ORIGINAL RESEARCH

10% Povidone-Iodine May Be a Practical Field Water Disinfectant

Jason D. Heiner, MD; David C. Hile, MD; Samandra T. Demons, PhD; Ian S. Wedmore, MD

From the Department of Emergency Medicine, Madigan Army Medical Center, Tacoma, WA (Drs. Heiner, Hile, and Wedmore); Department of Clinical Investigation, Madigan Army Medical Center, Tacoma, WA (Dr. Demons).

Objective.—A paucity of data exists regarding the use of iodophores such as povidone-iodine (PVI) to disinfect water. We sought to determine a practical minimal disinfecting concentration of 10% PVI over different contact times and temperatures when added to water inoculated with *E. coli*.

Methods.—1:100, 1:1,000, and 1:10,000 dilutions of 10% PVI were created. *Escherichia coli* was exposed to these dilutions for 5, 15, and 30 minutes at 10, 20, and 30°C. Bactericidal activity was neutralized with 0.5% sodium thiosulfate. Mean viable colony forming units (CFUs) was determined after triplicate plating on Luria-bertani agar and 24 hours of incubation at 37°C. Effective bactericidal activity was defined as a 5-log reduction.

Results.—Of the 200,000 *E. coli* plated, no CFUs were observed after exposure to the 1:100 dilution. After 5 minutes of contact time with the 1:1,000 dilution, at 10°C CFUs were too numerous to count (TNTC), at 20°C the mean CFU count was 92 (standard error ± 11), and at 30°C the mean CFU count was 25 (standard error ± 8). No CFUs were observed after 15 minutes of exposure to the 1:1,000 dilution across experimental temperatures. The 1:10,000 dilution always yielded CFU growth that was TNTC.

Conclusions.—The lowest disinfecting concentration of 10% PVI was the 1:1,000 dilution at 15 minutes of contact time. This supports the use of PVI for water disinfection against *E. coli*, the organism most commonly responsible for traveler's diarrhea. Further studies may assess its effectiveness against more virulent water borne pathogens.

Key words: water disinfection, povidone-iodine, *Escherichia coli*, traveler's diarrhea

Introduction

Even the most pristine appearing surface water may contain harmful infectious contaminants, and, without treatment, almost no surface water should be considered safe to drink.^{1–4} The main benefit to treating drinking water is to prevent gastrointestinal illness by enteric pathogens such as the bacterium *Escherichia coli*. *E. coli* is the most common causative agent of traveler's diarrhea and is recognized as an important waterborne pathogen and as an indicator organism for monitoring water quality.^{5–7} Enteric pathogens such as *E. coli* are found naturally in tropical, temperate, and cold waters. The diarrheagenic *E. coli* include such members as en-

terotoxigenic *E. coli*, enteropathogenic *E. coli*, enterohemorrhagic *E. coli*, enteroinvasive *E. coli*, and others.⁸

Iodophores such as povidone-iodine (PVI) are compounds consisting of iodine and inert polymers such as polyvinylpyrrolidone that have several advantages over elemental iodine preparations. As a topical disinfectant, iodophores tend to be less irritating to the skin, are more soluble in water, and are less staining, and yet maintain the antibacterial activity of iodine.⁹ Organic iodine and iodine containing filter products have been well established as effective, safe, and simple methods of water disinfection. These iodinated products have been used extensively by the United States Army, aboard the US Space Shuttle, and in situations necessitating the emergency disinfection of potable water supplies such as in austere environments and during times of natural disasters.^{10–15} The efficacy of iodophores to disinfect drinking water, however, has not been well established, and to our knowledge there are no previous studies that have

The abstract accompanying this work was presented at the Wilderness Medical Society: 2009 Wilderness Medicine Conference and Annual Meeting, Snowmass, CO, July 28, 2009.

Corresponding author: Jason Heiner, MD, Madigan Army Medical Center, Department of Emergency Medicine, 9040 Fitzsimmons Ave, Fort Lewis, WA 98431 (e-mail: jason.heiner1@us.army.mil).

effectively evaluated the potential for utilizing 10% povidone-iodine (PVI) as a practical field water disinfectant. We sought to experimentally determine a practical minimal disinfecting concentration of PVI when added to different temperatures of water inoculated with a known concentration of *E. coli*, and across water temperatures similar to those at which this organism is commonly found.

Methods

POVIDONE-IODINE SOLUTION

Betadine 10% Povidone-Iodine Topical Solution (1% available iodine, Purdue Frederick Company, Norwalk, CT, Lot #087-0215) was used in our experiment. Full-strength 10% PVI stock solution was added to sterile deionized water to create 1-L volumes of 1:100, 1:1,000, and 1:10,000 dilutions of 10% PVI. The PVI stock solutions and subsequent dilutions were used on the day that they were opened. The dilutions were kept at room temperature until placed in the temperature-controlled water bath as described below. The pH of each dilution was measured.

BACTERIA AND CULTURE MEDIUM

The clinical strain of *E. coli* used was ATCC 25922 and is our institution's laboratory quality control strain. Significant effective bactericidal activity was defined per convention as a 5-log reduction (99.999% bacterial kill).^{9,16} To detect this 10^5 colony-forming unit (CFU) reduction, an initial stock inoculum of 10^8 CFU/mL of bacteria was created, then washed, and suspended in phosphate-buffered water. Final viable bacteria CFU count was determined after incubation on Luria-bertani (LB) agar plates. All final plating was performed in triplicate, and final results were expressed as the mean CFU count of the 3 plates with calculated standard error.

NEUTRALIZATION

Neutralization is essential to ensure that, after timed exposure to PVI, the bactericidal action of PVI does not carry over into the final survivor culture medium. Sterile 0.5% sodium thiosulfate (STS) was used in our experiment (Sigma-Aldrich Company, St. Louis, MO, Product #S7026, Lot #106K0178). STS is well established as an appropriate neutralizing agent for PVI in experiments employing many different species of bacteria.^{17–20} Experimental controls as described in the “experimental procedure” section were performed to ensure that the neutralizing agent itself did not inhibit bacterial growth.

EXPERIMENTAL PROCEDURE

Temperature was a controlled variable in this experiment. The same protocol was performed with the PVI dilutions maintained in baths at 3 different temperatures: 10, 20, and 30°C. Sterile test tubes containing 9 mL of a given PVI dilution were placed in the water baths, and temperature equilibration was achieved in 30 to 60 minutes. One milliliter of our stock *E. coli* inoculum (at 10^8 CFU/mL) was then added to each of these test tubes with the PVI dilutions. One milliliter of this mixture (PVI + 10^7 CFU/mL of *E. coli*) was then withdrawn at set intervals of 5, 15, and 30 minutes, and added to 4 mL of the 0.5% sodium thiosulfate (STS) to neutralize the bactericidal activity of the PVI. This final solution (PVI + STS + 2×10^6 CFU/mL of *E. coli*) was gently agitated for 30 seconds, and the number of viable organisms present was determined by plating 0.1-mL samples containing 2×10^5 CFU of *E. coli* on LB plates in triplicate using standard surface plating techniques. Surviving CFUs from these 2×10^5 plated *E. coli* were counted after incubation at 37°C for 24 hours.

Experimental controls to assess for measurable antibacterial activity of the STS neutralizing agent were performed at each of the above temperatures and time intervals by substituting 9 mL of sterile deionized water for the 9 mL of PVI dilution. All other steps for the experimental control, including the details regarding the stock *E. coli* inoculums, addition of the STS, plating methods, incubation temperature, and incubation time, were otherwise identical to the steps that utilized PVI dilutions.

Results

Differences in disinfection ability were observed between the 3 concentrations of 10% PVI (Table). Of the 200,000 *E. coli* plated, no CFUs were observed at any sampling times after exposure to the 1:100 dilution of 10% PVI. After 5 minutes of contact time with the 1:1,000 dilution, at 10°C CFUs were too numerous to count (TNTC), at 20°C the mean CFU count was 92 (standard error ± 11), and at 30°C the mean CFU count was 25 (standard error ± 8). However, at 15 minutes of contact time, no CFUs were observed after exposure to the 1:1,000 dilution across experimental temperatures. The 1:10,000 dilution always yielded CFU growth that was TNTC. A trend toward faster disinfection at warmer experimental temperatures was observed with the 1:1,000 concentration. However, no significant difference in the time to effective disinfection (5-log reduction) relative to different temperatures for a given concentration of PVI was observed at any of our sampling times. The

Table. Final mean colony forming unit (CFU) results at all experimental temperatures for each 10% povidone-iodine (PVI) dilution plus control at the sample contact times

10% PVI dilution	Calculated free iodine concentration	pH	Number of CFUs after contact time		
			5 min	15 min	30 min
10°C					
1:100	100 mg/L	7.94	0	0	0
1:1,000	10 mg/L	7.83	TNTC	0	0
1:10,000	1 mg/L	7.91	TNTC	TNTC	TNTC
Control	0 mg/L	7.01	TNTC	TNTC	TNTC
20°C					
1:100	100 mg/L	8.13	0	0	0
1:1,000	10 mg/L	7.42	92 (SE: ±11)	0	0
1:10,000	1 mg/L	7.74	TNTC	TNTC	TNTC
Control	0 mg/L	7.02	TNTC	TNTC	TNTC
30°C					
1:100	100 mg/L	7.44	0	0	0
1:1,000	10 mg/L	7.53	25 (SE: ±8)	0	0
1:10,000	1 mg/L	7.48	TNTC	TNTC	TNTC
Control	0 mg/L	7.01	TNTC	TNTC	TNTC

From the original 2×10^5 plated bacteria, 2 or fewer final CFUs indicate a 5-log reduction of *E. coli* (TNTC = too numerous to count; SE = standard error).

control solution yielded CFU results that were TNTC at all experimental temperatures and sampling times.

Limitations

Some limitations from the experimental design deserve mention. A more accurate measure of the minimum effective concentration remains somewhat uncertain as we did not test a concentration between the minimally effective and consistently effective concentration points (such as a 1:5,000 concentration of 10% PVI). Also, while we chose 30 minutes as the longest contact time for practical disinfection, it is possible that a more dilute solution such as the 1:10,000 concentration of 10% PVI may have proven effective after a longer contact time. The disinfection constant (*Ct*) is a product of disinfectant concentration multiplied by time to disinfection, and is commonly used to compare the effectiveness of particular disinfectants against particular organisms. In practical terms, the *Ct* can be mathematically reached by increasing the disinfectant concentration or contact time. Although increasing the contact time of the 1:10,000 PVI dilution by a factor of 10 may seem prohibitively long for general field use, such an investigation may find that it then becomes as effective as the 1:1,000 dilution.

The frequency of our experimental sampling times may also limit our ability to draw conclusions. The chemical reactions that result in disinfection with halogens such as iodine are often faster and more effective at warmer tem-

peratures, and common water treatment protocols recommend that when treating colder waters either the halogen dose or its water contact time should be markedly increased to ensure disinfection.²¹ While we observed such trends in our experiment, it is possible that significant differences in speed to effective disinfection (5-log reduction) would have been observed if more frequent sampling times were performed or a wider range of temperatures were sampled. More frequent sample intervals could also refine the determination of the true time to effective disinfection of the 1:1,000 dilution, which may lie between the 5 and 15 minute sample times.

Other aspects of our experiments may pose limitations when anticipating actual field application. For example, while field water disinfection is often hindered by the presence of dissolved organic material, we did not attempt to characterize the disinfectant ability of PVI in the presence of such potentially interfering material. Also, while the high experimental concentration of *E. coli* was needed to ensure “effective disinfection” as it is commonly defined, these concentrations do not necessarily mirror nature. Studies regarding the potability of water not intended for drinking often report between zero and 10^2 CFUs/mL of bacteria such as *E. coli*, with a rarity of bacterial contamination nearing our experimental conditions of 2×10^5 CFU/mL of *E. coli*.^{1–4} It is therefore possible that lower experimental concentrations of PVI could at times be more effective in nature than in our study. However, we chose to use a robust and standard

definition of disinfection to investigate effective and practical use. In nature, it is unusual for one to know with certainty how safe a drinking source is, and water-borne organisms more resilient than *E. coli* are known to cause illness when ingested.^{1,6,22,23}

Discussion

PVI is a common component of portable first aid kits for use in wound care due to its excellent topical disinfecting profile. It is often carried into austere environments and other situations where water disinfection is essential prior to consumption. While field water disinfection often employs halogens such as iodine, little more than anecdotal experience supports the potential of PVI as an effective disinfectant for drinking water. Prior studies have demonstrated the susceptibility of *E. coli* to PVI, and a 10% PVI concentration falling between 1:1,000 to 1:10,000 has been suggested by past authors as being a possible, but relatively uninvestigated effective strength for field water disinfection.^{19,21,24,25}

PVI is a complex iodinated compound that acts as a predictable free iodine reservoir when diluted in water to the concentrations that we tested.⁹ Overall, our experiment supported the suggestion that PVI may be an effective field water disinfectant against *E. coli* at temperatures ranging from 10 to 30°C. The 1:100 concentration of 10% PVI was effective across all experimental conditions. However, this concentration of 10% PVI was selected primarily to vary experimental conditions and the associated amount of available iodine (100 mg/L water) would be not be practical as a water disinfectant. The 1:1,000 concentration of 10% PVI was effective at the 15 minute sampling time across the temperature ranges. A 1:1,000 concentration is equivalent to approximately 1 mL (approximately 20 drops) of 10% PVI solution per liter of water and results in about 10 mg of available iodine per 1 L of solution. This amount of available iodine per liter of treated water which demonstrated effectiveness in our study would generally be safe for emergency short term water disinfection.²⁵ The most dilute 1:10,000 concentration was not effective when tested following up to 30 minutes of contact time. Moreover, this concentration (approximately 2 drops of 10% PVI in 1 L of water) would yield about 1 mg of available iodine per 1 L of treated water, which is lower than that usually recommended for field water disinfection.

We did observe a trend toward faster disinfection at warmer experimental temperatures with the 1:1,000 dilution sampled at 5 minutes but did not observe a significant difference in time to effective disinfection (5-log reduction). This trend is consistent with past studies that have demonstrated decreased germicidal power of iodine in colder

water.^{26,27} One study examining the effect of free residual iodine at about 4 mg/mL against *Giardia* cysts found that the time to 100% kill was about 8 times faster when the experimental temperature was increased from 5 to 30°C.²⁶ Regarding halogens such as iodine, the *Ct* for *Giardia* may be several hundred times larger than for *E. coli*, with viruses demonstrating intermediate resistance.^{28,29} This concept is important when considering the milieu of potential infective organisms found in surface water.

While *E. coli* is an important waterborne pathogen, other bacteria such as *Salmonella* and *Campylobacter*, enteric viruses, and enteric protozoans are all threats to safe water consumption.^{6,23} Past studies examining other forms of iodinated disinfectants have supported the effectiveness of free iodine concentrations of up to 10 mg/L against a variety of potential pathogens such as bacteria, enteric viruses, and protozoal cysts.^{24,26,30–33} Direct comparison of our findings to past studies examining the effectiveness of iodine against *E. coli* is difficult at times due to experimental variations and differing thresholds for determining effective disinfection. However, our findings appear to be generally congruent. Past relevant iodine studies (primarily with tetraglycine hydroperiodide) have shown that 5 to 10 mg of iodine per liter of water is an effective disinfectant against *E. coli* in as little as 5 to 10 minutes, while 1 mg of iodine per liter of water may inactivate 99% of *E. coli* in 1 to 2 minutes.^{31–33} Importantly, iodine has demonstrated significant potential limitations when tested against very resistant organisms such as *Cryptosporidium parvum*.²² More studies are needed to investigate the behavior of PVI against a variety of other waterborne pathogens.

Our findings support the potential use of PVI as a practical field water disinfectant, and our results are compatible with past studies demonstrating the effectiveness of other iodine-based disinfectants against *E. coli*. While PVI was effective against *E. coli*, the organism most commonly responsible for traveler's diarrhea, its effectiveness against more virulent and hardy waterborne pathogens remains untested. The potential toxicity of PVI lies chiefly with the iodine component and not the inert polymer component. The primary inert polymer of PVI, polyvinylpyrrolidone, has been used as a blood plasma substitute and extender during World War II, and is widely used today in a number of industries such as a tablet binder in pharmaceuticals and as an additive and stabilizer to food and beverages.³⁴ Further studies may also assess the palatability of PVI when added to water as well as its ease of use as a field water disinfectant. This is paramount as a truly effective field water disinfectant must also be one that is consistently used by the individuals it is intended to protect.

Disclaimer

The views expressed herein are solely those of the authors and do not represent the official views of the Department of Defense or Army Medical Department.

References

1. Reynolds KA, Mena KD, Gerba CP. Risk of waterborne illness via drinking water in the United States. *Rev Environ Contam Toxicol*. 2008;192:117–158.
2. Levin RB, Epstein PR, Ford TE, et al. U.S. drinking water challenges in the twenty-first century. *Environ Health Perspect*. 2002;110(suppl 1):43–52.
3. Kimani-Murage EW, Ngindu AM. Quality of water the slum dwellers use: the case of a Kenyan slum. *J Urban Health*. 2007;84:829–838.
4. Derlet RW, Carlson JR. An analysis of wilderness water in Kings Canyon, Sequoia, and Yosemite national parks for coliform and pathologic bacteria. *Wilderness Environ Med*. 2004;15:238–244.
5. Travelers' diarrhea. NIH Consensus Development Conference. *JAMA*. 1985;253:2700–2704.
6. Sharma S, Sachdeva P, Virdi JS. Emerging water-borne pathogens. *Appl Microbiol Biotechnol*. 2003;61:424–428.
7. Plummer JD, Long SC. Monitoring source water for microbial contamination: evaluation of water quality measures. *Water Res*. 2007;41:3716–3728.
8. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev*. 1998;11:142–201.
9. Gottardi W. Iodine and iodine containing compounds. In: Block SS, ed. *Disinfection, Sterilization, and Preservation*. Philadelphia, PA: Lippincott Williams and Wilkins; 2001: 159–183.
10. McMonigal KA, Braverman LE, Dunn JT, et al. Thyroid function changes related to use of iodinated water in the U.S. Space Program. *Aviat Space Environ Med*. 2000;71: 1120–1125.
11. Morgan DP, Karpen RJ. Test of chronic toxicity of iodine as related to the purification of water. *US Armed Forces Med J*. 1953;4:725–728.
12. Goodyer L, Behrens RH. Safety of iodine based water sterilization for travelers. *J Travel Med*. 2000;7:38.
13. Laubusch EJ. Chlorination and other disinfection processes. In: American Water Works Association. *Water Quality and Treatment: A Handbook of Public Water Supplies*. New York: McGraw-Hill; 1971:158–223.
14. Atwater JE, Sauer RL, Schultz JR. Numerical simulation of iodine speciation in relation to water disinfection aboard manned spacecraft I. Equilibria. *Environ Sci Health A Environ Sci Eng Toxic Hazard Subst Control*. 1996;A31: 1965–1979.
15. Maberly GF, Eastman CJ, Corcoran JM. Effect of iodination of a village water-supply on goitre size and thyroid function. *Lancet*. 1981;2:1270–1272.
16. Cremieux A, Freney J, Davin-Regli A. Methods of testing disinfectants. In: Block SS, ed. *Disinfection, Sterilization, and Preservation*. 5th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2001:1305–1327.
17. Leung MP, Bishop KD, Monga M. The effect of temperature on bactericidal properties of 10% povidone-iodine solution. *Am J Obstet Gynecol*. 2002;186:869–871.
18. Berkelman RL, Holland BW, Anderson RL. Increased bactericidal activity of dilute preparations of povidone-iodine solutions. *J Clin Microbiol*. 1982;15:635–639.
19. Houang ET, Gilmore OJ, Reid C, Shaw EJ. Absence of bacterial resistance to povidone iodine. *J Clin Pathol*. 1976;29:752–755.
20. Ghogawala Z, Furtado D. In vitro and in vivo bactericidal activities of 10%, 2.5%, and 1% povidone-iodine solution. *Am J Hosp Pharm*. 1990;47:1562–1566.
21. Backer H. Water disinfection for international and wilderness travelers. *Clin Infect Dis*. 2002;34:355–364.
22. Butkus MA, Starke JA, Bowman DD, et al. Do iodine water purification tablets provide an effective barrier against *Cryptosporidium parvum*? *Mil Med*. 2005;170:83–86.
23. Leclerc H, Schwartzbrod L, Dei-Cas E. Microbial agents associated with waterborne diseases. *Crit Rev Microbiol*. 2002;28:371–409.
24. Lacey RW. Antibacterial activity of povidone iodine towards non-spore forming bacteria. *J Appl Bacteriol*. 1979;46:443–449.
25. Backer H, Hollowell J. Use of iodine for water disinfection: iodine toxicity and maximum recommended dose. *Environ Health Perspect*. 2000;108:679–684.
26. Fraker LD, Gentile DA, Krivoy D, Condon M, Backer HD. Giardia cyst inactivation by iodine. *J Wilderness Med*. 1992; 3:351–357.
27. Jarroll EL, Bingham AK, Meyer EA. Inability of an iodination method to destroy completely Giardia cysts in cold water. *West J Med*. 1980;132:567–569.
28. Hoff J. *Inactivation of Microbial Agents by Chemical Disinfectants: EPA/600/2-86/067*. Cincinnati, OH: US Environmental Protection Agency; July 1986.
29. Backer HD. Field water disinfection. In: Auerbach PS, ed. *Wilderness Medicine*. 5th ed. Philadelphia, PA: Mosby Elsevier; 2007:1368–1417.
30. Berg G, Chang SL, Harris EK. Devitalization of microorganisms by iodine. *Virology*. 1964;22:461–481.
31. Chang SL, Morris JC. Elemental iodine as a disinfectant for drinking water. *Ind Eng Chem*. 1953;45:1009–1012.
32. Safe Drinking Water Committee, Board on Toxicology and Environmental Health Hazards, Assembly of Life Sciences. The disinfection of drinking water. In: *Drinking Water and Health*. Washington, DC: National Academy Press; 1980;2:5–139.
33. Powers EM, Hernandez C, Boutros SN, Harper BG. Bactericidal efficacy of a flocculating emergency water purification tablet. *Appl Environ Microbiol*. 1994;60:2316–2323.
34. Robinson BV, Sullivan FM, Borzelleca JF, Schwartz SL. Introduction, absorption of PVP by various routes of administration, and excretion and metabolism of PVP. In: *PVP: A Critical Review of the Kinetics and Toxicology of Polyvinylpyrrolidone (Povidone)*. 1st ed. Chelsea, MI: Lewis; 1990:1–4, 29–68.