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Source water quality effects on monochloramine inactivation of adenovirus, coxsackievirus, echovirus, and murine norovirus

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ABSTRACT

There is a need for more information regarding monochloramine disinfection efficacy for viruses in water. In this study, monochloramine disinfection efficacy was investigated for coxsackievirus B5 (CVB5), echovirus 11 (E11), murine norovirus (MNV), and human adenovirus 2 (HAdV2) in one untreated ground water and two partially treated surface waters. Duplicate disinfection experiments were completed at pH 7 and 8 in source water at concentrations of 1 and 3 mg/L monochloramine at 5 and 15 °C. The Efficiency Factor Hom (EFH) model was used to calculate CT values (mg-min/L) required to achieve 2-, 3-, and 4-$\log_{10}$ reductions in viral titers. In all water types, monochloramine disinfection was most effective for MNV, with 3-$\log_{10}$ CT values at 5 °C ranging from 27 to 110. Monochloramine disinfection was least effective for HAdV2 and E11, depending on water type, with 3-$\log_{10}$ CT values at 5 °C ranging from 1200 to 3300 and 810 to 2300, respectively. Overall, disinfection proceeded faster at 15 °C and pH 7 for all water types. Inactivation of the study viruses was significantly different between water types, but there was no indication that overall disinfection efficacy was enhanced or inhibited in any one water type. CT values for HAdV2 in two types of source water exceeded federal CT value recommendations in the US. The results of this study demonstrate that water quality impacts the inactivation of viruses and should be considered when developing chloramination plans.

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1. Introduction

Disinfection is a critical step in the drinking water treatment process to reduce infectious virus concentrations, and chlorine is the most widely used disinfectant in the United States (AWWA, 2008). Monochloramine, which is formed by combining an ammonia source with free chlorine, is sometimes used to maintain a disinfectant residual in the distribution system because it is more stable than free chlorine and can help to minimize biofilm growth. However, monochloramine use in the US may increase as a result of the regulations set forth by the Stage 2 Disinfectants and Disinfection Byproducts Rule (Stage 2 DBPR) (USEPA, 2006b). The Stage 2 DBPR regulates the maximum levels of disinfection

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byproducts (DBPs) in drinking water. Both chlorine and monochloramine produce DBPs as a result of their interaction with organic and inorganic matter in water, but monochloramine is less reactive than chlorine and produces fewer regulated DBPs and at lower concentrations than chlorine. In addition, the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) (USEPA, 2006a) seeks to reduce the incidence of disease associated with pathogenic microorganisms in drinking water, particularly *Cryptosporidium*. Because *Cryptosporidium* oocysts are highly resistant to free chlorine, some water utilities may need to implement alternative disinfectants in order to comply with the LT2ESWTR treatment requirements.

In its Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources (Guidance Manual), the US Environmental Protection Agency (USEPA) recommended CT values of 1423 and 712 to achieve 3-$\log_{10}$ inactivation of viruses with monochloramine at pH8 and 5 and 15 °C, respectively (USEPA, 1990). The recommended CT values do not include a factor of safety, under the assumption that addition of ammonia will follow a period of free chlorine exposure, which could dramatically lower concentrations of viruses that may be resistant to monochloramine. However, CT value requirements for viruses using preformed monochloramine should be evaluated in order to determine whether they are sufficient for water treatment systems in which ammonia is added before chlorine, they are combined simultaneously, or there is an intrusion event into a distribution system where monochloramine is present.

The Guidance Manual CT value recommendations were obtained from disinfection experiments conducted with monodispersed hepatitis A virus (HAV) in buffered, reagent-grade water (RGW). Previous researchers found that chlorine disinfection of viruses in natural waters was significantly different than in RGW. Haas et al. (1996) found that the inactivation rates of MS2 were lower in surface waters than in RGW. The authors suggested that increased turbidities in the surface waters may have afforded some protection to the virions and resulted in the decreased inactivation rates. Similarly, Thurston-Enriquez et al. (2003a) found that higher CT values were required for inactivation of AdV40 in treated ground water and that it may have been due to ground water constituents that protected the viral particles via adsorption or enhanced aggregation. In another study, inactivation rates for HAdV2, E11, CVB5, and MNV in three source waters were significantly different between water types and compared to RGW, but could not be correlated to any measured water quality parameters (Kahler et al., 2010). Because water quality can impact chlorine disinfection of viruses, it is possible that monochloramine inactivation rates may also be different for RGW and natural waters.

While several studies have examined the disinfection efficacy of monochloramine for viruses in RGW (Baxter et al., 2007; Cromeans et al., 2010; Sirikanchana et al., 2008; Sobsey et al., 1991, 1988), there is little information in the literature regarding the disinfection efficacy of monochloramine in natural waters. The objective of this study was to examine the disinfection efficacy of monochloramine on selected viruses from USEPA's Contaminant Candidate List (CCL2) (USEPA, 2005) in one untreated ground water and two partially treated surface waters from distinct geographical regions. The impact of water quality was examined by comparing the inactivation rates of the study viruses in each water type, as well as to disinfection efficacy in RGW from a previous study (Cromeans et al., 2010).

2. Materials and methods

2.1. Virus propagation and assay

Clones of CVB5 (Faulker strain) and E1 (Farouk strain) were prepared from strains obtained from the American Type Culture Collection (ATCC, Manassas, VA) and propagated in BGM cells (Scientific Resources Program, CDC). MNV-1 was obtained from Karst et al. (2003) and propagated in RAW 264.7 cells obtained from ATCC. HAdV2 (strain 6) was obtained from CDC and propagated in A549 cells (Scientific Resources Program, CDC). Cell lines were maintained in either Eagle's Minimum Essential Medium (EMEM) or Dulbecco's Modified Eagle Medium (DMEM) as described previously (Cromeans et al., 2010). Viral titers were determined by plaque assay by inoculating 10-fold dilutions onto cell monolayers in 60 mm^2 dishes. After 1 h adsorption at 37 °C and 5% CO_2, the infected cells were overlaid with 5 mL maintenance medium (2×) containing 0.5% agarose. Following a 2-day incubation of MNV and enterovirus assays and a 5-day incubation of HAdV2, a second overlay containing 2% neutral red was added to visualize plaques within 4 h.

2.2. Cell associated virus (CAV) preparation

CAVs were prepared as described previously (Cromeans et al., 2010). Cell monolayers were infected at a multiplicity of infection of 0.5–1.0 and cultured in serum free medium until maximum virus titer was obtained based on replication studies of each virus. The culture medium was removed and replaced with chlorine-demand-free Dulbecco's PBS (CDF DPBS) before freezing at −70 °C. The CAV was purified by polyethylene glycol precipitation and chloroform extraction and the purified CAV (pCAV) was used on the same day as the experimental inoculum.

2.3. Reagents and glassware

CDF DPBS and CDF water were prepared according to Standard Method 4500-Cl C (APHA, 2005). A monochloramine stock solution was made by mixing equal volumes of 200 mg/L free chlorine and 800 mg/L ammonium chloride in pH 8 CDF water and was stored at 4 °C for 2 wk. Prior to each experiment, this stock was added to the experimental waters to achieve 1 or 3 mg/L monochloramine. Monochloramine residual was measured on a Hach DR/850 colorimeter using Hach Monochlor-F reagent pillows (Hach, Loveland, CO). CDF glassware was prepared by soaking in ≥5 mg/L free chlorine overnight. The glassware was rinsed 5 times with CDF water, covered with clean foil, and baked at 200 °C for 2 h. All glassware and water was pre-chilled at 5 or 15 °C before each experiment.

2.4. Test waters

Partially treated source water samples were obtained from Cobb County-Marietta Water Authority (CCMWA) in Marietta,