Biological Warfare Agents as Threats to Potable Water

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Nearly all known biological warfare agents are intended for aerosol application. Although less effective as potable water threats, many are potentially capable of inflicting heavy casualties when ingested. Significant loss of mission capability can be anticipated even when complete recovery is possible. Properly maintained field army water purification equipment can counter this threat, but personnel responsible for the operation and maintenance of the equipment may be most at risk of exposure. Municipal water treatment facilities would be measurably less effective. Some replicating (infectious) agents and a few biotoxins are inactivated by chlorine disinfection; for others chlorine is ineffective or of unknown efficacy. This report assesses the state of our knowledge of agents as potable water threats and contemplates the consequences of intentional or collateral contamination of potable water supplies by 18 replicating agents and 9 biotoxins known or likely to be weaponized or otherwise used as threats. *Key words*: bacteria, biological warfare, potable water, protozoa, rickettsia, toxin, virus. *Environ Health Perspect* 107:975–984 (1999). [Online 5 November 1999]

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Most biological warfare (BW) agents, whether replicating (infectious) agents or derived toxins, have been weaponized for an aerosol assault, and the threat is primarily to the respiratory tract, not to the digestive system. Infectious agents are more immediately incapacitating when respired and, as noted by Franz (*1*), "the effects of most toxins are more severe when inhaled than when they are consumed in food." Some of the infectious agents—*Shigella* spp. and *Vibrio cholerae*, for example—are obvious water threats, and most biotoxins would probably be effective threats to drinking water under suitable conditions. For some others, however, either there is no known infectious path through ingestion or the agent cannot survive in water. This report evaluates the potential threat to potable water supplies from recognized BW agents that are known or are likely to be weaponized. Agents were selected from various official documents, contractor reports, and reviews [most notably the text of Sidell et al. (*2*)]. This report is intended to provide guidance to field preventive medicine personnel as well as to identify deficiencies in the data. The emphasis is on concerns of the field army, but many findings apply to municipal systems as well. Other aspects of evaluation include clinical considerations, infective/toxic dose, environmental stability, disinfection efficacy, and removal by treatment systems.

Clinical Considerations

We emphasize signs and symptoms suggesting immediate or rapid loss of mission capability rather than long-term adverse effects. The nature of the illness may be different depending on whether the agent is inhaled or ingested, or as noted by Franz (*1*),

> Some toxins can elicit a significantly different clinical picture when the route of exposure is changed, a phenomenon that may confound diagnosis and delay treatment.

Diagnosis after the appearance of symptoms may be too late for effective treatment.

Infective/Toxic Dose

For most of the biotoxins, only rodent median lethal dose (LD_{50}) values are available to indicate relative toxicity. In the absence of more useful data, we used the screening dose, $LD_{50}/10^4$ expressed in milligrams per liter [derived by Layton et al. (*3*) and based on earlier studies by McNamara (*4*)], to represent the highest no-observed-adverse-effect level for soldiers ($NOAEL_s$) consuming 15 L/day of water. Thus,

$$\mathrm{NOAEL_s(mg/L)} = \frac{(\mathrm{LD_{50}} \times 0.004)}{100} \times \frac{70}{15} \sim \frac{\mathrm{LD_{50}}}{10^4}$$

where 0.004, in day^{-1}, is the ratio of the rat oral subchronic NOAEL (milligrams per kilogram per day) to LD_{50} (milligrams per kilogram) corresponding to the 10th cumulative percentile of the lognormal distribution for 33 representative substances (*4*), 70 is the standard weight of the soldier in kilograms, and 100 is the interspecies safety factor. For the few cases where LD_{50} values have been estimated for humans, the $NOAEL_s$ is taken to be $LD_{50}/100$, thereby eliminating the rodent-to-human safety factor. For the purpose of predicting performance decrement, it is assumed that no adverse effect will occur at a dose below this highly conservative number.

Individual susceptibility to infectious agents may vary widely. The incidence of shigellosis among infants and children, for example, is much higher than among adults. Differences in virulence of various agent strains, as well as differences in individual susceptibility, may account for the large (powers of 10) differences in infective dose quoted by different authors for many agents. Furthermore, for many replicating agents, the infective dose is known for inhalation but not ingestion, and it has been necessary to make the generally conservative assumption that they are equal. In this paper we made every effort to identify an infective dose applicable to healthy young adults, but it should be recognized that great differences in susceptibility can occur even within this select group. The drinking water equivalent of infective dose, in organisms per liter, has been calculated by dividing the literature value by 7, the maximum number of days personnel are presumed to occupy a nuclear/biological/chemical battlefield, and by 15 or 5 for consumption of 15 and 5 L/day of water, respectively. The derived values are highly conservative in that it is assumed that infectious organisms are accumulated over 7 days and are not cleared from the system. A general relation between the probability (p) of infection (but not necessarily illness), the average dose of organisms ingested (N), and the average infective dose (k) (*5*) can be derived as follows: $p = 1 - \exp(-N/k)$.

Environmental Stability

Data have been sought to indicate the stability of biotoxins or survival of infectious agents in water and to indicate the effects of heat, light, and drying on BW agents.

Disinfection Efficacy

Data have been sought to indicate whether BW agents are inactivated under normal

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conditions of field drinking water disinfection [i.e., 2.0 mg/L free available chlorine (FAC) for 30 min at temperatures > 15°C] or under the less rigorous conditions commonly used for municipal treatment. In general, such data are available only for the common waterborne pathogens. The knowledge that certain BW agents are inactivated by chlorine bleach or other disinfectants is useful for decontaminating surfaces, but not for developing water disinfection criteria. In a few cases, the efficacy of chemicals used for disinfecting personal water supplies, such as chlorine dioxide (*6*), iodine tablets [tetraglycine hydroperiodide or Globaline (Van Ben Industries, Long Island, NY)] (*7*), and sodium dichloro-isocyanurate (Chlor-Floc; Control Chemical Company, South Africa) (*7*), has been tested.

Removal by Treatment Systems

The army's mobile field water treatment system is the reverse osmosis water purification unit (ROWPU), in which the reverse osmosis (RO) membranes are preceded by coagulation/flocculation and multimedia filtration, similar (except for the membranes) to systems used by municipalities for treating surface waters. The membranes are additionally protected by spiral-wound cotton prefilters of 5-µm nominal pore size. Although removal by RO has been measured for only a few BW agents, it has been assumed that an intact RO membrane will exclude virtually all infectious agents and all but the smallest biotoxins because of size exclusion. By the same principle, microfiltration should exclude parasites and most bacteria, but not viruses or toxins, whereas ultrafiltration should exclude all infectious agents and possibly a few proteinaceous toxins. The principle of size exclusion has important exceptions, however. Adham et al. (*8*) found in bench studies that removal of MS-2 bacterial virus (a simulant for enteric viruses) by composite RO membranes can vary from < 2 $\log_{10}$ to > 7 $\log_{10}$, depending on the selection of membrane, apparently because of differences in quality control. Madaeni (*9*) documented other cases where size exclusion of microorganisms is violated in membrane filtration, and Ramachandran et al. (*10*) observed apparent passage of some *Bacillus subtilus* spores from heavily contaminated source water in field tests of the ROWPU.

Because the ROWPU rejects two-thirds of the intake raw water, bypassing the membranes to increase water production from fresh water sources has received serious consideration. However, almost no information was recovered concerning the removal of BW agents from water by conventional municipal treatment facilities. In the absence of RO membranes, the ROWPU multimedia filtration system can achieve 1–2 $\log_{10}$ reduction of *Bacillus globigii* spores and *Escherichia coli* and slightly < 1 $\log_{10}$ reduction of poliovirus 1, total aerobic bacteria, and total enteric bacteria (*11*). Well-operated municipal systems (omitting consideration of disinfection) should readily meet or exceed this performance (*12,13*). The standard cartridge filters do not appear to contribute substantially to overall microbial removals (*11,14*). There are many hand-held individual (*15*) and point-of-use drinking water treatment devices on the market; most use ceramic or depth filters, some include activated carbon, and several utilize RO. Some incorporate a disinfectant, commonly an iodinated resin or silver. Although many of these devices have excellent potential for treating BW agent-contaminated water, almost none have been adequately tested for this purpose.

Findings

Anthrax

Anthrax is a highly infectious disease of hooved animals that is readily transmitted to humans, although individual susceptibility varies widely. It is caused by a spore-forming bacterium, *Bacillus anthracis*, which has been weaponized for aerosol application by Iraq (*16–18*) and others, and was used by the Japanese Army during World War II to contaminate food and water supplies of Chinese cities (*19*).

The three recognized forms of disease in humans are cutaneous (the most common), pulmonary (the BW target), and gastrointestinal. Gastrointestinal anthrax is usually the result of consuming contaminated meat from infected animals and is much less common than the other forms in developed nations. According to Watson and Keir (*20*),

> Abdominal pain, fever, vomiting, bloody diarrhoea and shock are the principal manifestations of this form of the disease, which has an incubation period of 2–7 days. As with pulmonary anthrax, mortality is relatively high because of the failure to make a diagnosis in time for treatment to be effective.

The clinical aspects of anthrax are also discussed by Eitzen et al. (*18*), Friedlander (*21*), Benenson (*22*), BUMED (*23*), and Franz et al. (*24*).

Because anthrax can be transmitted among animals through ingestion of spore-contaminated water (*20*), we suspect that humans can be similarly infected, although cattle are much more susceptible. A worst-case critical dose via the inhalation route is estimated as 6,000 spores (*20*), corresponding to a drinking water concentration of 57 spores/L for consumption of 15 L/day over 7 days or 171 spores/L for consumption of 5 L/day. Less conservative estimates of infective dose [median infective dose (ID_{50}) values] are 20,000 spores (*25*) and 8,000–50,000 spores (*18,24*).

B. anthracis spores are indefinitely stable in the environment; they survive for 2 years in pond water (*26*) and 40 years or more in soil (*18,26*). The spores are also heat resistant but are inactivated in water after 25 min at 95°C (*26*).

Although the vegetative form of *B. anthracis* is deactivated by chlorine disinfection under field conditions (≥ 99.6% kill, 20 min, 5 mg/L FAC, ambient temperature) (*27*), the spore form is highly resistant. It is, however, readily destroyed on contaminated surfaces by application of 5–10% chlorine bleach or formaldehyde.

Anthrax spores are removed by any water treatment filter system with pore size < 1 µm, which would include the ROWPU, microfilters, and probably many individual water purifiers. If the ROWPU membranes are bypassed, the multimedia filter and standard prefilter (5-µm nominal pore size) will remove some but not all anthrax spores, and further reduction will not be achieved through disinfection. In the case of the ROWPU and many of the individual water purifiers, cleaning or replacing contaminated filter units could present a serious cutaneous exposure hazard to personnel performing this task.

Brucellosis

The causative agents of brucellosis are *Brucella melitensis* and *Brucella suis*; the latter has been weaponized for aerosol application (*19,24,28*). Because brucellosis is contracted through consumption of contaminated milk, it is prudent to consider water a potential route of infection (*29*).

Brucellosis is a highly infectious disease of animals; in humans it is known as undulant or Malta fever and is characterized by fever, sweating, malaise, aches, and pains (*30*), with an onset of 3 days to several weeks (*28*). Symptoms intensify, then diminish, with a gradual rise in intensity. The disease is incapacitating, but the case-fatality rate is low (*18,31*).

Eitzen et al. (*18*) and Franz et al. (*24*) list an aerosol infective dose of 10–100 *Brucella* organisms; McGeorge (*25*) quotes an ID_{50} by unspecified route of 10,000 organisms for *Br. suis*, and Jensen et al. (*32*) reference an effective oral dose (ED_{50}) of 10^6 for unspecified *Brucella*. A dose of 10,000 organisms would correspond to a drinking water concentration of approximately 100 organisms/L for consumption of 15 L/day or 300 organisms/L for consumption of 5 L/day for 7 days.

Br. melitensis may survive in soil for 7–69 days and in water for 20–72 days; it is inactivated by direct sunlight (*26*).

Br. melitensis is inactivated by 1% sodium hypochlorite (*26*), but we did not find any reference to its tolerance to hypochlorite under usual conditions of drinking water disinfection.

Cholera

Cholera is an acute infectious disease of humans that is caused by the ingestion of food or water contaminated by the bacterium *Vibrio cholerae. V. cholerae* was used by the Japanese Army during World War II to contaminate food and water supplies of Chinese cities (*19*) and is thought to have been utilized as a terrorist weapon to contaminate potable water (*25*).

Cholera is a disease of the intestinal tract, with an incubation period of 1–5 days, depending on the initial dosage and the resistance of the host. Signs and symptoms are a profuse watery diarrhea, rapid dehydration, and a state of collapse. Treatment consists of replacement of fluid and electrolytes; untreated, the victim may die within hours after the onset of symptoms (*30,31*).

An ingested dose of 10^3 organisms is infectious in a healthy individual (*33*), although Jensen et al. (*32*) quote an infective dose of 10^8 organisms. The lesser dose would correspond to a drinking water concentration of 10 organisms/L for consumption of 15 L/day or 30 organisms/L for consumption of 5 L/day for 7 days.

V. cholerae survives as long as 6 weeks in some waters containing organic matter, up to 24 hr in sewage, and 16 days in soil; it is sensitive to heat, sunlight, and drying, but resists freezing (*18,26*).

V. cholerae is easily killed by chlorine (*34*), and cholera is virtually unknown in situations where water supplies are properly disinfected. However, Rycus et al. (*35*) state, without documentation, that *V. cholerae* is"tolerant to residual chlorine" (*35*).

Clostridium Perfringens

Clostridium perfringens is a common organism in secondary sewage effluent. The spores may have potential for weaponization in aerosol form. No reference suggesting potential as an agent of drinking water contamination was recovered, although incidents of food poisoning are common (*31*), and investigators in Iraq have researched *C. perfringens* (*17*) or toxins thereof (*18*).

C. perfringens, an anaerobe, causes gas gangrene in wounds. Signs and symptoms of food poisoning, which include abdominal pain, diarrhea, and possibly nausea, usually appear 8–12 hr after consumption (*31*).

Freeman (*31*) suggests that an infective dose would involve foods (e.g., poultry) containing several million *C. perfringens* per gram, which could correspond to a drinking water concentration of approximately 10^6/L for consumption of 15 L/day over 7 days, or 10^7/L for consumption of 5 L/day.

C. perfringens is presumed to be indefinitely stable in sewage.

C. perfringens, a spore former used as an indicator organism, is relatively insensitive to inactivation by chlorine. It is reduced by < 1 $\log_{10}$ under treatment with a chlorine residual of 1.2 mg/L for 15 min at 20°C and a pH of approximately 7 (*36*).

Glanders

Glanders is primarily a debilitating disease of horses, but it is transmissible to humans. Glanders is caused by the bacillus *Burkholderia* (formerly *Pseudomonas*) *mallei*. Glanders may have been weaponized in aerosol form; a single reference suggesting its potential as an agent of drinking water contamination was recovered (*29*). According to Freeman (*31*), however, "meat from glandered animals has been ingested without resulting infection."

Inflammation of mucus membranes of the nose is common, and acute infection may result in death in a few days or weeks (*31*).

An ID_{50} of 3.2×10^6 organisms has been estimated (*32*), corresponding to a drinking water concentration of ~ 3×10^4 organisms/L for consumption of 15 L/day or 9×10^4 organisms/L for consumption of 5 L/day for 7 days.

Bu. mallei survives in water at room temperature for up to 30 days and in soil for more than 27 days (*26*), but it is apparently not naturally found in soil or water (*18*).

Bu. mallei is inactivated by 1% sodium hypochlorite (*26*), but no reference to its tolerance to hypochlorite under usual conditions of drinking water disinfection was recovered.

Melioidosis

Melioidosis, caused by the bacillus *Burkholderia* (formerly *Pseudomonas*) *pseudomallei*, is a disease of rodents and domestic animals, and also occurs in humans (*31*). It is cited as a potential adversary agent, presumably aerosolized, but is not known to have been used or acquired (*25*). *Bu. pseudomallei* is transmitted among rodents by biting insects; human disease can be caused by ingestion or by contact with contaminated water through overt or inapparent skin wounds or aspiration of contaminated water (*22,29*).

The most serious form of melioidosis in humans, an acute septicemic condition with diarrhea, has a high case-fatality rate if untreated (*31*).

The infective dose of meliodiosis is unknown; Parker et al. (*26*) state that it survives for years in soil and water.

Bu. pseudomallei is inactivated by 1% sodium hypochlorite (*26*), but no reference to its tolerance to hypochlorite under the usual conditions of drinking water disinfection was recovered.

Plague

Plague is a disease of rodents, both wild and domestic, caused by the bacillus *Yersinia pestis* and transmissible to humans. *Y. pestis* may have been weaponized in aerosol form and should be considered a threat in water as well (*25,32*). Cultures were used by the Japanese Army during World War II to contaminate food and water supplies of Chinese cities (*18,37*).

There are three major clinical forms of plague: bubonic, septicemic, and pneumonic. Signs and symptoms vary, but all forms appear to be characterized by high fever, toxemia, and prostration within 2–5 days after infection, with death a frequent outcome. Pneumonic plague can be spread by coughing (*18,30,31,38*).

An aerosolization ID_{50} of 3,000 organisms has been estimated (*25*); others suggest an infective dose of 100–500 organisms (*24*) or 1,000 (*26*) organisms by inhalation. Cooper et al. (*39*) presented a procedure for estimating the percentage of troops likely to become ill from ingestion of *Yersinia* spp. depending on the raw water source and the degree of treatment. They estimate a geometric mean infectious dose of 70 organisms by ingestion. A dose of 70 organisms would correspond to a drinking water concentration ≤ 1 organism/L for consumption of 15 L/day or 2 organisms/L for consumption of 5 L/day for 7 days.

Y. pestis survives in water for 16 days and in moist soil for > 60 days (*26*); it is viable for some time in dry sputum, flea feces, and buried bodies (*18*). It is inactivated in < 15 min by heating to 55°C (*26*) to 72°C (*18*) and it is killed after several hours of direct sunlight (*18*).

Y. pestis is inactivated by 1% sodium hypochlorite (*26*), but no reference to its tolerance to hypochlorite under usual conditions of drinking water disinfection was recovered. *Yersinia* is 100% inactivated by 0.25 mg/L chlorine dioxide (*39*).

Psittacosis

Psittacosis is a disease primarily of birds but also occurs in humans and in some lower mammals (*31*). It is caused by a rickettsia-like microorganism, *Chlamydia psittaci.* It is cited as a potential adversary agent (presumably aerosolized), although it is not known to have been used or acquired (*25*). A single reference suggesting a potential for waterborne infection was recovered (*29*).

Signs and symptoms of psittacosis include chills and fever, headache, sore throat, nausea, and vomiting; the case-fatality rate is ≤ 10% (*31*).

The infective dose of psittacosis is unknown.

Ch. psittaci is considered susceptible to heat, similar to *Rickettsia prowazekii*. It is stable for 18–24 hr in seawater (*26*).

Ch. psittaci is inactivated by 1% sodium hypochlorite (*26*), but no reference to its tolerance to hypochlorite under usual conditions of drinking water disinfection was recovered.

Coxiella (Q Fever)

Coxiella burnetii, a rickettsial (*18*) or rickettsia-like (*40*) organism common among domestic farm animals, causes Q fever in humans and is considered incapacitating rather than lethal. *Co. burnetii* has been weaponized in aerosol form (*19,40*) and is believed to have potential for infection through drinking water (*25,29*), although it is commonly transmitted through inhalation (*31*). Benenson (*22*) noted possible transmission from cattle through raw milk.

According to Eitzen et al. (*18*),

> Fever, cough, and pleuritic chest pain may occur as early as ten days after exposure. Patients are not generally critically ill, and the illness lasts from 2 days to 2 weeks.

More serious complications may arise (meningitis, inflammation of the heart), but they are uncommon (*40,41*).

A human ID_{50} (unspecified route) of 25 organisms has been estimated (*25*), corresponding to a drinking water concentration of < 1 organism/L for consumption of either 15 L/day or 5 L/day for 7 days. Others estimated an infective dose as low as 1–10 organisms by inhalation (*1,24,26*).

Co. burnetii survives in tap water for 160 days at 20–22°C and resists heat, drying, osmotic shock, and ultraviolet (UV) radiation (*18,26,40*).

Co. burnetii is inactivated by 1% sodium hypochlorite (*26*), but no reference to its tolerance to hypochlorite under usual conditions of drinking water disinfection was recovered. It is, however, resistant to "many standard antiseptic compounds" (*40*).

Lindsten and Schmitt (*42*) found that *Co. burnetii* was reduced to undetectable levels in water treated with the ERDLator, a now-discontinued item of army field equipment that combined ferric chloride and limestone coagulation with 0.8 mg/L residual chlorine disinfection, 20-min contact time, and diatomite filtration. Under the same conditions, but with a chlorine residual of 0.5 mg/L, inactivation of *Co. burnetii* was incomplete.

Salmonelloses

The Salmonellae are pathogens belonging to the enteric bacilli. *Salmonella typhimurium* is often found in outbreaks of food poisoning. *Salmonella* was used by the Japanese Army during World War II to contaminate food and water supplies of Chinese cities (*19*), and there are reports suggesting that it has been used by terrorists to contaminate drinking water (*25*) as well as food (*43*).

Of two types of salmonelloses, acute gastroenteritis is commonly caused by *S. typhimurium*, whereas typhoid fever is usually caused by *Salmonella typhi*. Acute gastroenteritis is characterized by vomiting and diarrhea, whereas the symptoms for typhoid fever are more general (*31*). Typhoid is readily spread through food and water and through contact with infected (sometimes asymptomatic) carriers. During the American Civil War, there were an estimated 75,000 military typhoid cases and 27,000 deaths (*34*). More recent data for acute gastroenteritis infections indicate an overall case-fatality rate of approximately 4%, with infants and persons older than 50 years of age most seriously affected (*31*).

The ED_{50} for *S. typhi* has been estimated as 10^5 organisms (*25*). Others quoted oral ED_{50} values for various *Salmonella* spp. in the range of 10^4–10^5 organisms; one reference listed the value as 15–20 cells for foodborne salmonellosis (*32*). A dose of 10^4 organisms would correspond to a drinking water concentration of ~ 100 organisms/L for consumption of 15 L/day or 300 organisms/L for consumption of 5 L/day for 7 days.

S. typhi survival in environmental media is 29–58 days in soil, 9 days in seawater, 8 days in fresh water (*26*), and up to 5 months in ice (*34*). *S. typhimurium* survival is about the same.

Since the introduction of chlorine treatment of municipal water, waterborne typhoid has virtually disappeared in the United States. *S. typhimurium* requires a UV radiation dose of 15.2 mW•sec/cm^2 at 253.7 nm to achieve > 99.9% inactivation (*44*).

Shigellosis

Shigellosis is a bacillary dysentery caused by the ingestion of various *Shigella* species, in particular *Shigella dysenteriae*. *Shigella* spp. are most commonly disseminated under conditions of poor hygiene through "direct or indirect fecal–oral transmission" (*22*). *Shigella* cultures were used by the Japanese Army during World War II to contaminate food and water supplies of Chinese cities (*19*). *Sh. dysenteriae* has been implicated in intentional food contamination (*45*) and should be considered a threat to potable water supplies as well (*31*)

The signs and symptoms of shigellosis are diarrhea, abdominal pain, and bloody stools after an incubation period of approximately 48 hr. Shigellosis is a common disease of field armies, and asymptomatic carriers are important in maintenance and spread of the infection (*31*).

The infective dose of *Shigella flexneri*, one of the dysentery bacilli, is 10^4–10^8 organisms, based on studies of human volunteers (*31*). The lower dose would correspond to a drinking water concentration of ~ 100 organisms/L for consumption of 15 L/day or 300 organisms/L for consumption of 5 L/day for 7 days. There are, however, great differences in individual susceptibility; one reference quotes an infective dose of 100 organisms by ingestion for *Sh. flexneri* (*26*), whereas another indicates ED_{50} values from 10 to 10^9 organisms for unspecified bacillary dysentery (*32*). Endemic incidence of shigellosis among infants and children is much higher than among adults (*31*). Cooper et al. (*39*) developed a procedure for estimating the percentage of troops likely to become ill with shigellosis depending on the raw water source and the degree of treatment.

Shigella spp. survive 2–3 days in water (*26*), and there have been recent outbreaks of shigellosis traced to recreational waters (*46*).

Sh. dysenteriae is 99.6–100% inactivated by an FAC residual of 0.05 mg/L in ~ 10 min at pH 7 and 20–29°C (*27*). UV radiation is also effective and practical for small systems, achieving > 99.9% inactivation at a dose of 3.4 mW•sec/cm^2 at 253.7 nm (*44*). Virtually all recent outbreaks of shigellosis have been limited to individual or noncommunity water supplies or recreational waters (*46*), suggesting that properly disinfected waters provide a reliable barrier to *Shigella*.

Tularemia

Tularemia is an epizootic disease of animals (especially rabbits and rodents), transmissible to humans, caused by the bacillus *Francisella tularensis* (formerly *Pasteurella tularensis*). *F. tularensis* has been weaponized in the aerosol form (*19*); contaminated water is also a source of disease (*22,29,47*).

Depending on the route of infection, there are several recognized clinical types of tularemia, among which are pulmonary, glandular, or ulceroglandular (commonly from handling an infected animal carcass), and typhoidal (from direct ingestion). Typhoidal tularemia produces influenza-like symptoms and may result in death if untreated (*18,30,31,47*).

An infective dose may involve as few as 10–50 organisms, or less, by inhalation (*18,24–26,48*) or as many as 10^8 by oral challenge (*18*). The larger number would correspond to a drinking water concentration of 10^6 organisms/L for consumption of 15 L/day or 3×10^6 for consumption of 5 L/day for 7 days.

F. tularensis can survive for months in water or mud and may even multiply under these conditions (*18,31*). It is inactivated by heat (*26*) but is resistant to freezing (*18*).

P. tularensis (*F. tularensis*) is 99.6–100% inactivated by 0.5–1.0 mg/L FAC at 10°C and pH 7 in approximately 5 min (*27*). However, other studies show that chlorine (0.5–2.0 mg/L) is ineffective against tularemia (*32*).

Epidemic Typhus

Epidemic or classic typhus is a louseborne disease caused by the rickettsial agent *R. prowazekii*, commonly associated with crowded conditions and poor hygiene. McGeorge (*25*) considers *R. prowazekii* a lethal agent suitable for aerosol dissemination. No reference was recovered suggesting a potable water threat.

After an incubation period of 5–18 days, signs and symptoms of epidemic typhus include high fever, chills, intense headache, back and muscle pains, and skin eruptions (*31*), as well as mental and physical depression (*30*). Although it is estimated that 315,000 persons died from epidemic typhus in Serbia in 1915 (*31*), the case-fatality rate increases with age and varies from 10 to 40% (*22*).

An infective dose of fewer than 10 organisms has been estimated, corresponding to a drinking water concentration of < 1 organism/L for consumption of either 15 L/day or 5 L/day for 7 days, if in fact epidemic typhus is transmissible through water (*26*).

R. prowazekii, unlike other rickettsia, is considered heat sensitive, surviving < 15 min at 50°C in milk (*26*).

R. prowazekii is inactivated by 1% sodium hypochlorite (*26*), but no reference to its tolerance to hypochlorite under usual conditions of drinking water disinfection was recovered.

Encephalomyelitis

Encephalomyelitis is any one of several viral, usually arthropodborne, diseases of animals to which humans may be susceptible. Venezuelan equine encephalomyelitis (VEE) has been weaponized in aerosol form (*18,19,49*). No reference suggesting potential as an agent of drinking water contamination was recovered.

According to Stedman (*30*), VEE in humans causes fever and severe headache after an incubation period of 2–5 days, and in a few cases there has been central nervous system involvement. Nausea, vomiting, diarrhea, and sore throat may follow. Full recovery takes 1–2 weeks, and case fatality is < 1% (*18*).

An ID_{50} of 25 virus particles is estimated (*25*), corresponding to a drinking water concentration of < 1 particle/L for consumption of either 15 or 5 L/day for 7 days. Franz et al. (*24*) indicate an infective dose of 10–100 organisms by aerosol. However, it is unlikely that VEE would be transmissible through water.

Parker et al. (*26*) state that VEE virus is sensitive to UV light, which may suggest that it would eventually be destroyed by sunlight.

VEE virus is inactivated by 1% sodium hypochlorite (*26*), but no reference to its tolerance to hypochlorite under usual conditions of drinking water disinfection was recovered. However, Eitzen et al. (*18*) note that VEE virus is "rather easily killed by heat and disinfectants."

Enteric Viruses

The enteric viruses, commonly transmitted by the fecal–oral route in infants, should be considered potable water threats. Iraq has researched enterovirus 17 (Picornaviridae) and human rotavirus (Reoviridae), both of which cause gastrointestinal disorders, but has apparently not weaponized either (*17*).

Signs and symptoms of rotaviral infections include vomiting, abdominal distress, diarrhea, and dehydration. Hospitalization and even death may occur among infants and elderly patients, but rarely among otherwise healthy young adults, almost all of whom give evidence of previous infection and carry some immunity (*50*). The incubation period of rotavirus infection is estimated as 19 hr to 2 days (*50*).

According to a risk model, members of the general public who ingested six rotavirus particles in a day (≤ 1/L) would have a 50% probability of becoming infected and a 25% probability of becoming ill; after 7 days these probabilities become 70 and 35%, respectively (*50*).

At least one strain of rotavirus may persist for 8–32 days in surface waters and > 64 days in tapwater (*50*).

Reported chlorine concentration–time values, from 0.01 to 0.5 for 99% inactivation at 5°C and neutral pH (*50*), indicate that rotavirus should be effectively destroyed by army field water disinfection practices, as well as by most municipal practices. Powers et al. (*7*) report better than 4-log reduction of simian rotavirus in ≤ 20 min at temperatures of 5–25°C by sodium dichloroisocyanurate or Globaline (*7*).

Powers et al. (*51*) reported the removal of ≥ 99.99% rotavirus by the hand-operated Survivor 35 RO unit (Recovery Engineering, Inc., Minneapolis, MN).

Viral Hemorrhagic Fever

The viral hemorrhagic fevers (VHFs) arise from several different viral families and include yellow fever, dengue fever, and Rift Valley fever (all arthropodborne) (*31*), as well as lassa fever, Ebola-Marburg viral diseases, and hantaviral diseases, among others (*18*). They may have been weaponized for aerosol application, but no reference was recovered suggesting a potable water threat.

According to *Stedman's Medical Dictionary* (*30*),

> the hemorrhagic fever syndrome is associated with high fever, scattered petechiae, gastrointestinal tract and other organ bleeding, hypotension, and shock; kidney damage may be severe…

In the case of Ebola virus, fatality rates may range from 50 to 90% (*18*) or greater (*52*).

Although Jensen et al. (*32*) suggested an ID_{50} by ingestion of 1 µg, which they equate to 10^5–10^{10} particles for all the VHF viruses (*32*), Franz et al. (*24*) proposed an infective dose by aerosol of 1–10 organisms. An ingested dose of 10^5 would correspond to ~ 1,000 particles/L for consumption of 15 L/day or 3,000/L for consumption of 5 L/day, if indeed VHFs are transmissible through water.

Lassa fever virus is rapidly inactivated at 56°C; the other viruses require 30 min exposure at that temperature. All are inactivated by UV light (*26*).

All of the listed VHF viruses are inactivated by 1–2% sodium hypochlorite and/or 1% iodine (*26*), but no reference to their chlorine tolerance under usual conditions of drinking water disinfection was recovered.

Variola (Smallpox)

Variola major, also known as smallpox, is a viral disease readily spread by person-to-person contact. The Japanese Army explored weaponization of variola virus during World War II (*18*), and McGeorge (*25*) considered it suitable for dissemination through contaminated items. Otherwise, no reference was recovered suggesting a potable water threat. One concern about variola is that troops have not been vaccinated since 1989 and civilians since 1980 (*18*).

After an incubation period of approximately 12 days, signs and symptoms include chills, fever, prostration, headache, backache, and vomiting, as well as pustule formation, with a case fatality rate among the unvaccinated of 25% or more (*18,31*).

The infective dose by aerosol is assumed low (10–100 organisms) (*24*) or is very small (*25*). At this time we cannot estimate an infective level for drinking water with confidence, if indeed variola is transmissable through water.

The variola virus is resistant to drying (*26*); viable variola virus has been recovered from scabs 13 years after collection (*53*).

Variola virus is inactivated by 1% sodium hypochlorite (*26*), but no reference to its tolerance to hypochlorite under usual conditions of drinking water disinfection was recovered.

Cryptosporidiosis

Cryptosporidiosis is a gastrointestinal infection resulting from ingestion of the oocysts of a protozoan, *Cryptosporidium parvum*. It is

commonly contracted from drinking water contaminated with cattle wastes. To the best of our knowledge, *Cr. parvum* has not been weaponized. However, it has been suggested as a potential agent for sabotaging potable water supplies by reason of its infectivity and ready availability (*54*).

The signs and symptoms of cryptosporidiosis are profuse watery diarrhea, nausea, and stomach cramps. The incubation period is 4–14 days, and for otherwise healthy individuals the symptoms last 10–15 days (*55*). For immune-compromised patients, symptoms last much longer and may be life-threatening.

The ID_{50} for healthy volunteers is 132 ingested viable oocysts (*55*); Haas et al. (*5*) determined the average infective dose as 238.6 oocysts for the general population, with 95% confidence limits of 132.0–465.4. However, the ID_{50} for some highly infectious strains of *Cr. parvum* may be reduced by half (*56*). Without considering whether oocysts are cleared from the gut before excysting, a dose of 132 oocysts would correspond to a drinking water concentration of approximately 1 oocyst/L for consumption of 15 L/day over 7 days, or about 3 oocysts/L for consumption of 5 L/day.

Cr. parvum oocysts are stable in water for days or more, but they are heat sensitive.

Cryptosporidium is an emerging pathogen for which disinfection regimens are still being developed. *Cr. parvum* oocysts are highly resistant to chlorine-based disinfection and to chlorine dioxide (*6*), but sequential combinations of chlorine and other disinfectants show promise (*57*). UV light systems of an advanced design have achieved > 4 $\log_{10}$ inactivation of *Cr. parvum* oocysts (*58*).

The ROWPU will remove 100% of *Cr. parvum* oocysts, which are 3–7 µm in size. Many individual water purifiers will reduce oocysts by at least 3 $\log_{10}$, as will 10-inch (25-cm) melt-blown polypropylene depth filters of 3-µm absolute pore size. However, < 90% removal of *Cr. parvum* oocysts is achieved by the 5.0-µm nominal pore size spiral-wound cotton prefilters of the ROWPU (*14*). Removal of oocysts by direct filtration will approach 3 $\log_{10}$ in well-operated municipal systems (*57*) and may exceed 5 $\log_{10}$ for slow sand filtration (*44*) or diatomaceous earth filtration (*57*), but chlorination of the product water provides no protection if filtration performance degrades.

Mycotoxins: Aflatoxins

Aflatoxins are metabolites of the mold *Aspergillus flavus*, which infects a variety of agricultural plants including peanuts. They are potent mutagens and carcinogens. Although not as acutely toxic as botulinum, staphylococcal enterotoxins, or even ricin, and lacking properties useful for biological warfare (*17*), they have been weaponized by Iraq for missile delivery (*16,17*).

Acute aflatoxin poisoning is characterized by jaundice, rapidly developing ascites (abdominal fluid), and portal hypertension, with a high mortality rate usually resulting from massive gastrointestinal bleeding. In children, aflatoxin produces Reye syndrome: disturbed consciousness, fever, convulsions, and vomiting (*59*).

The signs and symptoms described are produced by an average intake of 2–6 mg/day of aflatoxins (*59*). An ID_{50} of 10–100 mg/person has been estimated for aflatoxin B by an unspecified route of exposure (*32*). A safe exposure would need to be < 10 µg/L for consumption of 15 L/day.

The aflatoxins have limited water solubility and are probably heat stable.

Aflatoxins are probably chlorine tolerant under normal disinfection conditions, but this needs to be determined.

Anatoxin A

Anatoxin A, also known as the very fast death factor, is an alkaloid neurotoxin produced by the filamentous freshwater cyanobacteria *Anabaena flos-aquae*.

Wild and domestic animals poisoned by anatoxin through ingestion have been observed in the field to be staggering, gasping, and suffering convulsions (*60*). Death by respiratory arrest occurs in minutes to hours.

The LD_{50} (intraperitoneal) for mice is 200 µg/kg, with 4–7 min survival (*60*). The data are insufficient for developing a drinking water NOAEL for anatoxin A.

Anatoxin A is converted to a nontoxic form in water under ambient conditions in several days (*61*).

Chlorine is ineffective against cyanobacterial toxins at typical levels (*32*).

Alum flocculation, filtration, and chlorination are ineffective in the removal of cyanobacterial toxins, and Vuori et al. (*62*) found that an individual hand-operated drinking water purifier containing carbon, ion exchange resin, and silver achieved only an approximately 50% reduction in anatoxin A concentration, although RO was completely effective.

Botulinum Toxins

Botulinum toxins are derived from protein toxins produced by *Clostridium botulinum* and exist in seven neurotoxic forms. Botulinum toxins have been weaponized by Iraq and others for aerosol application (*16–19*). To successfully contaminate a potable water supply, a biotoxin even as lethal as botulinum toxins must be introduced downstream from treatment facilities and be able to survive contact with chlorine. The quantities involved make it impractical to poison large reservoirs (*54*).

Botulinum toxins are unusual in that they are more toxic when ingested than when inhaled (*18*), although they are less toxic than when injected (*63*). Symptoms may be experienced within 24–36 hr. A progressive paralysis from head to toe follows, usually seen first in drooping eyelids (*18,64*). The victim remains mentally alert for the duration of the illness, and death results from the inability to breathe.

The LD_{50} in mice by intraperitoneal or intravenous injection is approximately 0.001 µg/kg for most of the botulinum toxins (*1,63,65*). A human inhalation LD_{50} of 0.003 µg/kg has been estimated (*65*) and an oral LD_{50} of 0.4 µg/person (0.006 µg/kg) has been estimated based on 18 case reports of botulism, 16 of which were fatal (*66*). A conservative NOAEL would be 6×10^{-5} µg/L for water consumption of 15 L/day.

Sunlight inactivates botulinum toxins within 1–3 hr. They are detoxified in air within 12 hr. The toxins are destroyed after 30 min at 80°C, after several minutes at 100°C (*26*), and by boiling or sterilization (*61*).

Botulinum toxins are > 99.7% inactivated by 3 mg/L FAC in 20 min, similar to the army disinfection procedure, and are 84% inactivated by 0.4 mg/L FAC in 20 min (*67*), similar to municipal disinfection procedures.

In one RO field study > 99.988% removal of botulinum toxins from raw water spiked with the toxin was reported (*42*). Treatment systems using charcoal should effectively remove the toxin (*25*); thus, some individual water purifiers may provide protection.

Microcystins

The microcystins are hepatotoxic products of freshwater blooms of cyanobacteria of *Microcystis* spp., *M. aeruginosa* in particular. Microcystin-LR, also known as the fast death factor, is the most common of the microcystins (*68*) and presumably the toxin of choice to be weaponized. Although the aerosolized form of microcystin is the most likely threat, ingestion—even from natural sources—must be considered a significant hazard (*68*).

Microcystins have been implicated in the death of human dialysis patients (*69*). The toxic effect of microcystin administered intraperitoneally is rapid; it affects bile flow in < 10 min. Death in laboratory animals is due to hypovolemic shock caused by interstitial hemorrhage following liver necrosis (*60,66*).

Mice administered the aerosol LD_{50}, 67 µg/kg, died within hours (*66*). An ID_{50} of 1–10 mg (per person) for microcystin by an unspecified route of exposure has been estimated (*32*). A conservative drinking water

NOAEL for microcystin would be 10 µg/L for soldiers consuming 15 L/day; however, the World Health Organization has adopted a drinking water standard of 1.0 µg/L total microcystins for lifetime exposure.

The microcystins are all water soluble and temperature stable (*61,66*).

Microcystin is essentially unaffected by 30 min exposure to 100 mg/L FAC (*67*). Iodine has no effect at 16 mg/L.

Microcystin removal from water by RO is > 90%, but coagulation/flocculation was not effective (*67*), in agreement with earlier findings that alum flocculation, filtration, and chlorination are ineffective in the removal of cyanobacterial toxins. Vuori et al. (*62*) found that an individual hand-operated drinking water purifier containing carbon, ion exchange resin, and silver had no effect on microcystin concentration, although RO was completely effective.

Ricin

Ricin is derived from the bean of the castor plant. Waste from the production of castor oil is approximately 3–5% ricin (*1*). Although ricin is relatively easy to obtain, it does not pose a serious threat as a weapon of mass destruction, considering the quantity required to overwhelm a military or civilian infrastructure (*1*). Its preferred use seems to be as an assassin's weapon, and there are documented cases of umbrellas being used as a means of injecting a "ricin ball" into a human target (*70*). Ricin is considered a potential aerosol threat (*71*), and Iraq carries ricin in its BW inventory (*16,17*). Because ricin is significantly less toxic by oral ingestion than by other routes (*72*), it is a less credible threat in drinking water.

Signs and symptoms are most severe when ricin is injected. The central nervous system is affected soon after injection. Heart function is drastically decreased and convulsions occur after the toxin blocks synapse actions in the brain. Death occurs soon after. If ingested, ricin causes gastrointestinal hemorrhage (bloody diarrhea) with organ necrosis (*18*). If inhaled, ricin attacks the lungs, and damage begins within 8–12 hr, although signs may not become evident for 12–24 hr (*1*).

The oral LD_{50} for mice is 20 mg/kg (*72*). An estimated 100% lethal dose of 50–100 µg total dose per os (*66*) for humans is probably an outlier. A conservative NOAEL would be 2 µg/L for water consumption of 15 L/day.

Ricin is detoxified in 10 min at 80°C (*26*) and in ~ 1 hr at 50°C (pH 7.8); it is stable under ambient conditions (*61*).

Ricin is > 99.4% inactivated after 20 min treatment with FAC at 100 mg/L, but is essentially unchanged at 10 mg/L (*67*). Iodine has no measurable effect at 16 mg/L.

RO is > 99.8% efficient in removing ricin from product water, but coagulation/flocculation was not effective (*67*). A treatment system using charcoal should effectively remove ricin (*25*); thus, some individual or point-of-use water purifiers may provide protection.

Saxitoxin

Saxitoxin, the cause of paralytic shellfish poisoning, is produced by the marine dinoflagellate *Gonyaulax*, among others. Saxitoxin is highly toxic by ingestion, more toxic still by injection, and perhaps most toxic by aerosol administration (*66*). It has been weaponized for covert purposes (*19*).

Signs and symptoms occur within 30 min after ingestion and include abdominal distress, diarrhea, nausea, vomiting, vertigo, headache, rapid pulse, and numbness of the tongue and gums, leading to paralysis (*66,73*). Death can occur in 1–24 hr because of respiratory failure. The only treatments are general supportive care and artificial respiration.

The LD_{50} for saxitoxin by ingestion is estimated as 0.3–1.0 mg/person (*66*). A conservative NOAEL for saxitoxin would be 0.1 µg/L for a soldier consuming 15 L/day of potable water.

Saxitoxin is water soluble, acid stable, alkaline labile, and stable at normal atmospheric conditions (*26,61*).

Saxitoxin is essentially unaffected by 30 min exposure to 10 mg/L FAC but is > 99% inactivated at 100 mg/L FAC (*67*). Iodine (Globaline) has no effect at 16 mg/L.

Saxitoxin removal from water by RO is > 98.9%, but coagulation/flocculation is not effective (*67*). Removal by charcoal may be marginal at the normal pH of drinking water, at which saxitoxin is strongly cationic.

Staphylococcal Enterotoxins

Staphylococcal enterotoxin B (SEB), which has been weaponized (*19*), is one of a number of protein toxins produced by bacteria such as *Staphylococcus aureus*. SEB can be either inhaled (aerosolization) or ingested from contaminated water or food, and could be used to sabotage food or low volume water supplies (*18*).

SEB is an incapacitating toxin, causing severe gastrointestinal pain, projectile vomiting, and diarrhea if ingested, and fever, chills, headache, muscle aches, shortness of breath, and nonproductive cough if inhaled (*18,24,31,74*). Signs and symptoms develop within several hours, but diminish after several more. Full recovery is likely, but soldiers could be incapacitated for up to 2 weeks (*18*).

Franz et al. (*24*) state that 30 ng/person is incapacitating and 1.7 µg/person is lethal by aerosol (*75*). ED_{50} values for humans are variously reported as 1–4 µg (*31*), 20–50 µg (*32*), and 20–25 µg/person (*66*) by ingestion. SEB can be lethal, but doses causing incapacitation are at least 100 times less (*1*). A conservative NOAEL would be 0.01 µg/L for water consumption of 15 L/day.

SEB is stable in both acidic and basic solutions. It is inactivated at 100°C after a few minutes and does not survive for long at room temperature (*26*).

The disinfection efficacy of SEB is unknown.

Water treatment systems using charcoal should remove SEB (*25*); thus, some individual and point-of-use water purifiers may provide protection.

Mycotoxins: T-2

T-2 toxin is one of several trichothecene mycotoxins isolated from cereal grains infected with *Fusarium* and some other genera of fungi. Russian experience with infected agricultural products indicates that ingested trichothecenes could impose a deadly threat (*18,66,76*). Unconfirmed and controversial findings suggest the use of trichothecenes as BW agents in Laos, Cambodia, and Afghanistan, and Iraq has investigated the weaponization of trichothecenes (*17*). Other trichothecenes, viz., nivalenol, 4-deoxynivalenol, and diacetoxyscirpenol, may be present in crude preparations; their toxicities are probably similar to but no greater than that of T-2 (*66*).

Topical exposure to T-2 toxin causes blistering, skin necrosis, and other effects. Sublethal effects of ingested T-2 toxin include lightheadedness, nausea, vomiting, and diarrhea (*18,66*). Eitzen et al. (*18*) described a protracted lethal illness after an accidental ingestion of bread contaminated by *Fusarium*.

The short-term U.S. Department of Defense Tri-Service field water quality standards for T-2 toxin are 26 and 8.7 µg/L for the consumption of 5 and 15 L/day, respectively (*77*).

The stability of T-2 toxin in water at room temperature is such that breakdown does not occur fast enough over the course of 7 days to negate potential health concerns for military personnel (*78*).

T-2 toxin is < 3% inactivated by 100 mg/L FAC after 30-min exposure at room temperature (*67*). Iodine has no effect at 16 mg/L.

T-2 toxin removal from water by RO is > 99.9%, but coagulation/flocculation was not effective (*67*). A treatment system using charcoal should effectively remove T-2 toxin (*25*); thus, some individual or point-of-use water purifiers may provide protection.

Tetrodotoxin

Tetrodotoxin is a potent neurotoxin that has caused the deaths of many humans as a result of consumption of improperly prepared

pufferfish (*66*). It was likely investigated as a potential BW weapon (*25*) and may be sufficiently soluble to present a drinking water threat (*61*).

The toxic signs of tetrodotoxin poisoning appear 10 min to 4 hr after ingestion and include numbness of the lips, tongue, and fingers, as well as anxiety, nausea, vomiting, and other symptoms, progressing with time to paralysis. Death, usually within 6 hr, is due to respiratory failure (*66,73*). The only treatment is artificial respiration, and recovery can be complete in 24 hr.

The oral lethal dose in humans has been estimated as 1–2 mg (*66*), and the LD_{50} has been estimated as 30 µg/kg (*32*). A conservative drinking water NOAEL for tetrodotoxin would be 0.1 µg/L for consumption of 15 L/day.

Tetrodotoxin is soluble in slightly acid water and is temperature stable (*61,66*).

Tetrodotoxin is rapidly inactivated by 50 mg/L chlorine at pH < 3 and > 9 (*66*), but no reference to the effect of chlorine under normal drinking water conditions was recovered.

Summary

BW agents as waterborne threats. The threat potentials for 18 replicating agents are summarized in Table 1. Although they are intended primarily for aerosol application, many of the bacterial agents have strong potential as waterborne threats. The rickettsial diseases, which are spread primarily by biting insects in nature, are less credible, with the possible exception of Q fever. The viral diseases evaluated as BW agents, except for the enteric viruses and possibly variola, appear to have virtually no potential as waterborne threats, and many adults have acquired immunity to the enteric viruses through childhood exposure. Given sufficient quantities, the nine biotoxins (Table 2) are potential waterborne threats, and they, like the infectious agents, are generally stable enough in the environment to be used as drinking water contaminants. There will doubtless be additions to both lists with time. The practicality of using BW agents as potable water contaminants has been addressed elsewhere (*54,70,79–81*). With few exceptions, the dose of any BW agent required to cause adverse health effects is of such magnitude as to make essential the targeting of water supplies closest to the consumer; these might include finished water storage facilities, vulnerable points in the distribution system, or even bottled water. Targeting of large bodies of water such as water supply reservoirs would be impractical, even though intentional or collateral water contamination could result from an aerosol attack. In terms of quantities required to present a credible threat, any or all of the replicating agents indicated as positive water threats ("yes" in Table 1) may be available from domestic sources or rogue nation intercession. Among the biotoxins, only BTX, ricin, SEB, T-2 mycotoxin, and possibly aflatoxin could likely be procured in sufficient quantities at the present time. Alternative synthetic techniques may make some of the rare biotoxins available in quantity in the future. In a nuclear/biological/chemical environment, the greatest risk of injury could be to those who service the water treatment equipment and are exposed to the agents at the highest concentrations.

Incapacitating effects. The percentage of troops disabled and the intensity of symptoms are largely unpredictable, but most infectious agents cause extreme gastrointestinal distress, with severe stomach pains, diarrhea, and vomiting. Some cases will need hospitalization, and without prompt medical care some may die. Symptoms of biotoxin ingestion are also variable, although the

Table 1. Summary of threat potential of replicating agents.

Agent/disease	Weaponized	Water threat	Infective dose[a]	Stable in water	Chlorine tolerance[b]
Anthrax	Yes	Yes	6,000 spores (inh)	2 years (spores)	Spores resistant
Brucellosis	Yes	Probable	10,000 organisms (uns)	20–72 days	Unknown
Cholera	Unknown	Yes	1,000 organisms (ing)	Survives well	Easily killed
Clostridium perfringens	Probable	Probable	10^8 organisms (ing)	Common in sewage	Resistant
Glanders	Probable	Unlikely	3.2×10^6 organisms (uns)	Up to 30 days	Unknown
Melioidosis	Possible	Unlikely	Unknown	Unknown	Unknown
Plague	Probable	Yes	500 organisms (inh)	16 days	Unknown
Psittacosis	Possible	Possible	Unknown	18–24 hr, seawater	Unknown
Q fever	Yes	Possible	25 organisms (uns)	Unknown	Unknown
Salmonella	Unknown	Yes	10^4 organisms (ing)	8 days, fresh water	Inactivated
Shigellosis	Unknown	Yes	10^4 organisms (ing)	2–3 days	Inactivated, 0.05 ppm, 10 min
Tularemia	Yes	Yes	10^8 organisms (ing)	Up to 90 days	Inactivated, 1 ppm, 5 min
Typhus	Probable	Unlikely	10 organisms (uns)	Unknown	Unknown
Encephalomyelitis	Probable	Unlikely	25 particles (aer)	Unknown	Unknown
Enteric viruses	Unknown	Yes	6 particles (ing)	8–32 days	Readily inactivated (rotavirus)
Hemorrhagic fever	Probable	Unlikely	10^5 particles (ing)	Unknown	Unknown
Smallpox	Possible	Possible	10 particles (uns)	Unknown	Unknown
Cryptosporidiosis	Unknown	Yes	132 oocysts (ing)	Stable days or more	Resistant

Abbreviations: aer, aerosol; ing, ingestion; inh, inhalation; uns, unspecified.
[a]Total infective dose used to calculate water values. [b]Ambient temperature, ≤ 1 ppm free available chlorine, 30 min or as indicated.

Table 2. Summary of threat potential of biotoxins.

Biotoxin	Weaponized	Water threat	NOAEL, 2 L/day[a]	Stable in water	Chlorine tolerance[b]
Aflatoxin	Yes	Yes	75 µg/L	Probably stable	Probably tolerant
Anatoxin A	Unknown	Probable	Unknown	Inactivated in days	Probably tolerant
Botulinum toxins	Yes	Yes	0.0004 µg/L	Stable	Inactivated, 6 ppm, 20 min
Microcystins	Possible	Yes	1.0 µg/L[c]	Probably stable	Resistant at 100 ppm
Ricin	Yes	Yes	15 µg/L	Stable	Resistant at 10 ppm
Saxitoxin	Possible	Yes	0.4 µg/L	Stable	Resistant at 10 ppm
Staphylococcal enterotoxins	Probable	Yes	0.1 µg/L	Probably stable	Unknown
T-2 mycotoxin	Probable	Yes	65 µg/L[d]	Stable	Resistant
Tetrodotoxin	Possible	Yes	1 µg/L	Probably stable	Inactivated, 50 ppm

NOAEL, no-observed-adverse-effect level.
[a]Estimated as 7.5 times the NOAEL calculated for consumption of 15 L/day. [b]Ambient temperature, ≤ 1 ppm free available chlorine, 30 min or as indicated. [c]World Health Organization drinking water standard. [d]Derived from short-term U.S. Department of Defense Tri-Service standard (*77*).

range of individual susceptibilities is generally narrower than for infectious agents. Extreme gastrointestinal distress, often accompanied by hemorrhaging and/or paralysis, is common. Again, many victims may die without immediate medical care. A procedure has been developed for estimating the percentage of troops likely to become ill from ingestion of enteric pathogens depending on the raw water source and the degree of treatment (*39*), and it may be possible to expand this approach to include most or all BW pathogens.

Efficacy of water treatment. A properly functioning RO system with quality membranes should, by size exclusion, reduce any BW agent to a safe level in the product water (*42*), although low molecular weight toxins such as the mycotoxin T-2 may in time penetrate an intact RO membrane. Such a sustained challenge would probably be beyond an adversary's capability, however. In general, failure of the ROWPU to consistently provide BW agent-free water would be an indication of failed membranes or leaking seals; however, several reports have documented apparent exceptions to size exclusion of microbes from RO permeate (*8–10*). When RO is not used for water purification (as with diatomaceous earth filters, most individual water purifiers, or pickup water) or when the membranes of the ROWPU are deliberately bypassed, the ultimate protection against infectious agents may be disinfection. A well-designed and well-operated municipal water treatment plant with filtration can be expected to achieve 2–3 $\log_{10}$ removal of bacteria (omitting consideration of disinfection) (*12,13*) and 2–4 $\log_{10}$ removal of protozoan cysts (*13,57*). For the proteinaceous biotoxins, i.e., BTX, ricin, and SEB, as much as 1 $\log_{10}$ removal may be possible under optimal conditions of coagulation and flocculation. Some agents, including anthrax spores, *Cryptosporidium* oocysts, and most biotoxins, are nearly impervious to FAC used for disinfection, whereas for many others there is no useful information. Only limited indication of the efficacy of iodine compounds or silver (used in some individual water purifiers) was found. Some individual and point-of-use water purifiers are potentially capable of removing infectious agents or biotoxins from water, and some may remove both, but few have been rigorously tested.

Research needs. There is a critical need for rapid online and field methods for detecting and quantifying both infectious agents and biotoxins in water and in other environmental samples. Indeed, much promising research, such as use of the polymerase chain reaction to amplify bacterial DNA, is directed toward addressing this deficiency. Of equal importance is the requirement to determine the tolerance of BW agents for chlorine and other water disinfectants, because disinfection may be the ultimate barrier. Finally, there is a need to determine the efficacy of available individual and point-of-use water purifiers with respect to removal or inactivation of BW agents.

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