# Contaminant Candidate List Viruses: Evaluation of Disinfection Efficacy [Project #3134]

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### PRINCIPAL INVESTIGATORS:

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# **OBJECTIVES:**

The goal of this project was to investigate the disinfection efficacy of free chlorine and monochloramine for Contaminant Candidate List 2 (CCL2) viruses, including human adenovirus (HAdV), coxsackievirus, echovirus, and calicivirus.

### **BACKGROUND:**

The current standards for chlorine and monochloramine disinfection of drinking water were established using information obtained from bench-scale disinfection experiments of monodispersed hepatitis A virus. It is not clear whether these standards are sufficient for the four virus types listed on the CCL2. Several disinfection studies have been conducted on representative coxsackieviruses and echoviruses, but much less is known about adenoviruses and caliciviruses. No previous study has investigated this suite of virus types under the same disinfection conditions. Further, there is a lack of comprehensive information regarding the disinfection efficacy of chlorine and monochloramine in source drinking water. Lastly, few studies have attempted to examine disinfection of aggregated viruses.

# APPROACH

Baseline disinfection experiments were performed in pH 7 and pH 8 demand-free reagent grade water with 0.2 mg/L free chlorine or 1 mg/L monochloramine at 5°C. These baseline experiments were performed using several human adenoviruses (HAdV2, HAdV40, and HAdV41), two coxsackieviruses (coxsackievirus B3 [CVB3] and coxsackievirus B5 [CVB5]), two echoviruses (echovirus 1 [E1] and echovirus 11 [E11]) and murine norovirus (MNV, studied as a surrogate for human norovirus). The most resistant representative of each virus type for each disinfectant was selected for additional virus disinfection experiments using three distinct types of source water collected from drinking water treatment plants. Experiments were performed in source water at pH 7 and 8 using 0.2 and 1 mg/L free chlorine or 1 mg/L and 3 mg/L monochloramine at 5 and 15°C. Free chlorine and monochloramine disinfection experiments were then performed for aggregated preparations of HAdV2 in source water from one drinking water treatment plant. Viral titers before and after disinfection were determined by virus-specific plaque assays. The efficiency factor Hom model was used to calculate Ct values (disinfectant concentration in mg/L x exposure in min) required to achieve 2-, 3-, and 4-log<sub>10</sub> reductions in viral titers.

# **RESULTS/CONCLUSIONS:**

In all water types, chlorine and monochloramine disinfection were most effective for MNV, with 3-log<sub>10</sub> Ct values at 5°C ranging from <0.02–0.03 for chlorine and 53–111 for monochloramine. Chlorine disinfection was least effective for CVB5 for all water types, with 3-log<sub>10</sub> Ct values at 5°C ranging from 2.3–7.6. Monochloramine disinfection was least effective for HAdV2 and E11, depending on pH and water type. At 5°C, 3-log<sub>10</sub> Ct values for HAdV2 ranged from 1044–3308, while those for E11 ranged from 814–2288. Overall, chlorine was much more effective than monochloramine, and disinfection proceeded faster at 15°C and at pH 7 for all water types. Disinfection susceptibility was markedly different between water types for some viruses, but no single environmental water type had consistently different inactivation rates. Ct values for chlorine and monochloramine disinfection of aggregated HAdV2 were 2 and 1.4 times higher than for monodispersed HAdV2, respectively.

# APPLICATIONS/RECOMMENDATIONS:

The results of this project can be used by water utilities to ensure that current and planned free chlorine and chloramination systems are designed and operated to meet specific disinfection goals. In addition, the data from this project can be used to model the survival of these viruses in distribution systems, whether associated with a treatment system breakthrough or distribution system intrusion scenario. In particular, Ct values for CVB5 can be used, in conjunction with disinfection results from other studies, to guide planning and operation of free chlorine treatment systems (as free chlorine was least effective for CVB5 in the present study). The results from this project indicate that a Ct value of 10 (or 20, if incorporating a 2x factor of safety for aggregated virus) may be needed to achieve a 4-log<sub>10</sub> inactivation of CVB5 with free chlorine at 5°C, pH 8, which is above the Ct value of 8 recommended in the USEPA's Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources (Guidance Manual) to achieve a 4-log<sub>10</sub> inactivation with chlorine at 5°C, pH 6–9. The Guidance Manual recommended Ct of 8 included a safety factor of 3x to account for potential virus aggregation. However, Ct values for the study viruses, including CVB5, were below the 2-log<sub>10</sub> Ct value of 12 reported by the World Health Organization (WHO) as an expected performance level for chlorine disinfection of viruses in water at  $5^{\circ}$ C, pH 7–7.5.

For chloramination systems, Ct values from this project for HAdV2 and E11 can be used in conjunction with disinfection results from other studies to guide system planning and operation. The results from this project indicate that a Ct value of 4,400 (or 6,200, if incorporating a 1.4x factor of safety for aggregated virus) may be needed to achieve a 4-log<sub>10</sub> inactivation of HAdV2 with monochloramine at 5°C, pH 8, which is above the Ct value of 1,988 recommended in the Guidance Manual to achieve a 4-log<sub>10</sub> inactivation with chloramines at pH 8. The Guidance Manual recommended Ct of 1,988 does not include a safety factor to account for potential virus aggregation. When compared to WHO guidelines for enhanced water treatment processes, which suggest that monochloramine disinfection should achieve 2-log<sub>10</sub> virus inactivation at a Ct = 430 (15 °C, pH 6-9), the results from the present study suggest that a Ct value as high as 1400 (for

HAdV2 without incorporating an aggregation factor) would be needed to achieve the same virus inactivation level under similar temperature and pH conditions.

### **Future Research**

The results of this project show that the water source can have a significant effect on disinfection Ct values for free chlorine and monochloramine. Additional research would be useful to evaluate which water quality parameters are associated with reduced or increased efficacy of these common drinking water disinfectants. This study demonstrated that different virus types (and, indeed, different representatives of a single virus type) can have significantly different susceptibility to free chlorine and monochloramine disinfection. Future research on these disinfectants should incorporate multiple virus types and/or multiple representatives of each virus type, and include other viruses that have been included on the CCL3 (e.g., hepatitis A virus). The results of this study also indicate that MNV is highly susceptible to inactivation by free chlorine and monochloramine, relative to the other viruses studied. Additional research is needed to develop a tissue culture system for human norovirus so that these viruses can be studied directly. In the absence of such a culture system for human norovirus, additional research is needed to evaluate other potential surrogates for human norovirus.

The results of the aggregated virus disinfection experiments provide further evidence that aggregated viruses are less susceptible than monodispersed viruses to free chlorine and monochloramine disinfection. However, the virus aggregation research in this project also indicated that commonly used aggregated virus preparation procedures do not consistently result in production of aggregated virus preparations for water disinfection experiments. In addition, these experiments demonstrated that the degree of virus aggregation (measured as percent aggregated and size distribution of aggregates) can decrease substantially when aggregated virus preparations are added to environmental water samples. Further research is needed on the effectiveness of free chlorine and monochloramine for other viruses, including research on the degree of aggregation achieved for different viruses using different aggregated virus preparation procedures and the stability of these preparations in water of different quality.

### **RESEARCH PARTNER:**

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