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# Inactivation of bacterial biothreat agents in water, a review

**L. J. Rose** and

Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, GA, USA

**E. W. Rice**

National Homeland Security Research Center, US Environmental Protection Agency, Cincinnati, OH, USA

L. J. Rose: lrose@cdc.gov

## Abstract

Water supplies and water distribution systems have been identified as potential targets for contamination by bacterial biothreat agents. Since the 2001 *Bacillus anthracis* bioterrorist attacks, additional efforts have been aimed at research to characterize biothreat organisms in regards to their susceptibility to disinfectants and technologies currently in use for potable water. Here, we present a review of research relevant to disinfection of bacteria with the potential to pose a severe threat to public health and safety, and their potential surrogates. The efficacy of chlorine, monochloramine, chlorine dioxide, and ultraviolet light to inactivate each organism in suspension is described. The complexities of disinfection under varying water conditions and when the organisms are associated with biofilms in distribution systems are discussed.

### Keywords

biothreat agents; drinking water; Tier 1 agents; water disinfection; water treatment

## INTRODUCTION

Concerns regarding the security of drinking water supplies and associated infrastructure have increased over the last decade in response to potential vulnerabilities to intentional contamination with biological agents (Meinhardt 2005; Gleick 2006; Nuzzo 2006). Five bacterial genera belonging to the US Department of Health and Human Services and the Department of Agriculture Tier 1 listed agents and one genus previously on the category B Select Agent list (National Select Agent Registry 2013), have the potential to pose a severe threat to public health and safety, to animal or plant health, or to animal or plant products (Centers for Disease Control and Prevention 2012). Some of these agents were shown to survive in or to be transmitted by water or both (Sinclair *et al.* 2008; Pumpuang *et al.* 2011; Gilbert & Rose 2012).

Correspondence to: L. J. Rose, lrose@cdc.gov.

Drinking water can be contaminated at multiple points along the treatment and distribution chain. These locations include the source (surface water or ground water), the treatment facility, or after treatment such as in a storage tank or within the distribution system (Khan *et al.* 2001; Gleick 2006; Nuzzo 2006). Most medium to large drinking water utilities (those serving a population of ≥ 10,000) use a multiple-barrier approach to treatment, which employs various unit processes for the physical removal and chemical inactivation of pathogens. The treatment regimen can vary significantly between utilities: depending upon the source of the water (ground or surface), the source water quality (the organic load, pH, hardness, etc.), and organizational characteristics of a municipality (such as funding availability). Because water quality can vary seasonally, treatment scenarios can also vary seasonally at the same facility (American Water Works Association Disinfection Systems Committee 2000a, 2000b, 2008a, 2008b; Seidel *et al.* 2005). Each treatment facility employs a strategy suited to its needs. Primary pathogen removal and inactivation occurs within the treatment facility and includes physical removal processes such as flocculation, sedimentation, and filtration that are coupled with disinfection, including the use of ultraviolet (UV) irradiation, and/or various chemical disinfectants (free chlorine, monochloramine, chlorine dioxide, and ozone). Secondary disinfection provides a residual protection by preventing or controlling regrowth or recontamination during water storage and distribution. Chlorine dioxide, ozone, and UV light are used as primary disinfectants, while free chlorine and monochloramine are commonly used for both primary and secondary disinfection.

The water treatment industry typically uses concentration-time (Ct) values to calculate microbial inactivation and to evaluate the effectiveness of water treatment. The Ct value (mg $\cdot$ min $L^{-1}$) is the product of the concentration of a disinfectant (C, mg $L^{-1}$) and the time of exposure to the disinfectant (t, min), and is calculated for each organism of concern for a value that will describe the conditions necessary to achieve 2, 3, or 4 $\log_{10}$ inactivation of that organism (Hoff 1986; Connell 1996). References were selected for inclusion in this review if the test conditions were presented clearly, and if the data were presented in Ct values or graphically in a manner in which the Ct values could be estimated. The data presented typically were collected in laboratory settings with relatively clean potable water or ultra-purified water, and at temperatures and pH levels typical of most water distribution systems in the United States. The application of the results, however, must be qualified by saying that the efficacy of the disinfectants may not be comparable to what is presented if used in water with more organic matter, different pH levels, and widely different temperatures from those employed in the studies presented.

This review summarizes the findings of recent research on disinfection of bacterial threat agents in water with commonly used primary and secondary disinfectants, and discusses the knowledge gaps in this field.

## CHLORINE

According to a 2007 American Water Works Association (AWWA) survey of 312 water utilities, chlorine is the most used disinfectant for secondary disinfection of potable water (American Water Works Association Disinfection Systems Committee 2008a). Free chlorine

is known to react with organic substances to produce trihalomethanes and other hazardous halogenated disinfection by-products (DBPs). Water treatment plants must prevent elevated levels of DBPs to meet US Environmental Protection Agency (EPA) limits (US Environmental Protection Agency 2006a), yet still ensure that water has been adequately disinfected. Some utilities use alternate disinfectants over the year to address seasonal changes in source water quality or to comply with regulatory limits for DBPs (US Environmental Protection Agency 2006a, b).

Chlorine dissociates in water to form hypochlorous acid and hypochlorite ion in a pH-dependent reaction with hypochlorous acid predominating at or below pH 7.5. The inactivation efficacy of free available chlorine (FAC) on any microorganism is dependent upon both the pH and temperature of the water (Hoff 1986; Connell 1996). Hypochlorous acid is the most effective disinfectant of the chemical species in the water–chlorine mixture. In the 2007 AWWA survey (American Water Works Association 2008a), the mean reported distribution system water pH was 7.4, although the values ranged from 4.9 to 9.0. Considering this range of pH levels possible at any given time, the Ct values reported in Tables 1 and 2 can be considered a best case scenario. Most data reported in this review are the result of testing at pH 7.0 and 8.0.

The earliest systematic chlorine disinfection study with *Bacillus anthracis* spores was conducted in 1958 by Brazis *et al.* (1958). The work evaluated FAC efficacy on *B. anthracis* at several pH levels, and found that as the pH was increased from 6.2 to 10.5, increasing concentrations of FAC were needed to achieve the same 4 $\log_{10}$ inactivation. This finding was confirmed in subsequent work by Rice *et al.* (2005), in which Ct values (3 $\log_{10}$ reduction) for *B. anthracis* Sterne at 23 °C increased from 68 to 191 when pH was elevated from 7 to 8 (Table 1).

Water temperature also affects the efficacy of chlorine disinfection by influencing kinetics of the chemical reactions above and the interaction of the disinfectant with the cells (Haas 1980). An example from Rose *et al.* (2005) of this is the increase of the Ct (3 $\log_{10}$ inactivation) for *B. anthracis* Sterne from 86 to 271 with water temperatures of 25 °C to 5 °C, respectively (Table 1). Brazis *et al.* (1958) also demonstrated the effect of temperature on free chlorine disinfection of *B. anthracis;* a 4 $\log_{10}$ inactivation at 4 °C required at least three times the FAC concentration than that needed at 22 °C.

Not surprisingly, *B. anthracis* spores are significantly more resistant to chlorine than all of the non-sporulating bacteria tested (Rose *et al.* 2005), with 3 $\log_{10}$ inactivation Ct values ranging from 68 to 339 at pH 7, (depending upon water temperature and strain), and up to 478 at pH 8 (Table 1). Differences in susceptibility were seen between the virulent Ames strain (more resistant – Ct (3 $\log_{10}$ reduction) of 102 at pH 7, 25 °C), and the avirulent Sterne strain (less resistant – Ct of 86 at pH 7, 25 °C) (Table 1). Cho *et al.* (2006) demonstrated a synergistic effect when chlorine dioxide or ozone was followed by free chlorine treatment of *B. subtilis* spores that enhanced inactivation significantly. *B. anthracis* may behave similarly to *B. subtilis* in susceptibility to the combined treatment, though testing has yet to be done.

The Gram-negative vegetative biothreat agents (Table 2) are substantially more susceptible to chlorine than the *Bacillus* spores (Table 1), as evidenced by the significantly lower Ct values. Few differences in Ct values were seen between 5 ° and 25 °C when *Brucella suis, Brucella meilitensis, Burkholderia mallei,* and *Yersinia pestis* were challenged with FAC. These four bacteria were very susceptible to low levels of FAC, with 3 $\log_{10}$ inactivation Ct values below 0.7 (Table 2). Hence, if the water contained 1.0 mg $L^{-1}$ FAC, noted by the AWWA as being the mean and median concentration reported by the 2007 AWWA survey participants (American Water Works Association 2008a), then these four Gram-negative organisms would be inactivated by three orders of magnitude within 0.7 min, assuming a first order reaction rate.

*Burkholderia pseudomallei,* which is endemic to Southeast Asia and northern Australia, has been linked to disease transmitted by a community water supply (Currie *et al.* 2001). There appears to be much variation in tolerance to disinfectants within this species, though little is known about the resistance mechanism (Howard & Inglis 2003, 2005; O'Connell *et al.* 2009). Some strains produce increased amounts of mucoid polysaccharide, which is readily observed in their colonial morphology, and has been reported to affect resistance to UV light, but was not directly correlated to FAC resistance (Howard & Inglis 2005). One study conducted with Australian isolates found some cells in a test suspension survived 1,000 mg $L^{-1}$ FAC, using a broth-based most probable number (MPN) culture method (Howard & Inglis 2003). In contrast, using the same MPN culture method as well as a standard plate count culture method, O'Connell *et al.* (2009) tested 11 strains of various origins and morphologies (but not the same Australian isolates mentioned previously) and found that all strains were inactivated within 10 minutes with a FAC concentration of 1 mg $L^{-1}$ (Table 2). These findings suggest that a wide range of susceptibility exists within the species.

*Francisella tularensis* is another Gram-negative organism that demonstrates a greater tolerance to FAC as compared to vegetative cells of other biothreat organisms. *F. tularensis* possesses a surface capsule that has not been well characterized, but is known to protect the bacteria from serum complement (Sandstrom *et al.* 1988; McLendon *et al.* 2006), and may also protect the cell from disinfectants. Some variability in FAC tolerance is seen between strains, especially at 5 °C and pH 8 where 4 $\log_{10}$ reduction Ct values ranged from 24.3 for the LVS strain to 103.4 for the MA00-2987 strain (Table 2). Interestingly, no statistical differences in 4 $\log_{10}$ reduction Ct values were seen between strains at 25 °C and pH 7, with values ranging from 0.7 to 1.7 (O'Connell *et al.* 2010) (Table 2). Under the best conditions (pH 7, 25 °C) and 1 mg $L^{-1}$ FAC, the most tolerant strain of planktonic *F. tularensis* would require <1 min for inactivation by four orders of magnitude. However, at elevated pH (pH 8) and low temperature (5 °C) the most tolerant strain of *F. tularensis* would require up to 1.7 h for the same 4 $\log_{10}$ inactivation (O'Connell *et al.* 2010). Although the environmental reservoir(s) for *F. tularensis* is not yet fully understood, tularemia outbreaks have been associated with natural (untreated) water most likely due to the presence of infected animals or animal carcasses in or near the water (Karpoff & Antonoff 1936; Grunow *et al.* 2012). In natural waters and in potable water distribution systems, free-living amoeba are common, and the coexistence of *F. tularensis* with amoeba may contribute to its persistence in the environment and potentially to its resistance to disinfectants in water, as discussed later.

## MONOCHLORAMINE

Chloramines are created by the mixing of chlorine with ammonia. Chloramines are less effective against most organisms when compared to free chlorine, but are more stable than free chlorine in distribution systems and produce fewer of the regulated DBPs associated with chlorine disinfection (US Environmental Protection Agency 1999). Monochloramine is the predominate form used in drinking water disinfection and it is used in approximately 30% of US utilities for secondary disinfection, making it second only to free chlorine (American Water Works Association Disinfection Systems Committee 2008b). Monochloramine is most stable at pH 8, and most disinfection testing has been performed using preformed monochloramine at pH 8. However it may not be possible to consistently maintain this pH level in water distribution systems and the method of chloramination preparation (ammoniation prior to or after chlorination) varies among utilities.

All of the organisms tested were more tolerant of monochloramine than of free chlorine as evidenced by the larger Ct values (Tables 1 and 2). *B. anthracis* spores were, as expected, significantly more resistant than the non-spore forming organisms. Differences were seen between strains of *B. anthracis* spores, with the Ct values for the Sterne strain 1.5 to three times greater than those of the Ames strain, depending upon temperature (Table 1). To achieve a 2 $\log_{10}$ inactivation of planktonic *B. anthracis* Ames spores at 25 °C with 2 mg $L^{-1}$ monochloramine, 6.5 hours of contact time is necessary (785 mg · min $L^{-1}$ ÷ 2 mg $L^{-1}$ ÷ 60 min $hr^{-1}$), and 10 hours contact time for a 3 $\log_{10}$ reduction.

*B. pseudomallei, B. mallei, B. melitensis, B. suis, F. tularensis,* and *Y. pestis* demonstrated 2 $\log_{10}$ reduction Ct values of 21.9 to 104.4 if suspended in water maintained at 25 °C and pH 8 (Table 2). These Ct values can be interpreted by considering that if the target concentration of 2 mg $L^{-1}$ monochloramine (American Water Works Association Disinfection Systems Committee 2000a) is maintained in a distribution system, a 2 $\log_{10}$ inactivation of these Gram-negative bacteria will be achieved within 52 min (104 mg · min $L^{-1}$ ÷ 2 mg $L^{-1}$). As with chlorine, lower water temperature (5 °C) reduced the disinfection efficacy of monochloramine as evidenced by three to five times greater Ct values than at 25 °C (Table 2). *B. melitensis* was the most resistant of these vegetative Gram-negative organisms and when challenged with 2 mg $L^{-1}$ monochloramine in water held at 5 °C, 250 min (4.2 hours) was required to achieve a 2 $\log_{10}$ reduction in viable organisms (Ct = 501.8, Table 2).

## CHLORINE DIOXIDE

Chlorine dioxide ($ClO_2$) is generated by reacting chlorine gas with sodium chlorite in solution or solid form. About 8% of US water utilities were using chlorine dioxide in 2007, according to an AWWA survey (American Water Works Association Disinfection Systems Committee 2008a). $ClO_2$ dissipates quickly, and does not produce substantial amounts of DBPs. It is typically used as a primary disinfectant, with an average concentration of 1.18 mg $L^{-1}$ and 13.8 min contact time within the treatment facility (American Water Works Association Disinfection Systems Committee 2000b, 2008a).

As seen with chlorine and monochloramine, *B. anthracis* spores were more tolerant of $ClO_2$ than the remaining biothreat bacteria tested, with Ct values ranging from 57 to 738 for

spores and 0.02 to 2.0 for the remaining organisms (all Gram-negative), depending upon temperature, pH, and strain (Tables 1 and 2). At 25 °C, $ClO_2$ Ct values for *B. anthracis* Sterne spores were comparable to chlorine Ct values (3 $\log_{10}$ Ct at pH 7: 81 vs. 86, $ClO_2$ vs. FAC, respectively, Table 1) but at 5 °C, $ClO_2$ was less efficacious than chlorine (667 vs. 271, $ClO_2$ vs. FAC, respectively, Table 1).

$ClO_2$ was seen to be more effective at inactivating most of the Gram-negative organisms at pH 8 than at pH 7 (two exceptions: *B. suis* and *B. mallei* M-9 at 25 °C), although the effect was not as distinct as seen with FAC disinfection where better inactivation was observed at pH 7. Researchers have reported slight differences in the efficacy of $ClO_2$ on *Escherichia coli* and *Legionella pneumophila* with changes in water pH (Botzenhart *et al.* 1993, Junli *et al.* 1997). In contrast, changes in pH made no significant difference in inactivation of *B. anthracis* spores (Shams *et al.* 2011), *B. subtilis* spores (Cho *et al.* 2006), *B. stearothermophilus* spores, or *B. cereus* spores (Foegeding *et al.* 1986).

The Gram-negative organisms were more susceptible to $ClO_2$ at 25 °C than at 5 °C, with all showing a 3 $\log_{10}$ reduction at 25 °C Ct ≤0.7, and at 5 °C Ct ≤2.0. Differences in $ClO_2$ susceptibility between the Gram-negative organisms were most evident at pH 7 and 5 °C, with *F. tularensis, B. melitensis,* and *B. suis* demonstrating slightly more tolerance to $ClO_2$ than *Y. pestis, B. pseudomallei,* and *B. mallei.*

Regardless of these differences, Ct values for all of the Gram-negative organisms were ≤2, therefore at a concentration of 1 mg $L^{-1}$ $ClO_2$, all would be inactivated within 2 min under any of the water conditions tested.

## UV IRRADIATION

In US drinking water treatment facilities, UV light is used for primary treatment, but only 2% (5 of 218) of utilities reported using UV disinfection in 2007 (American Water Works Association Disinfection Systems Committee 2008b). This technology is expected to become more wide-spread because of a new EPA water treatment rule and guidance released in 2007 (US Environmental Protection Agency 2006a, c). The five utilities responding to the survey reported the designed fluence (dose) to be 40–45 mJ $cm^{-2}$. Point-of-use UV devices are also available to treat water at distal ends of the distribution system, which will deliver 40 mJ $cm^{-2}$ (class A device) or 16 mJ $cm^{-2}$ (class B device). The doses required for the given $\log_{10}$ inactivation of the bacterial biothreat agents are reported in Table 3. The data presented are from laboratory experiments conducted with a low-pressure lamp with a wavelength of 254 nm.

The radiant energy doses required for 4 $\log_{10}$ inactivation of the non-spore forming organisms tested ranged from 4.1 mJ $cm^{-2}$ (*Y. pestis* Harbin) to 10.5 mJ $cm^{-2}$ (*B. suis*). These fluences are similar to other non-spore forming waterborne pathogens such as *E. coli, Shigella sonnei, Yersinia enterocolitica,* and *Campylobacter jejuni* (Chang *et al.* 1985; Butler *et al.* 1987). When examining the UV susceptibility of the Gram-negative organisms in Table 3, little variation in UV susceptibility was seen between isolates of the same species (≤3 mJ $cm^{-2}$ in fluence for a 4 $\log_{10}$ inactivation).

*B. anthracis* spores were significantly more resistant to UV than the Gram-negative vegetative organisms with a 2 $\log_{10}$ inactivation requiring >36 mJ $cm^{-2}$, depending upon strain and experimental parameters (Table 3). In addition, the slope of the inactivation curve leveled off so that providing additional UV dose did not inactivate more spores proportionally. Knudson (1986) found *B. anthracis* Sterne spores to be more tolerant of UV than two more recent studies with *B. anthracis* Sterne spores (Nicholson & Galeano 2003; Rose & O'Connell 2009), in that a 2 $\log_{10}$ inactivation required approximately 135 mJ $cm^{-2}$, and a 3 $\log_{10}$ inactivation was not achieved with a fluence of 189 mJ $cm^{-2}$ (Table 3). The disparate susceptibilities reported may be explained by the differences in sporulation and/or storage media used, or slight differences in experimental conditions. Some researchers noted that susceptibility can vary with the growth media or physiological conditions of the cells when sporulation occurs (Nicholson & Law 1999; Rose & O'Connell 2009). Mamane-Gravetz & Linden (2005) also noted that when *B. subtilis* spores were challenged with UV light, the dose-response curve tailed off at fluences greater than 60 mJ $cm^{-2}$, and after testing hydrophobicity and particle sizes, found that the tailing was due to aggregates of spores providing protection of spores within the aggregate from UV light. Spores that are more hydrophobic demonstrate more aggregation and their UV fluence–response curves are more likely to tail off at the higher fluence applications (Mamane-Gravetz & Linden 2005). In another study, Nicholson & Galeano (2003) found no difference in UV susceptibility between *B. anthracis* Sterne spores and two *B. subtilis* spore strains.

If utilities design their UV treatment systems to deliver fluences of 40–45 mJ $cm^{-2}$, this should inactivate >4 $\log_{10}$ of all planktonic Gram-negative bacterial biothreat organisms present in non-turbid water, but only 1 to 2 $\log_{10}$ of *B. anthracis* spores (depending upon strain). Combining ozone treatment with UV treatment was shown to enhance reduction of *B. subtilis* spores by 33% (Jung *et al.* 2008), and may prove effective for *B. anthracis* spores as well.

## BOILING

Advisories to 'boil water' are often issued to the public if potable water is found unsuitable for consumption. Bringing water to a rolling boil for 1 min will inactivate most bacteria, viruses, and protozoa (Geldreich 1989). *B. anthracis* Sterne spores were found to require 3 min of boiling in a covered vessel for complete inactivation of 4.95 $\log_{10}$ spores. In an open vessel, however, 2.13 $\log_{10}$ and 2.01 $\log_{10}$ spores remained viable after 3 min and 5 min, respectively (Rice *et al.* 2004). In another study, *B. subtilis*, a surrogate for *B. anthracis*, was found to be present in the steam arising from a boiling flask containing a suspension of spores (Weber & Dunahee 2003). These two studies demonstrate that the boiling water in an open vessel does not sufficiently inactivate *Bacillus* spores in 3 to 5 minutes, and may aerosolize the spores, possibly creating an inhalation risk. Data are not available to demonstrate if steam escaping from a covered pot may also pose a possible inhalation risk.

## USE OF SURROGATES

Most laboratories do not have the security, containment, and protection needed to work with fully virulent biothreat agents, and surrogate organisms are commonly used for fate,

transport, and disinfection studies. The use of appropriate surrogates is essential so that the resulting data can be applied during a response to an actual biothreat event. In disinfection testing, use of a more resistant organism as a surrogate is often desired, since this would provide even more assurance that the treatment is effective and allow for some deviation in water quality or strain-to-strain susceptibility differences. The Gram-negative biothreat organisms tested are similar in disinfectant and UV susceptibility to other Gram-negative organisms and coliforms of concern to the water industry such as *E. coli* (Tables 1 and 3). In addition, low virulence strains that can be manipulated safely in biosafety level 2 laboratories are available for use as surrogate organisms (i.e., *Yersinia pestis* A1122). For these reasons, more attention has been given to finding appropriate disinfection surrogates for *B. anthracis* spores.

*Bacillus atrophaeus* var. *globigii* (BG; previously *B. globigii, B. subtilis* var. *niger*, and *B. atrophaeus* var. *niger*) is a commonly used surrogate for *B. anthracis*, partly because of the work of Brazis *et al.* (1958). His work demonstrated that BG is more resistant to FAC than the virulent *B. anthracis* (Ohio State University) in buffered water adjusted to pH 6.2, 7.2, and 8.6, but if the water was adjusted to pH 10.5, the two species are equivalent in susceptibility. Sivaganesan *et al.* (2006) also demonstrated that BG is more resistant to FAC than *B. anthracis*, with a 2 $\log_{10}$ Ct at 5 °C, pH 7 of 372 for BG as compared to 220 for *B. anthracis* Ames (Table 1). These data suggest that BG would be an appropriate conservative surrogate for *B. anthracis* FAC disinfection testing. In another study, *B. cereus* spores were found to be very close in FAC susceptibility to *B. anthracis* Sterne spores, while *B. thuringiensis* spores were more resistant than both *B. anthracis* Sterne and *B. cereus* spores, but comparable in susceptibility to *B. anthracis* Ames spores (pH 7, 23 °C and 5 °C) (Rice *et al.* 2005). *B. thuringiensis* may, therefore, be another choice of a surrogate to ensure adequate disinfection for *B. anthracis*, if the water of concern is maintained at pH 7. *B. subtilis* ATCC 6633 was investigated as a potential surrogate for FAC testing (Barbeau *et al.* 1999), and when the data were compared from tests conducted at similar, though not exactly the same pH and temperatures, the *B. subtilis* Ct values were three times greater than those for *B. anthracis* Sterne (2 $\log_{10}$ reduction, 20 °C and 23 °C, pH 7; 148 and 45, respectively), and almost twice the Ct value reported for *B. anthracis* Ames tested at 25 °C and pH 7 (Ct = 79) (Table 1). The greater Ct values for *B. subtilis* as compared to *B. anthracis* were also seen when testing was performed at pH 8 (Table 1, 2 $\log_{10}$ inactivation, *B. subtilis* vs. *B. anthracis* Sterne, 368 and 127, respectively). These data also point to *B. subtilis* as a potential conservative surrogate for *B. anthracis* when conducting disinfection studies.

Dow *et al.* (2006) conducted monochloramine testing on *B. subtilis* in water containing small amounts of organic and inorganic matter (<0.05 NTU, <0.3 mg $L^{-1}$ DOC), and found a 2 $\log_{10}$ inactivation Ct value of approximately 5,900, which is four to seven times higher than seen for *B. anthracis* spores tested under similar pH and temperature conditions (Table 1, pH 8, 22 °C–25 °C). More work is required to determine if this higher Ct is due to the differences in the spores' susceptibility to monochloramine, or due to the differences in water quality.

Cho *et al.* (2006) found *B. subtilis* to be slightly more susceptible to $ClO_2$ than *B. anthracis* spores, with a 2 $\log_{10}$ Ct of approximately 35 (25 °C, pH 8.2), as compared to 57 and 73 for

*B. anthracis* Sterne and Ames, respectively (Table 1). Hosni *et al.* (2009) conducted $ClO_2$ susceptibility testing of *B. globigii* at a slightly lower temperature (20 °C, pH 8), and reported a 2 $\log_{10}$ Ct of 76, comparable to *B. anthracis* spores (57 and 73 for *B. anthracis* Stern and Ames, respectively, Table 1). Side-by-side comparisons of the surrogate spores and *B. anthracis* spores should be conducted before selecting an appropriate surrogate spore for $ClO_2$ disinfection.

The UV susceptibility of non-spore forming bacteria that can compromise water quality, such as *E. coli, C. jejuni*, and *Y. enterocolitica* (Butler *et al.* 1987), appear to be close to that of the vegetative bacterial biothreat agents with a 4 $\log_{10}$ inactivation requiring fluences of 2.1–8.4 as compared to 4.1–10.5 mJ $cm^{-2}$ (Table 3).

*B. subtilis* has historically been used as a very conservative surrogate for *Cryptosporidium* and *Giardia* when validating a water system's UV reactor (Sommer *et al.* 1998; US Environmental Protection Agency 2006c). Several researchers have compared the UV susceptibility of *B. subtilis* spores to that of other microorganisms (Setlow 1988; Nicholson & Galeano 2003). Most of these studies, with one exception (Cho *et al.* 2006), found similar UV fluences for *B. subtilis* inactivation (36–48 mJ $cm^{-2}$ for 2 $\log_{10}$ inactivation). Furthermore, Nicholson & Galeano (2003) found no difference in UV susceptibility between *B. anthracis* Sterne spores and two *B. subtilis* spore strains (Table 3).

## OZONE

Ozone was used by 9% of respondents to a 2007 survey of US treatment facilities (American Water Works Association Disinfection Systems Committee 2008a), and is effective in inactivating many waterborne bacteria and viruses (White 1999). No ozone efficacy data were found, however, for the bacterial biothreat agents of concern. Larson & Mariňas (2003) challenged *Bacillus subtilis* ATCC 6051 spores (a possible surrogate for *B. anthracis* spores) with ozone and found that at pH 7 and 20 °C, the 3 $\log_{10}$ inactivation Ct value was about 8.2. Lower temperature and higher pH reduced the efficacy of the ozone on the *B. subtilis* spores. Driedger *et al.* (2001) tested *B. subtilis* ATCC 6633 under the same pH and temperature conditions as Larson *et al.*, and reported a 3 $\log_{10}$ Ct value of approximately 10 (estimated from a plot). Vegetative bacteria are more susceptible to ozone than spores, with reported 99% inactivation Ct values of 0.02 for *E. coli* (5 °C and pH 6–7, Hoff 1986), <1– < 5 for *L. pneumophila* and <1–13 for *Mycobacterium fortuitum* (25 °C and pH 7, Jacangelo *et al.* 2002). These values may be representative of many vegetative bacteria, although more work is needed to confirm the efficacy of ozone on the biothreat bacteria.

## BIOFILMS AND AMOEBA

The data presented in Tables 1, 2, and 3 are specific to planktonic organisms, but it is also important to consider the efficacy of disinfectants on organisms attached to surfaces and associated with biofilms. Potable water distribution system pipes are universally colonized with biofilms in spite of the low nutrient conditions and the presence of residual disinfectants (LeChevallier *et al.* 1988b). Many pathogenic bacteria, such as *L. pneumophila* (Murga *et al.* 2001), *Helicobacter pylori* (Park *et al.* 2001; Bunn *et al.* 2002), and *Salmonella typhimurium* (Armon *et al.* 1997), have been demonstrated to survive and persist within

model biofilms and in drinking water system biofilms. Bio-film-associated microorganisms, including pathogens, attached to surfaces and particles are also known to be more resistant to disinfection than planktonic organisms (Herson *et al.* 1987; LeChevallier *et al.* 1988a). Little information is available regarding how actual bacterial biothreat agents interact with biofilms, although some investigations have been conducted using surrogate agents.

Szabo *et al.* (2007) demonstrated that *B. atrophaeus* var. *globigii* spores, a surrogate for *B. anthracis* spores, were able to persist in a model drinking water biofilm on corroded iron coupons. In the model system, a concentration of 10 mg $L^{-1}$ free chlorine for 6 days reduced viable spores by 2 $\log_{10}$, but close to $4 \times 10^3$ CFU $cm^{-2}$ remained on the coupons. Additional increases in chlorine concentration (25 and 70 mg $L^{-1}$) provided little additional inactivation (Szabo *et al.* 2007). One reason for the inability of high concentrations of chlorine to inactivate biofilm associated spores is that the chlorine was measured to be 40–70% lower at the surface of the biofilm or the iron surface, as compared to the bulk fluid surrounding the biofilm (Szabo *et al.* 2006). The surface material, the microbial community, exopolysaccharide produced by biofilm associated organisms, microbial metabolites, and other substances that become trapped in the biofilm can also create a demand for the chlorine and reduce the amount that actually comes in contact with the spores. Rough or corroded pipe surfaces can also provide protective areas that the chlorine cannot reach (Szabo *et al.* 2007). Additionally, the surface composition can influence the efficacy of the disinfectant. When spores in a biofilm on a copper surface were challenged with chlorine and monochloramine, the Ct values were consistently higher than if the spores were in a biofilm established on a PVC surface when challenged with the same disinfectants (Morrow *et al.* 2008). Morrow *et al.* (2008) also demonstrated monochloramine to be more effective at disinfecting *B. anthracis* Sterne and *B. thuringiensis* spores in a biofilm than chlorine, corroborating previous reports that monochloramine is more stable and is less reactive toward the biofilm matrix (LeChevallier *et al.* 1990; Griebe *et al.* 1994). Hosni *et al.* (2009), using the same experimental method as Szabo *et al.* (2007), found that $ClO_2$ was able to penetrate the biofilm matrix much better than chlorine and inactivate 4 $\log_{10}$ CFU of biofilm associated BG spores with 25 mg $L^{-1}$ within 8 days.

Addition of a germinant (50% Trypticase™ soy broth) into a model distribution system, followed by flushing, was found to enhance the efficacy of chlorine (5 mg $L^{-1}$) and encourage detachment of BG spores from established biofilms on concrete and iron surfaces, resulting in no detectable spores (>4 $\log_{10}$ CFU $cm^{-2}$ reduction) (Szabo *et al.* 2012). Morrow & Cole (2009) also demonstrated enhanced chlorine efficacy after addition of germinant (1 mM inosine and 8 mM l-alanine) to a biofilm reactor containing *B. anthracis* Sterne spores associated with an established biofilm. Efficacy was improved from 0.4 $\log_{10}$ to 3.4 $\log_{10}$ inactivation when the reactor was treated with 10 mg $L^{-1}$ free chlorine (Morrow & Cole 2009).

Szabo *et al.* (2006) demonstrated that *Klebsiella pneumoniae*, a potential surrogate for any of the Gram-negative biothreat organisms, was protected from chlorine by association with a mixed species biofilm. In addition, without a chlorine challenge, *K. pneumoniae* was unable to colonize the iron surface for more than 2 weeks, indicating that the microbe may have had trouble competing with the established municipal water biofilm organisms. Whether any of

the Gram-negative biothreat bacteria are able to persist and multiply within a municipal water system biofilm has yet to be determined.

Another challenge to disinfection of biothreat agents are their potential association with free-living amoeba, common in natural waters and in potable water distribution systems (Howard & Inglis 2005; Marciano-Cabral *et al.* 2010). Several of these agents, such as *F. tularensis* (Abd *et al.* 2003; El-Etr *et al.* 2009), *B. pseudomallei* (Inglis *et al.* 2000), *Y. pestis* (Nikul'shin *et al.* 1992), and *Bacillus anthracis* spores (Dey *et al.* 2012) have been shown to co-exist with amoeba. Protozoa phagocytize the bacteria, yet several bacterial species are able to resist digestion, and some are capable of multiplying within the amoeba. *B. pseudomallei* was demonstrated to survive endocytosis and to subsequently escape from three *Acanthamoeba* spp. (Inglis *et al.* 2000). The coexistence of *L. pneumophila* and several coliforms was shown to contribute to their resistance to disinfectants in water (King *et al.* 1988; Kilvington & Price 1990). Similarly, when *B. pseudomallei* was co-cultured with *Acanthamoeba, B. pseudomallei* was 1,000- to 10,000-fold more resistant to FAC, and *B. pseudomallei* was found to replicate within *Acanthamoeba* during long FAC exposure times (Howard & Inglis 2005).

## CONCLUSION

The vulnerability of drinking water supplies to acts of bioterrorism continues to be a matter of concern for public health authorities and water utilities. While the potential use of these agents for intentional contamination has been recognized since the cold war era (Berger & Stevenson 1955), it has only been within the last decade that there has been a concerted effort to evaluate water treatment practices for countering such threats. The current review provides a summary of recent studies designed to determine the efficacy of common water treatment practices for inactivation of bacterial biothreat agents. The vegetative biothreat bacteria were found to be susceptible to all disinfectants as currently used in modern water treatment systems, although some strains of *F. tularensis* and *B. pseudomallei* were reported to be slightly more tolerant of free chlorine than other vegetative cells. The *Bacillus anthracis* spores were significantly more resistant to all disinfectants than the vegetative cells, and a range of susceptibility was seen between strains. While these studies were conducted under ideal or oxidant demand-free conditions, they provide important information on the innate resistance of these organisms. Future studies in this area should be aimed at evaluating the effect that varying water quality conditions might have on these processes.

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**Table 1**

Ct values for inactivation ($log_{10}$) of *Bacillus anthracis* spores and surrogate spores with free available chlorine, monochloramine, and chlorine dioxide

| | | | Ct (mg • min $L^{-1}$) $log_{10}$ inactivation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 2 | 3 | 2 | 3 | |
| **Isolate** | **pH** | **Temp (°C)** | **Free available chlorine** | | | **Monochloramine** | | **Chlorine dioxide** | | **References** |
| *Bacillus anthracis* Ames | 7 | 5 | 220 | 339 | | – | – | 579 | 738 | Rose *et al.* (2005, 2007); Shams *et al.* (2011) |
| | | 25 | 79 | 102 | – | – | – | 60 | 81 | |
| | 8 | 5 | – | – | – | 3,499 | 6,813 | 569 | 712 | |
| | | 25 | – | – | – | 785 | 1,204 | 73 | 84 | |
| *Bacillus anthracis* no.811 Ohio State Univ. | 7.2 | 4 | – | – | 463[a] | – | – | – | – | Brazis *et al.* (1958) |
| | | 22 | – | – | 118[a] | – | – | – | – | |
| **Surrogate** | | | | | | | | | | |
| *Bacillus anthracis* Sterne | 7 | 5 | 190 | 271 | – | – | – | 491 | 667 | Rose *et al.* (2005, 2007); Shams *et al.* (2011) |
| | | 25 | 60 | 86 | – | – | – | 68 | 81 | |
| | 8 | 5 | – | – | – | 10,532 | 15,164 | 481 | 606 | |
| | | 25 | – | – | – | 1,442 | 1,847 | 57 | 69 | |
| *Bacillus anthracis* Sterne | 7 | 5 | 140 | 210 | 280 | – | – | – | – | Rice *et al.* (2005) |
| | | 23 | 45 | 68 | 90 | – | – | – | – | |
| | 8 | 5 | 319 | 478 | 637 | – | – | – | – | |
| | | 23 | 127 | 191 | 254 | – | – | – | – | |
| *Bacillus cereus* ATCC 7039 | 7 | 5 | 117 | 175 | 233 | – | – | – | – | Rice *et al.* (2005) |
| | | 23 | 41 | 62 | 82 | – | – | – | – | |
| | 8 | 5 | 340 | 510 | 680 | – | – | – | – | |
| | | 23 | 132 | 199 | 264 | – | – | – | – | |
| *Bacillus atrophaeus*, var globigii (Dugway) | 7 | 5 | 372 | 446 | – | – | – | – | – | Sivaganesan *et al.* (2006) |
| | | 23 | 108 | 136 | – | – | – | – | – | |
| | 8 | 5 | 943 | 1144 | – | – | – | – | – | |
| | | 23 | 367 | 438 | – | – | – | – | – | |
| *Bacillus atrophaeus*, var globigii (Dugway) | 8 | 20 | 282 | 351 | – | – | – | 76 | – | Hosni *et al.* (2009) |

| | | | Ct (mg • min $L^{-1}$) $log_{10}$ inactivation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 2 | 3 | 2 | 3 | |
| **Isolate** | **pH** | **Temp (°C)** | **Free available chlorine** | | | **Monochloramine** | | **Chlorine dioxide** | | **References** |
| *Bacillus globigii* no. 102 (Ohio State University) | 7 | 4 | – | – | 845[a] | – | – | – | – | Brazis *et al.* (1958) |
| | | 22 | 72[b] | – | 206[a] | – | – | – | – | |
| *Bacillus subtilis* ATCC 6633 | 7 | 20 | 148 | – | – | – | – | – | – | Barbeau *et al.* (1999) |
| *Bacillus subtilis* ATCC 6633 | 8.2 | 25 | 368 | – | – | – | – | 35 | – | Cho *et al.* (2006) |
| *Bacillus subtilis* ATCC 6633 | 8.2 | 22 | – | – | – | ~5,900[c] | – | – | – | Dow *et al.* (2006) |
| *Bacillus subtilis* ATCC 6015 | 8.0 | 20 | – | – | – | 10,400[d] | 10,800[d] | – | – | Larson & Marinas (2003) |
| *Bacillus thuringiensis* subsp. israelensis ATCC 35646 | 7 | 5 | 229 | 344 | 458 | – | – | – | – | Rice *et al.* (2005) |
| | | 23 | 66 | 99 | 132 | – | – | – | – | |
| | 8 | 5 | 481 | 721 | 961 | – | – | – | – | |
| | | 23 | 246 | 369 | 492 | – | – | – | | |

[a]Estimated from Brazis *et al.* (1958), Table 1.

[b]Estimated value (estimated by Barbeau *et al.* (1999), from Brazis *et al.* (1958) data).

[c]Estimated from Dow *et al.* (2006), Figure 7. Test water contained slight amounts of dissolved organic matter (<0.3 mg $L^{-1}$) and inorganic matter (turbidity <NTU).

[d]Estimated from Larson & Mariňas (2003), Figure 9.

**Table 2**

Ct values for inactivation ($log_{10}$) of vegetative biothreat bacteria and surrogates with free available chlorine, monochloramine, and chlorine dioxide

| | | | Ct (mg • min $L^{-1}$) $log_{10}$ Inactivation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 2 | 3 | 2 | 3 | |
| **Isolate** | **pH** | **Temp (°C)** | **Free available chlorine** | | | **Monochloramine** | | **Chlorine dioxide** | | **References** |
| *Brucella suis* MO562 | 7 | 5 | – | – | | – | – | – | – | Rose *et al.* (2007) |
| | | 25 | – | – | | – | – | – | – | |
| | 8 | 5 | – | – | | 134.3 | 156.8 | – | – | |
| | | 25 | – | – | | 47.8 | 56.1 | – | – | |
| *Brucella suis* EAM562 | 7 | 5 | 0.3 | 0.4 | | – | – | 0.7 | 1.0 | Rose *et al.* (2005); Shams *et al.* (2011) |
| | | 25 | 0.1 | 0.2 | | – | – | 0.2 | 0.3 | |
| | 8 | 5 | – | – | | – | – | 0.3 | 0.4 | |
| | | 25 | – | – | | – | – | 0.2 | 0.2 | |
| *Brucella melitensis* ATCC 23456 | 7 | 5 | 0.3 | 0.5 | – | – | – | 1.6 | 2.0 | Rose *et al.* (2005, 2007); Shams *et al.* (2011) |
| | | 25 | 0.1 | 0.2 | – | – | – | 0.6 | 0.7 | |
| | 8 | 5 | – | – | – | 501.8 | 579.5 | 1.0 | 1.3 | |
| | | 25 | – | – | – | 104.4 | 116.6 | 0.3 | 0.3 | |
| *Burkholderia mallei* M-9 | 7 | 5 | 0.2 | 0.2 | – | – | – | 0.3 | 0.3 | Rose *et al.* (2005, 2007); Shams *et al.* (2011) |
| | | 25 | 0.1 | 0.2 | – | – | – | 0.1 | 0.1 | |
| | 8 | 5 | – | – | – | 158.6 | 194.1 | 0.3 | 0.4 | |
| | | 25 | – | – | – | 52.2 | 64.6 | 0.1 | 0.1 | |
| *Burkholderia mallei* M-13 | 7 | 5 | 0.2 | 0.2 | – | – | – | 0.5 | 0.6 | Rose *et al.* (2005); Shams *et al.* (2011) |
| | | 25 | 0.1 | 0.2 | – | – | – | 0.2 | 0.3 | |
| | 8 | 5 | – | – | – | – | – | 0.3 | 0.3 | |
| | | 25 | – | – | – | – | – | 0.1 | 0.1 | |
| *Burkholderia pseudomallei* ATCC 23343 | 7 | 5 | 1.0 | 1.4 | 1.8 | – | – | 0.3 | 0.4 | O'Connell *et al.* (2009); Shams *et al.* (2011) |
| | | 25 | 0.7 | 0.9 | 1.1 | – | – | 0.1 | 0.2 | |

| | | | Ct (mg • min $L^{-1}$) $log_{10}$ Inactivation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 2 | 3 | 2 | 3 | |
| **Isolate** | **pH** | **Temp (°C)** | **Free available chlorine** | | | **Monochloramine** | | **Chlorine dioxide** | | **References** |
| | 8 | 5 | 0.9 | 1.9 | 2.8 | 190 | 226 | 0.2 | 0.3 | |
| | | 25 | 0.5 | 1.1 | 1.8 | 49 | 73 | 0.1 | 0.1 | |
| *Burkholderia pseudomallei* CA 652 | 7 | 5 | 2.3 | 3.7 | 5.0 | – | – | 0.3 | 0.4 | O'Connell *et al.* (2009); Shams *et al.* (2011) |
| | | 25 | 0.8 | 1.3 | 1.7 | – | – | 0.3 | 0.4 | |
| | 8 | 5 | 3.7 | 5.8 | 7.8 | 234 | 281 | 0.4 | 0.5 | |
| | | 25 | 0.9 | 1.4 | 1.9 | 70 | 88 | 0.1 | 0.2 | |
| *Burkholderia pseudomallei* AU631 | 7 | 5 | 0.1 | 0.2 | 0.3 | – | – | – | – | O'Connell *et al.* (2009) |
| | | 25 | 0.1 | 0.1 | 0.1 | – | – | – | – | |
| | 8 | 5 | 0.2 | 0.3 | 0.4 | 240 | 266 | – | – | |
| | | 25 | 0.1 | 0.1 | 0.1 | 42 | 49 | – | – | |
| *Burkholderia pseudomallei* TH694 | 7 | 5 | 0.1 | 0.2 | 0.4 | – | – | – | – | O'Connell *et al.* (2009) |
| | | 25 | 0.1 | 0.1 | 0.2 | – | – | – | – | |
| | 8 | 5 | 0.1 | 0.3 | 0.5 | 404 | 477 | – | – | |
| | | 25 | 0.1 | 0.2 | 0.4 | 99 | 113 | – | – | |
| *Burkholderia pseudomallei* SC763 | 7 | 5 | 0.2 | 0.3 | 0.5 | – | – | – | – | O'Connell *et al.* (2009) |
| | | 25 | 0.2 | 0.3 | 0.4 | – | – | – | – | |
| | 8 | 5 | 0.5 | 0.8 | 1.1 | 302 | 382 | – | – | |
| | | 25 | 0.1 | 0.2 | 0.3 | 53 | 60 | – | – | |
| *Francisella tularensis* LVS | 7 | 5 | 5.0 | 6.7 | 8.5 | – | – | 0.8 | 1.0 | Rose *et al.* (2007); O'Connell *et al.* (2010); Shams *et al.* (2011) |
| | | 25 | 0.7 | 1.0 | 1.2 | – | – | 0.2 | 0.2 | |
| | 8 | 5 | 15.9 | 20.1 | 24.3 | 76.0 | 97.9 | 0.8 | 1.0 | |
| | | 25 | 2.0 | 2.7 | 3.5 | 26.3 | 30.4 | 0.1 | 0.2 | |
| *Francisella tularensis* Schu S4 | 7 | 5 | 13.4 | 16.8 | 20.3 | – | – | – | – | O'Connell *et al.* (2010) |
| | | 25 | 0.9 | 1.3 | 1.7 | – | – | – | – | |
| | 8 | 5 | 47.4 | 62.3 | 77.2 | – | – | – | – | |
| | | 25 | 3.7 | 4.5 | 5.2 | – | – | – | – | |
| *Francisella tularensis* NY98 | 7 | 5 | 11 | 16 | – | – | – | 1.2 | 1.5 | Shams *et al.* (2011); Rose *et al.* (2007); |

| | | | Ct (mg • min $L^{-1}$) $log_{10}$ Inactivation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 2 | 3 | 2 | 3 | |
| **Isolate** | **pH** | **Temp (°C)** | **Free available chlorine** | | | **Monochloramine** | | **Chlorine dioxide** | | **References** |
| | | | | | | | | | | FAC data[a] |
| | | 25 | 2.0 | 3.9 | – | – | – | 0.2 | 0.2 | |
| | 8 | 5 | 47 | 70 | – | 84.0 | 116.0 | 0.9 | 1.1 | |
| | | 25 | 4.3 | 6.5 | – | 31.3 | 37.1 | 0.1 | 0.2 | |
| *Francisella tularensis* MA00-2987 | 7 | 5 | 13.6 | 16.9 | 20.2 | – | – | – | – | O'Connell *et al.* (2010) |
| | | 25 | 0.9 | 1.3 | 1.6 | – | – | – | – | |
| | 8 | 5 | 64.1 | 83.8 | 103.4 | – | – | – | – | |
| | | 25 | 2.7 | 3.4 | 4.2 | – | – | – | – | |
| *Francisella tularensis* NM99-1823 | 7 | 5 | 14.4 | 17.7 | 21.0 | – | – | – | – | O'Connell *et al.* (2010) |
| | | 25 | 0.4 | 0.5 | 0.7 | – | – | – | – | |
| | 8 | 5 | 45.4 | 60.5 | 75.7 | – | – | – | – | |
| | | 25 | 2.9 | 3.7 | 4.5 | – | – | – | – | |
| *Yersinia pestis* A1122 | 7 | 5 | 0.5 | 0.7 | – | – | – | 0.4 | 0.5 | Rose *et al.* (2005, 2007); Shams *et al.* (2011) |
| | | 25 | 0.4 | 0.6 | – | – | – | 0.2 | 0.2 | |
| | 8 | 5 | – | – | – | 92.0 | 115.6 | 0.2 | 0.3 | |
| | | 25 | – | – | – | 27.6 | 33.1 | 0.02 | 0.03 | |
| *Yersinia pestis* Harbin | 7 | 5 | 0.03 | 0.04 | – | – | – | 0.4 | 0.5 | Rose *et al.* (2005, 2007); Shams *et al.* (2011) |
| | | 25 | 0.03 | 0.04 | – | – | – | 0.3 | 0.3 | |
| | 8 | 5 | – | – | – | 80.7 | 91.4 | 0.1 | 0.2 | |
| | | 25 | – | – | – | 21.9 | 25.0 | 0.04 | 0.06 | |
| **Surrogates** | | | | | | | | | | |
| *Escherichia coli* | 7 | 25 | – | – | – | – | – | 0.28 | – | Natl. Res. Council (1980) |
| | 9 | 25 | – | – | – | 40 | – | – | – | |
| | 10 | 5 | 0.92 | – | – | – | – | – | – | |
| *Escherichia coli*[b] | 7 | 5 | – | – | <2 | – | – | – | – | Rice *et al.* (1999) |
| *Escherichia coli* | 7 | 25 | 0.4 | – | – | – | – | – | – | King *et al.* (1988) |

| | | | Ct (mg • min $L^{-1}$) $log_{10}$ Inactivation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 2 | 3 | 2 | 3 | |
| **Isolate** | **pH** | **Temp (°C)** | **Free available chlorine** | | | **Monochloramine** | | **Chlorine dioxide** | | **References** |
| *Enterobacter cloacae* | 7 | 25 | 0.4 | – | – | – | – | – | – | King *et al.* (1988) |
| *Klebsiella pneumoniae* | 7 | 25 | 0.5 | – | – | – | – | – | – | King *et al.* (1988) |
| *Yersinia enterocolitica* | 7 | 25 | 0.5 | – | – | – | – | – | – | King *et al.* (1988) |

[a] Free available chlorine data for *F. tularensis* NY98, pH 7 and pH 8 from unpublished work conducted with identical methods as references Rose *et al.* (2005) and O'Connell *et al.* (2009).

[b] Multiple strains.

**Table 3**

UV dose (mJ/cm$^2$) required for given $\log_{10}$ inactivation of biothreat organisms and surrogates

| | **$\log_{10}$ inactivation** | | | | |
|---|---|---|---|---|---|
| **Biothreat organism** | **1** | **2** | **3** | **4** | **References** |
| *Bacillus anthracis* Ames spores | 25.3 | ~40 | >120[a] | >120[a] | Rose & O'Connell (2009) |
| *Brucella suis* MO562 | 1.7 | 3.6 | 5.6 | 7.5 | Rose & O'Connell (2009) |
| *Brucella suis* KS528 | 2.7 | 5.3 | 7.9 | 10.5 | Rose & O'Connell (2009) |
| *Brucella melitensis* ATCC 23456 | 2.8 | 5.3 | 7.8 | 10.3 | Rose & O'Connell (2009) |
| *Brucella melitensis* IL195 | 3.7 | 5.8 | 7.8 | 9.9 | Rose & O'Connell (2009) |
| *Burkholderia pseudomallei* ATCC 11688 | 1.7 | 3.5 | 5.5 | 7.4 | Rose & O'Connell (2009) |
| *Burkholderia pseudomallei* CA650 | 1.4 | 2.8 | 4.3 | 5.7 | Rose & O'Connell (2009) |
| *Burkholderia mallei* M-9 | 1.0 | 2.4 | 3.8 | 5.2 | Rose & O'Connell (2009) |
| *Burkholderia mallei* M-13 | 1.2 | 2.7 | 4.1 | 5.5 | Rose & O'Connell (2009) |
| *Francisella tularensis* NY98 | 1.4 | 3.8 | 6.3 | 8.7 | Rose & O'Connell (2009) |
| *Yersinia pestis* Harbin | 1.3 | 2.2 | 3.2 | 4.1 | Rose & O'Connell (2009) |
| **Surrogates** | | | | | |
| *Bacillus anthracis* Sterne spores | 23.0 | ~40 | >120[a] | >120[a] | Rose & O'Connell (2009) |
| *Bacillus anthracis* Sterne spores[b] | 27.5 | 36 | 53 | >60[c] | Nicholson & Galeano (2003) |
| *Bacillus anthracis* Sterne spores[b] | 81 | 135 | >189 | >189 | Knudson (1986) |
| *Bacillus subtilis* ATCC 6633 spores[b] | 24.5 | 40 | 50 | 60 | Nicholson & Galeano (2003) |
| *Bacillus subtilis* ATCC 6633 spores[b] | 16 | 22 | 28 | >34[d] | Cho *et al.* (2006) |
| *Bacillus subtilis* spores[b] | 12 | 24 | 60 | 120 | Kruithof *et al.* (2007) |
| *Bacillus subtilis* ATCC 6633 spores[b] | 28 | 39 | 50 | >60[c] | Sommer *et al.* (1998) |
| *Bacillus subtilis* WN624 spores[b] | 24.5 | 36 | 52 | 60 | Nicholson & Galeano (2003) |
| *Escherichia coli*[b] | 3.0 | 4.8 | 6.7 | 8.4 | Chang *et al.* (1985) |
| *Escherichia coli*[b] | 2.5 | 4.0 | 5.2 | 6.7 | Butler *et al.* (1987) |
| *Francisella tularensis* LVS | 1.3 | 3.1 | 4.8 | 6.6 | Rose & O'Connell (2009) |
| *Campylobacter jejuni*[b] | 1.0 | 1.5 | 1.8 | 2.1 | Butler *et al.* (1987) |
| *Cryptosporidium* | 2.5 | 5.8 | 12 | 22 | USEPA (2006c) |

| Biothreat organism | $Log_{10}$ inactivation | | | | References |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| *Giardia* | 2.1 | 5.2 | 11 | 22 | USEPA (2006c) |
| MS2 bacteriophage[e] | 58 | 100 | 143 | 186 | USEPA (2006c) |
| *Yersinia enterocolitica*[b] | 1.1 | 2.3 | 3.0 | 3.6 | Butler *et al.* (1987) |
| *Yersinia pestis* A1122 | 1.4 | 2.6 | 3.7 | 4.9 | Rose & O'Connell (2009) |

[a] 3 and 4 log10 inactivation not achieved with a dose of 120 mJ/cm.

[b] Some UV doses estimated from a graph.

[c] 4 log10 inactivation not achieved with a dose of 60 $mJ/cm^2$.

[d] 4 log10 inactivation not achieved with a dose of 34 $mJ/cm^2$.

[e] Reduction equivalent dose bias values for virus inactivation credit.