited study has suggested that a high dose is required (U.S. EPA, 2006; Janda and Abbott, 2010). In an early volunteer feeding study, Morgan et al. (1985) reported that only 2 of 57 individuals developed diarrhoea following ingestion of *A. hydrophila* strains at doses of up to 10^{10} CFU. It has been speculated that the concentrations required to cause illness are much higher than the numbers that would typically be found in treated drinking water supplies (U.S. EPA, 2006).

Recently, in a large survey of clinical and waterborne strains of *Aeromonas* collected from across the United States and worldwide, Khafanchi et al. (2010) reported detecting 3 isolates belonging to the *A. caviae* group that were genetically indistinguishable and possessed the same virulence factors. The authors suggested that these findings provided the first evidence of human infection and colonization by a waterborne *Aeromonas* strain (Khafanchi et al., 2010).

B.2.3.2 *Health effects*

Aeromonas-associated diarrhoea has been encountered worldwide, mostly in normally healthy persons across all age groups (Janda and Abbott, 2010). Having low stomach acidity, receiving antimicrobial therapy or having compromised immune function (e.g., from HIV infection or through underlying disease – especially liver disease) are thought to be associated

risk factors (Merino et al., 1995; Percival, 2004, von Gravenitz, 2007; Janda and Abbott, 2010). The distinction of *Aeromonas* as a cause of gastrointestinal illness is controversial (von Gravenitz, 2007; Janda and Abbott, 2010). Case reports and a small number of foodborne outbreaks have linked the presence of *Aeromonas* to cases of diarrhoeal disease (U.S. EPA, 2006; Janda and Abbott, 2010). However at the present, no outbreaks of gastrointestinal illness have been reported for which a strain of *Aeromonas* has been definitely identified as the causative agent (Janda and Abbott, 2010). Furthermore, researchers have been unable to find an animal model in which *Aeromonas*-mediated gastrointestinal illness can be replicated (U.S. EPA, 2006; Janda and Abbott, 2010).

Where *Aeromonas* species have been associated with gastroenteritis, the most common symptom is watery diarrhoea that has been accompanied by fever and abdominal pain (Janda and Abbott, 2010). Far less commonly, *Aeromonas* has also been identified in association with other forms of gastrointestinal illness ranging from a dysenteric type of illness with bloody stools, to a chronic, or subacute watery diarrhoea (Janda and Abbott, 2010). *Aeromonas* infections can also be asymptomatic, with individuals shedding the bacteria in their stools, but not showing any symptoms of disease (Percival, 2004).

Aeromonas species have been positively isolated from skin, wound and soft-tissue infections (Percival, 2004; Janda and Abbott, 2010). These can range in scope from mild irritations (e.g., pus-filled lesions) to cellulitis (inflammation below the skin), to, in extreme cases, necrotizing fasciitis (flesh-eating disease) (Janda and Abbott, 2010). These are often the result of trauma or penetrating injury from occupational or recreational water exposure, and are generally seen more frequently in adults than children. *Aeromonas* has also recently been implicated in respiratory infections. However these have been rare and have largely been caused by near drownings or aspirations of contaminated waters unrelated to drinking water supplies (Janda and Abbott, 2010)

Factors responsible for the pathogenicity and virulence of *Aeromonas* species or strains are poorly understood. A number of potential virulence components have been identified that would appear to enable the organisms to behave as human pathogens. These include such things as pili, fimbriae and flagella for attachment and colonization; external lipopolysaccharides, capsules or surface layers to assist in evading host defenses; and toxins, hemolysins, proteases and other enzymes for causing damage to host cells (von Gravenitz, 2007; Janda and Abbott, 2010). Current studies have been unable to specifically pinpoint which combination of factors would make a strain of *Aeromonas* behave as an enteropathogen (Janda and Abbott, 2010). Research has identified that a known diarrhoea-causing strain of *A. hydrophila* possesses four prospective virulence factors: two hemolysins (named Act and HlyA), a heat-stable enterotoxin (Ast) and a heat-labile enterotoxin (Alt) (Erova et al., 2007; Janda and Abbott, 2010). Despite such findings, the role and relative significance of each remains uncertain, as studies have also found these factors distributed among numerous clinical and environmental strains in different combinations (Erova et al., 2007; von Gravenitz, 2007; Castilho et al., 2009; Janda and Abbott, 2010). It has been proposed that only certain subsets of *Aeromonas* strains have the ability to cause disease (Janda and Abbott, 2010).

Aeromonas is not a reportable organism in North America, or in most countries worldwide (PHAC, 2011; CDC, 2011; Janda and Abbott, 2010). Of the case reports or outbreaks of *Aeromonas*-related illness encountered in the literature, most have been tied to food, hospitals, travel, non-water environments or are unknown. At present, no epidemiological evidence has been provided linking an *Aeromonas* outbreak to ingestion, inhalation or skin contact with treated drinking water supplies (U.S. EPA, 2006; von Gravenitz, 2007; Janda et al., 2010).

As *Aeromonas*-related gastrointestinal illness is mild and self-limiting, treatment for infection is generally not necessary. However, for other presentations of infection, antibiotic therapy is usually implemented. Aeromonads are resistant to ampicillin and a variety of other β-lactam antibiotics including penicillin and some cephalosporins (Percival, 2004; Janda and Abbott, 2010).

B.2.3.3 Treatment technology

As mentioned previously, aeromonads are ubiquitous in many water environments. Consequently, they will be present in most source waters used for drinking water production. Nonetheless, existing evidence indicates that current treatment and disinfection methods can effectively remove *Aeromonas* from treated drinking water. Data from pilot-scale (Harrington et al., 2003; Xagoraraki et al., 2004) and full-scale (Chauret et al., 2001; El-Taweel and Shaban, 2001; Yu et al., 2008) investigations have demonstrated that well-operated conventional filtration systems (coagulation-flocculation-sedimentation-rapid rate gravity sand filtration) are capable of *Aeromonas* removals of up to 4-log. In a pilot-scale conventional treatment study, Xagoraraki et al. (2004) observed that reducing filter effluent turbidity to less than 0.2 NTU resulted in *A. hydrophila* removals of greater than 3-log to just under 4-log (median: 3.5 log). Yu et al. (2008) investigated the effectiveness of different water treatment processes in removing *Aeromonas* as measured using both culture-based and real-time PCR detection methods. Conventional filtration (3 full-scale plants) reported removals of culturable *Aeromonas* ranging from > 0.3 log to 4 log (Yu et. al., 2008). The authors further reported that no culturable *Aeromonas* could be detected after sedimentation (Yu et al., 2008). Log removals as measured by real-time PCR detection correlated well with, but were routinely lower than, those demonstrated by the culture-based detection method (Yu et al. 2008).

For slow sand filtration, the authors examined 2 full-scale and 1 pilot-scale plants, reporting log removals of greater than1 log, greater than 1.8 log for the full-scale plants and greater than 1 log for the pilot-scale plant. Culturable *Aeromonas* was not detected in samples collected post-filtration (Yu et al., 2008). Meheus and Peters (1989) reported similar results for slow sand filtration, observing *Aeromonas* removals of 98-100%.

With membrane filtration, the full-scale plants included in the Yu et al. (2008) study demonstrated a capability of removing culturable *Aeromonas* by greater than 3.8 and 4 logs.

Aeromonads are susceptible to inactivation by disinfectants commonly used in the drinking water treatment, such as chlorine, monochloramine, chlorine dioxide, ozone and UV (Knoechel et al., 1991; Medema et al., 1991, Sisti et al., 1998; U.S. EPA, 2002; 2006). For chlorination, Sisti et al. (1998) reported *Aeromonas* T_{95} values (5°C, inoculum ~10^4 cells) of 5 min at 0.6 mg/L, and of 68 min at 0.05 mg/L free chlorine in a lab-scale chlorination experiment. The authors also found *Aeromonas* (clinical strains) to be more susceptible to chlorine than *E. coli* (clinical strains). Free chlorine concentrations of 0.14 mg/L (10°C) and greater than 0.5 mg/L (20-37°C) were sufficient to produce a 5-log inactivation of clinical and nosocomial strains of *Aeromonas* within 5 min in an experiment conducted by Chamorey et al. (1999). In contrast, de Oliveira Scoraris et al. (2008) observed that the majority of *Aeromonas* strains (water and culture collection strains) were not killed after 1 minute exposure to free chlorine at 1.2 mg/L.

Chauret et al. (2001) conducted a study at both full-scale and pilot-scale simultaneously to assess the presence of *Aeromonas* in source water and at various sites within the treatment plant and distribution system and to assess biofilm formation. The authors noted no detectable *Aeromonas* in treated water immediately after secondary disinfection with chloramine

(concentration range: 2-3 mg/L), despite observing counts ranging from <1 to 490 CFU/100 mL after chlorine disinfection (pre-filtration) and post-GAC filtration.

With chlorine dioxide, Medema et al. (1991) reported CT_{99} values of 0.04-0.14 mg·min/L for a drinking water strain of *A. hydrophila*. In the same study, a naturally occurring *Aeromonas* population (predominantly *A. sobria*) was observed to be slightly more sensitive with a reported CT_{99} of 0.1 mg·min/L.

For UV disinfection, data produced by the U.S. EPA suggested the capability for a 1 and 2 log inactivation of *A. hydrophila* at doses of 3 and 8 mWs/cm^2 respectively (equivalent to 3 and 8 mJ/cm^2) - doses significantly less than those commonly employed in water treatment (U.S. EPA, 2002).

In the distribution system, maintaining an adequate disinfectant residual should provide control of *Aeromonas* in the finished water. The potential exists for *Aeromonas* to regrow in the distribution system, however. During a year-long survey of a major drinking water distribution system in Scotland, Gavriel et al. (1998) reported that although *Aeromonas* was not detected in water samples collected downstream from chlorination prior to the distribution network; it could be occasionally recovered from distribution samples, even at locations maintaining substantial residual chlorine doses (> 0.2 mg/L). Similarly, other studies have demonstrated that *Aeromonas* could be detected in municipal distribution systems at locations having temperatures below 14°C and chlorine residuals greater than 0.2 mg/L (Chauret. al, 2001; Pablos et al., 2009)

Elimination of *Aeromonas* in the distribution system once the organisms become established in biofilms can be difficult (Holmes and Nicholls, 1995; Gavriel et al., 1998; Langmark et al., 2007). Elements important for helping to control *Aeromonas* growth include limiting the number of organisms entering the distribution system through effective treatment and maintenance, maintaining low water temperatures, providing appropriate free chlorine residuals, and limiting the levels of organic carbon compounds (WHO, 2010).

B.2.3.4 Assessment

Some studies have been undertaken to determine if the indicators currently used in the drinking water industry, including *E. coli*, total coliforms, and HPC, can be used as surrogates for the presence of *Aeromonas*. Several studies have showed no evidence of a relationship between *Aeromonas* incidence and coliforms, *E. coli*, or HPCs (Holmes et al., 1996; Gavriel et al., 1998; Fernandez et al., 2000; Pablos et al., 2009). Although no direct correlation exists between *Aeromonas* populations and total HPC counts, the organisms do make up a portion of HPC bacteria found in water, and are detected by HPC tests (Pablos et al., 2009). The Netherlands have established drinking water standards for *A. hydrophila*, consisting of a median value (1 yr period) of 20 CFU/100 mL in water leaving the treatment plant and a 90th percentile value (1 yr period) of 200 CFU/100 mL in distribution system water (van der Kooij, 2003; Pablos et al., 2009). These values have been based on an assessment of achievability and motivated by a precautionary approach, rather than on the public health significance of their occurrence in drinking-water (WHO, 2002).

Aeromonas is not considered an indicator of fecal contamination or treatment failure (U.S. EPA, 2002). The organisms have been proposed as a possible supplemental indicator of drinking water quality by relating to the presence of biofilm. Therefore, if there are significant increases in *Aeromonas* concentrations in a drinking water supply, this indicates a general deterioration of bacteriological quality.

When looking at the overall public health significance of *A. hydrophila* in drinking water, further epidemiological studies are needed for a better understanding of the relationship between

Aeromonas illness and the presence of these organisms in drinking water. Based on the current evidence, treated drinking water likely represents a very low risk. It has been proposed that in comparison to other possible pathogens that can potentially be acquired through drinking water, *Aeromonas* is at the low end of the scale in terms of relative risk (Rusin et al., 1997; Janda and Abbott, 2010). Nevertheless, it is advisable to minimize *Aeromonas* in drinking water supplies as much as is practical until its public health significance has been fully investigated.

B.2.4 *Helicobacter pylori*

Helicobacter pylori is a recognized human pathogen that can colonize the human stomach. The understanding of how this organism is spread is still quite limited, however it is believed that there are a few routes of transmission, including through drinking water (Percival and Thomas, 2009). Disease is benign for the most part in the majority of persons infected, but more serious disorders such as peptic ulcers or stomach cancer can develop in a small percentage of cases.

Helicobacters are Gram-negative, motile small curved rods that are closely related to *Campylobacter*. The organisms have two distinct forms, a spiral rod shape and a shorter coccoid form, which is taken on under conditions of stress. To date, the coccoid form has been found to be non-culturable. The genus *Helicobacter* has at least 25 species as determined by DNA sequencing – of which *H. pylori* is the species of relevance for the water industry. Other *Helicobacter* species have been detected in humans that have been associated with gastric illness; however these are not considered to be as prevalent.

B.2.4.1 Sources and exposure

The primary reservoir identified for *H. pylori* is the human stomach (Dunn et al., 1997; Brown, 2000). There has been evidence that some animals can be infected by *H. pylori* (cats, dogs, sheep, primate monkeys) but presently the consensus is that they do not hold a significant role as reservoirs in transmitting this organism to humans (Baele et al., 2009; Haesebrouck et al., 2009). Although *H. pylori* has been cultured from human faeces, at present isolation from water using culture methods has not been successful (Percival and Thomas, 2009). It is believed that the spiral culturable form rapidly transforms into a viable, non-culturable state (coccoid form) in the water environment. This is thought to be a stress response to environmental changes which can include: temperature, low nutrient availability and differences in osmolarity (Adams et al., 2003; Percival and Thomas, 2004).

Exact details on the transmission of *H. pylori* remain unclear (Bellack et al., 2006). Based on epidemiological findings, a higher risk of *H. pylori* infection exists among persons of low economic status living in crowded conditions or unhygienic environments (Brown, 2000; Gomes and Demartinis, 2004). Transfer mechanisms that have been proposed include gastric-oral, oral-oral and fecal oral (Percival and Thomas, 2009). Overall, it is speculated that person-person transfer is the most likely route of transmission (Brown, 2000). The fact that it has not yet been possible to culture viable Helicobacters from the water environment has raised questions regarding the possibility of waterborne transmission. Nevertheless, there has been significant evidence provided in support of water as an important source of infection. Molecular techniques (PCR, Fluorescent in-situ DNA hybridization [FISH]) have been used to confirm the presence of *H. pylori* in natural waters (Hegarty et al., 1999; ; Sasaki et al., 1999; Horiuchi et al., 2001; Moreno et al., 2003; Benson et al., 2004). As well, in the laboratory, *H. pylori* has been shown to survive for days, up to weeks, in sterile river water, stream water, saline solution, and distilled water at a wide variety of pH levels and in temperatures ranging from 4°C to 25°C (West et al.,

1992; Shahamat et al., 1993; Adams et al., 2003; Azevedo et al., 2008). As with *Legionella* and mycobacteria, evidence has been supplied that biofilms and free-living waterborne amoebae may provide environmental niches where *H. pylori* can persist (Park et al., 2001; Winiecka-Krusnell et al., 2002; Watson et al., 2004; Braganca et al., 2007)

 Waterborne transmission has been suggested as an important source of infection in developing countries (Bellack et al., 2006). Supporting evidence has come from epidemiological studies showing that individuals consuming untreated or more-contaminated waters had a high risk of infection (Klein et al, 1991; Goodmane et al, 1996; McKeown et al., 1999; Herbarth et al., 2001; Brown, 2002). There has been less evidence of the importance of waterborne transmission in developed countries (Percival and Thomas, 2009). However, findings of *H. pylori* in drinking water distribution systems suggest it still can play an important role (Baker and Hegarty, 2001; Watson et al., 2004; Giao et al., 2008; Percival and Thomas, 2009). Additional research is required to provide further insight into the persistence, viability and associated risk of *H. pylori* in drinking water systems.

 The infectious dose necessary for colonization of humans is not known. Results of challenge studies suggest that it is less that 10^4 cells, and related to stomach pH (Solnick et al., 2001; Graham et al., 2004). However, given the high percentage of infected individuals among the population and the evidence from cases of accidental infection (e.g., from laboratory work, use of improperly maintained endoscopes), the dose could be much lower (Langenberg, 1990; Matysiak-Budnik et al., 1995).

B.2.4.2 Health effects

 Human infection with *H. pylori* leads to gastritis, or inflammation of the stomach lining (Dunn et al., 1997; Kusters et al., 2006). The organism colonizes the human stomach, stimulating the immune system and inflammatory cells; and it is this response that brings about gastritis. In the majority of *H. pylori* infections there are no outward telltale signs of disease (Kusters et al., 2006). It has been well established that infections with *H. pylori* are generally acquired during childhood, with a lower frequency of infection in adults (Allaker et al., 2002; Ernst and Gold, 2006). Further, infection, once established is considered to be lifelong unless treatment is pursued (Blaser et al., 1992; Kusters et al., 2006). Broad estimates of the risk of infected persons developing these advanced diseases have been put at 10-20% for peptic ulcer disease and 1-2% for gastric cancer (Ernst and Gold, 2000; Kusters et al., 2006). *H. pylori* is also the primary cause of peptic ulcers (Kuipers et al., 1995). It has been estimated that 85-95% of ulcers are the result of infection with this organism (Kuipers et al., 1995). Carriage of *H. pylori* has also been recognized as an important risk factor for the development of gastric cancer (i.e., gastric lymphoma and adenocarcinoma) (Dunn et al., 1997; Pinto-Santini et al., 2005).

 Infection with *H. pylori* is treatable (Scott et al., 1998; Vakil et al., 2007); and researchers have indicated that data from animal and human infection studies suggests that an *H. pylori* vaccine is possible (Graham et al., 2004; Del Guidice et al., 2009). This area of research is currently being explored.

B.2.4.3 Treatment technology

 Similar to other bacteria, a proportion of the *H. pylori* present in the source water will be removed using physical methods, such as coagulation, sedimentation, and filtration. *H. pylori* is also susceptible to disinfectants commonly used in drinking water treatment (e.g., chlorine, UV, ozone and monochloramine)

 Literature regarding the disinfection of *H. pylori* is limited when compared to that

available for other waterborne bacterial pathogens. Investigations have been made difficult due to the fact that cells of *H. pylori* become viable but non-culturable in the environment, and this form cannot be detected easily by regular culture methods (Moreno et al., 2007). With chlorination, data provided from the few reported studies suggested log reductions of culturable *H. pylori* cells ranging from 0.3 log at 0.1 mg/L chlorine for 1 minute (Baker et al., 2002) to greater than 4 log at 0.5 mg/L chlorine for 80 seconds (Johnson et al., 1997), to approximately 7 log at 1 mg/L chlorine for 5 minutes (Moreno et al., 2007). Moreno et al (2007) conducted research using a combination of direct viable count and fluorescent in-situ DNA hybridization methods (DVC-FISH) specifically to study the effects of chlorination on *H. pylori* cell viability. The researchers demonstrated that viable *H. pylori* cells could be detected after 3 hours of exposure to 1.0 mg/L chlorine, but not after 24 hours of exposure. For UV disinfection, Hayes et al. (2006) reported greater than 4 log inactivation of culturable *H. pylori* cells at fluences of less than 8 mJ/cm^2. Disinfectant CT$_{99}$ values for *H. pylori* reported by Baker et al. (2002) were ozone: 0.24 mg/L min; chlorine: 0.299 mg/L min; and monochloramine 9.5 mg/L min. In terms of response to disinfection as compared to *E. coli*, Baker et al. (2002) reported that *H. pylori* was statistically more resistant to chlorine and ozone, but not to monochloramine. Other authors have similarly reported *H. pylori* having greater resistance to chlorine as compared to *E. coli* (Johnson et al., 1997; Moreno et al., 2007).

Association with biofilms has also been shown to protect *H. pylori* from disinfectants, similar to other bacterial pathogens. Giao et al. (2010) observed *H. pylori* cells (measured by peptide nucleic acid [PNA] probe) remained viable for at least 26 days following exposure to 0.2 and 1.2 mg/L chlorine. Also, in contrast to findings provided by other researchers, the authors observed that *H. pylori* cells in suspension did not lose culturability after 30 minutes exposure to chlorine at an initial concentration of 1.2 mg/L (Giao et al., 2010). The current body of research suggests that the CT provided by a typical water treatment plant is sufficient to inactivate *H. pylori* in the finished water. However, if *H. pylori* does enter the distribution system, potentially through a break in treatment or infiltration into the system, disinfectant residuals maintained in the distribution system are probably insufficient for inactivation (Baker et al., 2002). Successful distribution system control of *Helicobacter* would similarly be aided by management steps to reduce the biofilm formation and the presence of free-living amoebae in this environment.

B.2.4.4 Assessment

Overall, the predominant transmission route for *H. pylori* seems to be situation dependent, with person-to-person transmission playing a key role in many circumstances. Water and food appear to be of lesser direct importance, but they can still play a significant role in situations with improper sanitation and lax hygiene

Much is still unknown regarding the ecology and behaviour of *H. pylori* in water systems. However sufficient information has been provided to suggest *H. pylori* can be regarded as a potential human pathogen with the potential for waterborne transmission. Illness associated with *H. pylori* infection is of a mild or benign nature in the majority of cases, and outbreaks of illness have not been linked to the presence of *H. pylori* in drinking water supplies. Further research is needed to provide clarity on such topics as its presence in source waters, its susceptibility to treatment and disinfection; and its overall significance for drinking water systems in Canada.

B.3.   Issues of Emerging Interest

B.3.1. Disinfection and antibiotic resistance in organisms

Disinfectants and antibiotics exert action on bacteria through very different mechanisms. Antibiotics characteristically act against specific target sites within the bacteria – interfering with a particular specific component of an essential process or pathway. In contrast, disinfectants act in a general manner against multiple targets which are fundamental components of the bacterial cell (e.g., proteins and DNA/RNA). Free chlorine, chloramine, chlorine dioxide and ozone are all very strong oxidizers which inactivate bacterial cells by destroying the activity of cell proteins that can be involved with cell structure or metabolism. UV light inactivates bacterial cells by altering the DNA in such a way that the cell can no longer multiply. Because of the fundamental differences in the way these two types of antibacterial strategies operate, antibiotic-resistant bacteria are not expected to show increased resistance to the action of drinking water disinfectants.

Bacterial resistance to antibiotics can be brought about in a variety of ways. Some examples are that cells may not allow the antibiotic to penetrate the cell, they may lack the required target site, or they or may possess enzymes that can modify or destroy the antibiotic. There are numerous types of antibiotics which can be categorized into different classes based on their structure or mode of action. Bacteria having a particular resistance mechanism may be unaffected by antibiotics of a similar class or that target the same site. These same bacteria may be vulnerable to different antibiotics, or may possess mechanisms that make them resistant to multiple classes of antibiotics.

Very little data has been generated to date regarding the effects of disinfectants on antibiotic-resistant bacteria in drinking water. Some early work found that a greater proportion of heterotrophic plate count bacteria in treated water are antibiotic-resistant bacteria as compared to those in untreated water (Armstrong et al., 1981; 1982). Templeton et al., (2009) conducted an investigation on the susceptibility of ampicillin and trimethoprim-resistant strains of *E. coli* to free chlorine and UV disinfection. The authors observed no differences in UV inactivation between antibiotic-resistant and antibiotic-sensitive E. coli under the doses and contact times tested. The trimethoprim-resistant *E. coli* strain did show slightly greater resistance to free chlorine than antibiotic-sensitive *E. coli*; however the authors concluded that the difference was likely to be negligible under chlorine doses and contact times typically observed in routine drinking water treatment. It was further concluded that these disinfectants did not likely select for ampicillin or trimethoprim resistance during drinking water treatment. No drinking water studies were found pertaining to the inactivation rates for other disinfectants such as ozone or chlorine dioxide against antibiotic-resistant bacteria.

Presently there is no evidence to indicate that the use of disinfectants in drinking water systems favours the selection of antibiotic-resistant bacteria in any way (Templeton et al., 2009). Additional study in this area is needed. The evidence at present, though limited, suggests that antibiotic resistance in bacteria is not an important factor in chlorine and UV treatment effectiveness at doses and contact times typically applied in drinking water treatment systems.

B.3.2. Residential-scale and private drinking water systems

The presence of *E. coli* in a residential-scale or private drinking water system demonstrates that the source or the system has been impacted by recent faecal contamination; as a result, the water is unsafe to drink. The absence of *E. coli* during routine verification should be an

adequate indication of the sufficient removal and inactivation of enteric bacterial pathogens. Where applicable, testing frequencies for residential-scale[1] systems will be determined by the responsible authority and should include times when the risk of contamination is greatest, for example, in early spring after the thaw, after an extended dry spell, or following heavy rains. For owners of private supplies, existing wells should be tested two to three times per year and during these same periods. New or rehabilitated wells should also be tested before use to confirm microbiological safety.

Other bacterial pathogens that occur naturally in the water environment can be found in groundwater, though typically at a lower frequency and in lower numbers than in surface waters. The levels of these organisms necessary to cause disease in healthy individuals are uncertain, though limited study has suggested that reasonably elevated numbers beyond those typically found in source waters are required. These organisms are most likely to be found in distribution system biofilms, and can survive and grow there to reach significant populations. In smaller systems, distribution system biofilms are less of a concern than in municipal systems because distribution systems are smaller or non-existent and the retention time for the finished water is shorter. Nevertheless, private homeowners should also be aware that in the case of *Legionella*, domestic hot water systems have been identified as being contaminated with this organism and as a result should keep the water heater at a suitable temperature (60ºC) to protect against the potential for the growth of this organism. The National Plumbing Code of Canada includes requirements of a minimum of 60°C for water temperature in hot water storage tanks, to address the growth of *Legionella* (NRCC, 2010). Homeowners should also take appropriate safety measures to reduce the risk of scalding at the tap. These measures include installing thermostatic or pressure-balanced mixing valves to control the water temperature at the tap (Bartram et al., 2007; Bentham et al., 2007).

B.3.3. Point of entry and point of use treatment devices.

The information on treatment, disinfection and inactivation of the organisms in this document is more relevant to municipal-scale systems. Municipal treatment of drinking water is designed to reduce microbial contaminants to levels below typically shown to be associated with disease. The use of residential-scale treatment devices on municipally treated water is generally not necessary but primarily based on individual choice. In cases where small systems or individual households obtain drinking water from private wells or surface water supplies such as lakes, treatment devices can be used as an additional barrier for reducing pathogen concentrations in drinking water.

Point of entry systems (installed where water enters the home) and point of use systems (installed at the faucet) have received interest for use in treatment and disinfection of drinking water in small, rural or remote communities, particularly those using a groundwater source. The most common types of treatment devices available for the removal and inactivation of waterborne pathogens (including bacteria) are UV disinfection and membrane filtration (reverse osmosis, nanofiltration). These technologies have been shown to effectively remove waterborne pathogens from drinking water (LeChevallier and Au, 2004; MWH, 2005).

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[1] For the purposes of this document, a residential-scale water supply system is defined as a system with a minimal or no distribution system that provides water to the public from a facility not connected to a municipal supply. Examples of such facilities include schools, personal care homes, day care centres, hospitals, community wells, hotels, and restaurants. The definition of a residential-scale supply may vary between jurisdictions.

Health Canada does not recommend specific brands of treatment devices, but it strongly recommends that consumers use devices that have been certified by an accredited certification body as meeting the appropriate NSF International (NSF)/American National Standards Institute (ANSI) drinking water treatment unit standards. Homeowners should ensure that the selection and installation of treatment devices comply with applicable local regulations. Homeowners should also follow the proper procedures for operation and maintenance found in the manufacturer's instructions. These instructions should be consulted regarding the device's performance capabilities.

Part C. References and acronyms

C.1   References

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## C.2	List of acronyms

AIDS	acquired immune deficiency syndrome
ANSI	American National Standards Institute
AWWA	American Water Works Association
CCL	Contaminant Candidate List
CDC	Centers for Disease Control
CFU	colony-forming unit
CT	concentration × time
DAEC	diffuse adherent *E. coli*
DNA	desoxyribonucleic acid
EAEC	enteroaggregative *E. coli*
EHEC	enterohaemorrhagic *E. coli*
EIEC	enteroinvasive *E. coli*
EPA	Environmental Protection Agency (United States)
EPEC	enteropathogenic *E. coli*
ETEC	enterotoxigenic *E. coli*
HIV	human immunodeficiency virus
HPC	heterotrophic plate count
LP	low pressure
Mac	*Mycobacterium avium* complex
MP	medium pressure
NSF	NSF International
NTU	nephelometric turbidity units
PCR	polymerase chain reaction
PHAC	Public Health Agency of Canada
POE	point of entry
POU	point of use
RNA	ribonucleic acid
UV	ultraviolet
VNC	viable-non-culturable
WHO	World Health Organization