United States
Environmental Protection
Agency

Municipal Environmental
Research Laboratory
Cincinnati OH 45268

Research and Development

EPA-600/S2-84-094 Sept. 1984

EPA

Project Summary

Effect of Particulates on Disinfection of Enteroviruses in Water by Chloramines

Pasquale V. Scarpino

The inactivation kinetics of chloramines (monochloramine and dichloramine) on an enterovirus, poliovirus 1 (Mahoney), and on an enteric indicator of fecal pollution, *Escherichia coli* 11229, were examined in laboratory bench-scale studies using the kinetic (stirred beaker) apparatus. The disinfecting ability of chloramines in the presence of viral aggregates and organic particulates was compared with viral inactivation in pure buffer systems with unassociated viruses and without added particulates. In addition, comparisons of chloramines, hypochlorous acid, and hypochlorite ion were made in a variety of test situations involving, for example, (1) several types of particulates (enterovirus-associated animal cells, solids-associated primary effluents, and fecal suspensions), (2) aggregated and unassociated single viruses, (3) different temperatures of reactivity, (4) different pH's, and (5) different disinfectant combinations.

Aggregated poliovirus was more resistant to both monochloramine and dichloramine than were the unassociated viruses. Almost doubling the monochloramine dose from 12 to 22 mg/L at 5 C and pH 9 did not double the rate of virus disinfection. Dichloramine inactivated poliovirus 1 less effectively than did monochloramine. Monochloramine formed at pH 9 and then adjusted to pH 7 gave a stable solution of mostly monochloramine. Viral disinfection rates then examined at both pH 7 and 9 were similar, but monochloramine killed the test bacterium *E. coli* 10 times more rapidly at pH 7 than at pH 9. Forming monochloramine was about 1.2 times more effective as a disinfectant than newly made, preformed monochloramine at 5 C and pH 9.

Poliovirus 1 survivors that had been exposed eight times to monochloramine at 15 C and pH 9 were 2.3 times more resistant to monochloramine than both the initially used, unexposed virus and those viruses exposed fewer than eight times to monochloramine.

Human epidermoid carcinoma (HEp-2) and Buffalo Green Monkey (BGM) cells were used to study the effects of cell-associated turbidity on the disinfection process. The object was to mimic the natural state of viruses as they are freshly discharged in feces. The rate of disinfection was influenced by both the disinfectant used and the cell-induced turbidity of the system. Both of the cell-associated viruses were more susceptible to hypochlorous acid than to monochloramine. Increasing the turbidity increased the resistance of the cell-associated viruses to monochloramine.

Total coliforms in fecal suspensions disinfected with hypochlorous acid showed an initial rapid die-away of greater than 99.9% during the first minute of interaction, followed by a protracted period of survival. The turbidity of primary effluents also gave protection to naturally occurring coliforms disinfected with monochloramine.

This Project Summary was developed by EPA's Municipal Environmental Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Background

Knowledge about virus inactivation in water is assuming greater importance as streams, rivers, and lakes that serve as drinking water sources for many cities become more and more contaminated with sewage. Enteric viruses infective for man are the most important viral agents known to be present in water and wastewater, and more than 100 different types may be present in human feces. Enteric viruses include the enteroviruses (primarily polioviruses, coxsackieviruses, and echoviruses), hepatitis type A, Norwalk type agents, rotaviruses, reoviruses, adenoviruses, and parvoviruses. Since enteric viruses are found in the feces of infected persons and are readily isolated from urban sewage, they may enter water supplies and present health hazards to humans. Virologists in several countries have reported the presence of enteroviruses in drinking water samples obtained from public water supply systems that use conventional treatment methods of filtration followed by disinfection. These studies all involve water that is bacteriologically safe and contains a chlorine residual considered to be virucidal. The passage of viruses through a water treatment plant's treatment train could be due to an enhanced viral resistance to chlorine, the presence of natural particulate matter, the association of the viruses with the alum used for flocculation, or virus association with organic matter.

Most viruses in the natural environment are associated with solids and do not occur in a free state. The association of viruses with solids does not necessarily mean virus inactivation. In fact, clay solids do not appear to have any deleterious effect on the viruses. Concern exists that particulates (turbidity) in drinking water may interfere with disinfection. Thus, turbidity in drinking water may alter the virus minimal infectious dose by protecting the viruses. This possibility resulted in the National Interim Primary Drinking Water Regulations (NIPDR), which allow a maximum contaminant level of 1 Nephelometric Turbidity Unit (NTU), or up to 5 NTU if that level does not interfere with achieving and maintaining disinfection.

Studies suggest the occurrence of an evolutionary or adaptive alteration that increases the resistance of the virus population after repeated sublethal exposures to free chlorine. Virus inactivation in water appears to be favored by acid conditions.

Objectives

Because chloramine (combined chlorine) is commonly used in place of chlorine to disinfect waters with high levels of trihalomethane precursors (organics), more precise data are needed on the efficiency of chloramine disinfection alone and in the presence of particulate matter (turbidity) in drinking water. The main objective of this study was, therefore, to investigate the effects of particulates in water on the disinfection of enteroviruses by chloramines. The complete study objectives are outlined below:

1. To determine the effect of turbid water on the disinfection of test microbes (primarily poliovirus 1 and the reference bacterium *E. coli*) using combined available chlorine (the chloramines). These results were then compared with those of free chlorine (hypochlorous acid and hypochlorite ion). The relationship of particulate material to disinfection efficiency was then examined. Particulates included human fecal solids, sewage-primary effluent solids, and animal-cell-associated poliovirus 1.
2. The disinfection ability of chloramines (both monochloramine and dichloramine) was studied at various chloramine concentrations, temperatures, contact times, and pH values; at various concentrations and types of particulates; and with single versus aggregated preparations of test virions. Comparisons were also made of disinfection efficiencies for monochloramine used as a preformed dose and as forming doses. Also studied were the virus inactivation with double monochloramine doses and the addition of multiple doses of poliovirus 1 during the progress of the experiment.
3. To select a monochloramine-resistant poliovirus 1 mutant.

Methods

All of the disinfection studies were performed using the kinetic apparatus. The poliovirus 1 (Mahoney strain) stocks used in these studies were prepared as either aggregates or singles. Enterovirus-associated animal cells were prepared to simulate naturally found cell-associated viruses that can be excreted from the intestinal tract of humans. Two cell lines were used — human epidermoid carcinoma (HEp-2) and Buffalo Green Monkey (BGM) cells. Primary effluent from a municipal sewage treatment plant and human feces were used to prepare solids-associated, naturally occurring coliforms. Animal viruses were titered by the plaque technique in BGM continuous cell lines. *E. coli* survivors were recovered and enumerated with surface-inoculated trypticase soy agar plates, whereas primary effluent studies used the most probable number multiple-tube fermentation method through the confirmed test.

Results and Discussion

Monochloramine and Dichloramine Disinfection of Poliovirus 1 Singles and Aggregates

A number of studies have implicated aggregates in the viral inocula as the cause of aberrations in survival curves when viruses are exposed to destructive chemical and physical agents such as disinfectants. This study showed aggregated poliovirus 1 to be 1.7 times more resistant to the disinfectant than the singles preparation (Figure 1). Similar results were found in the dichloramine studies. The disinfectant apparently penetrates slowly into the aggregated viral mass, thus enabling some viruses to survive and develop in the tissue culture recovery system.

Effects of Temperature On Inactivation of Poliovirus 1 Singles by Monochloramine and Dichloramine

Since chemical disinfection is a rate process, the chemical reaction rate increases with increasing temperatures. The empirical rule of thumb is that the rate of the reaction increases by a factor of 2 to 3 for each 10-degree rise in temperature. The temperature coefficient for a 10-degree change (Q_{10}) when destroying virus by free chlorine has been observed to increase the rate of virus inactivation by a factor of 2 to 3 (200 to 300 times). Studies for 99% inactivation of poliovirus 1 singles by monochloramine at pH 9 and temperatures of 5, 15, and 25 C yielded an average Q_{10} value of 2.75, whereas at the 90% level, the average value was 1.95. The 90% inactivation kinetics of poliovirus 1 singles by dichloramine at 5 and 15 C yielded a Q_{10} of 2.5, which was within the 2 to 3 factor increase noted earlier.

Effects of pH on Inactivation of Poliovirus 1 Singles and E. coli by Monochloramine

Most finished drinking waters in the United States are maintained below pH 9 — usually between 7 and 9. At pH 9 and above, the chloramine that is formed when hypochlorous acid reacts with ammonia is predominantly monochloramine. Thus many research studies are done at pH 9 or above. However, pH values lower than 9 can be encountered in drinking water treatment. To determine the disinfectant quality of a still predominantly monochloramine system at a pH below 9, monochloramine levels were first preformed at pH 9, and the pH was immediately adjusted to 7. Initial concentrations

of monochloramine were stable at this latter pH for more than 4 hr. The change in pH had no apparent effect on the disinfection of the poliovirus 1 singles, but it increased the rate of monochloramine disinfection of *E. coli* about 10 times (Figure 2). The effect of pH on transport mechanisms across the bacterial cell membrane may have influenced the greater monochloramine destructive effect at pH 7. Earlier work has demonstrated that *Entamoeba histolytica* cysts take up more chlorine and have lower survival rates at low pH. Other research using the surrogate animal virus, bacteriophage f2 (instead of an animal virus like poliovirus 1) produced greater virus inactivation at lower pH. The fact that the present study did not produce a similar effect points up the need for caution when using a surrogate animal virus. These studies should be continued using other animal viruses to determine whether inactivation by monochloramine is greater at pH 7 than at pH 9.

Disinfection of E. coli *Using Preformed and Forming Monochloramines*

Chloramine research studies usually use preformed monochloramines as the disinfectant. For many years, ammonia (NH_3) has been combined with chlorine (Cl_2) to form chloramines for the treatment of drinking water. Ammonia is still deliberately added to some chlorinated public water supplies to provide a combined available chlorine residual (i.e., chloramines). Monochloramine is the principal chloramine that is encountered in drinking water treatment, but in recent years, chloramines have not been recommended as a primary disinfectant because of their perceived low germicidal efficiency. Another concern is whether forming monochloramines are better disinfectants than preformed monochloramines. Thus the present study attempts to cast more light upon the real-world situation in which monochloramines are formed during the process of disinfection and are not added in the preformed state.

The disinfection efficiencies of preformed monochloramine (NH_2Cl) and forming monochloramine (free chlorine and NH_3) against the test bacterium *E. coli* were compared along with reference to the disinfection ability of free chlorine alone. The forming monochloramine (Figure 3) was about 1.2 times more effective than the preformed monochloramine. Split-second exposure of the *E. coli* inoculum to the hypochlorite-hypochlorous acid mixture that existed at pH 9 in the forming monochloramine study may have been responsible for the initial faster kill of the test bacteria.

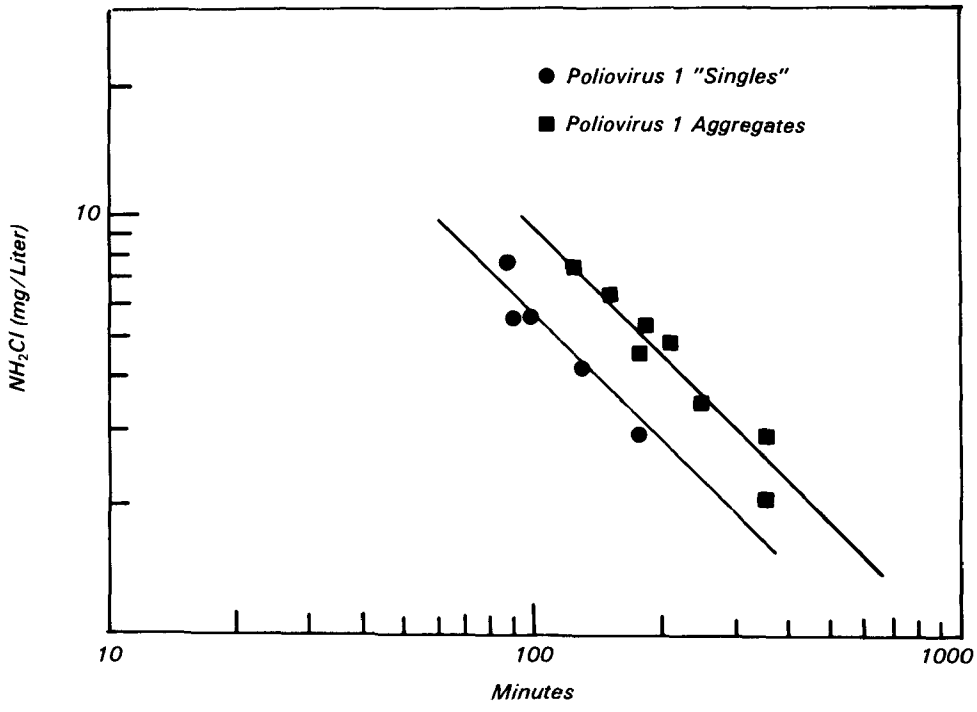


Figure 1. Concentration-time relationship for 99% activation of poliovirus 1 singles and aggregates by monochloramine at pH 9 and 15 C.

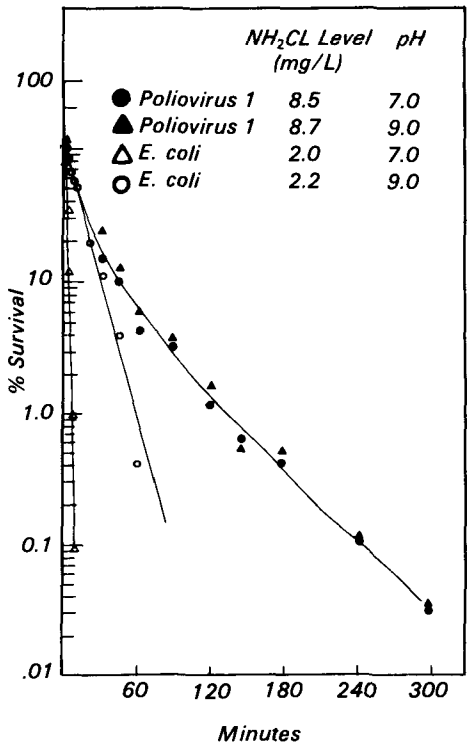


Figure 2. The inactivation of poliovirus 1 singles and Escherichia coli *at 5 C by monochloramine at pH 9 and 7 (preformed at pH 9).*

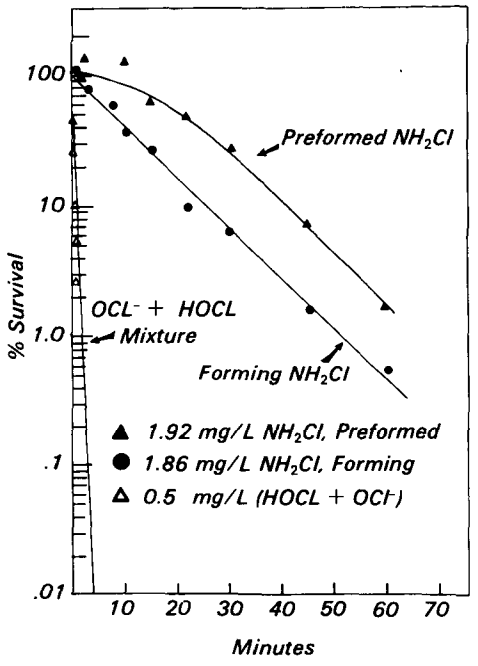


Figure 3. Disinfection of E. coli *11229 at 5 C and pH 9 by forming and preformed monochloramines compared with a 0.5-mg/L mixture of hypochlorous acid (HOCl) and hypochlorite ion (OCl^-).*

The disinfection rates for the forming and preformed monochloramines were the same after the first 15 min of the study; the observed differences in the positioning of the lines can be attributed to the differences in bacterial numbers after the first 3 min of the study. Although the original bacterial inocula were similar, there was a greater initial kill of the bacteria in the forming monochloramine study than in the preformed monochloramine. After the first 3 min of the forming monochloramine experiment, the ability of the newly formed monochloramine to kill the remaining bacteria was the same as that encountered in the preformed study, but there was more bacteria to disinfect in the preformed study. The brief initial exposure of the bacteria in the inoculum to the free chlorine present before the monochloramine was completely formed appears to account for the differences between the two monochloramine survival curves.

Sequential Addition of Poliovirus 1 to Determine the Extent of Monochloramine Disinfecting Efficiency

Survival curves in these studies often show retardant die-away and inactivation patterns. Although the disinfectant level was never depleted during the course of the experimentation, the question arose as to whether changes had nevertheless occurred in the disinfectant's efficiency to account for retardant curves. Thus a second inoculum of poliovirus 1 was added 2 hr after the first virus administration to determine whether the inactivation rate of this subsequent virus inoculum would mimic the first portion of the curve. The rapid initial inactivation rate reappeared, indicating that the disinfection efficiency of the original monochloramine had not been affected or altered and that this monochloramine was still capable of inactivating the additional inoculum.

Effect of Increasing Concentrations of Monochloramines on the Inactivation of Poliovirus 1 Singles

During this research, it was noted that increasing the monochloramine concentration did not proportionally increase the rate of inactivation of poliovirus 1 singles. Though 12 mg/L of monochloramine was about four times more effective at the 99% inactivation point than the lower concentration of 5.4 mg/L, 22 mg/L was as effective as 12 mg/L. This result is contrary to that of some researchers who used *E. coli* as the test organism and consistent with results of other investigators who used viruses.

Effect of Chloride Ions on Monochloramine Disinfection of Poliovirus 1 Singles and E. coli

Poliovirus 1 has been found to be inactivated more rapidly by chlorine in the form of hypochlorite ion (OCl^-) at pH 10 than by hypochlorous acid (HOCl) at pH 6. The borate buffer system (containing KCl) is believed to have an influence on the hypochlorite ion and hypochlorous acid virucidal relationships. Since 0.2 N HCl was used in this study to prepare preformed monochloramine at pH 9, the effect of the chloride ion on the disinfection process was investigated. The addition of 0.2 N HCl made the 0.05 M borate buffer system (without KCl) about 0.02 M with respect to chloride ions. In a similar study with *E. coli* at 5 C, 3.2 mg/L monochloramine was formed at pH 7, 0.02 M chloride ions were added as the sodium salt, and disinfection was compared with that at the same level of monochloramine at pH 7, but without the added chloride ions. No effect of the added chloride ions was observed. Thus the observed difference in disinfection at pH 9 and pH 7 for *E. coli* (Figure 2) was due to the pH change to 7 and not to the addition of chloride ions when the 0.2 N HCl was added to the buffer system.

Selection for Monochloramine-Resistant Poliovirus 1

Studies have suggested that viruses become resistant to free chlorine after repeated sublethal exposures. Thus a test was undertaken to determine whether successive exposures of virus to monochloramine would have the same effect. Two procedures were used to prepare virus singles (aggregates could increase virus resistance). Polioviruses prepared by both procedures were exposed separately under the same test conditions for similar time periods. After exposure to monochloramine, the more resistant plaques representing survivors were isolated, regrown, and re-exposed to monochloramine. Eight repetitive monochloramine exposure cycles were performed for viruses prepared by both procedures. The viruses prepared by one method developed no resistance to monochloramine, whereas those prepared by the other method gradually did. The poliovirus prepared by the latter method and exposed eight times were 2.3 times more resistant to monochloramine than either unexposed poliovirus or virus exposed seven times to monochloramine (Figure 4).

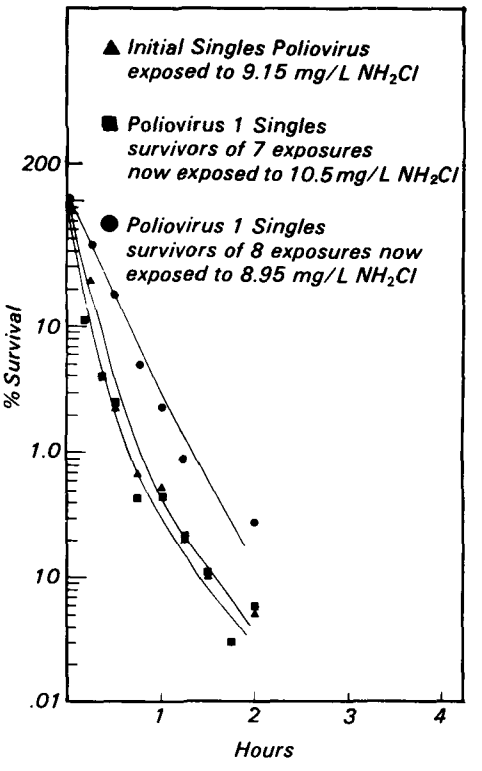


Figure 4. Inactivation of prepared poliovirus at pH 9 and 15 C before and after repeated exposure to monochloramine.

Disinfection of HEp-2-Cell-Associated Poliovirus 1 With Monochloramine

Disinfection studies with animal-cell-associated poliovirus 1 were performed using two continuous cell lines, HEp-2 and BGM kidney cells. The cell-associated virus system approximates the state of viruses as they are excreted from the body into domestic sewage. The viruses are protected by their cell association (Figure 5). This effect is more dramatic when the viruses are 99.9% through 99.99% inactivated.

The effect of organic turbidity on the disinfection of HEp-2 cell-associated poliovirus was studied with monochloramine concentrations ranging from 4.15 to 21.0 mg/L at 5 C and pH 7 and 9 (Figure 6). Nearly doubling the monochloramine dosage (from 12.2 to 21.0 mg/L) at pH 9 in the presence of almost the same turbidity reduced the time required for 90% virus inactivation from 50 to 30 min. Increasing the turbidity from 0.8 NTU to 2.0 NTU at monochloramine levels of 10.35 and 11.3 mg/L, respectively, and pH 7 significantly decreased disinfection efficiency. Turbidity caused by the presence of animal cells interfered with the disinfec-

tion process. The pH change from 9 to 7 had no apparent effect on the rate of poliovirus 1 inactivation whether or not the virus was associated with animal cells.

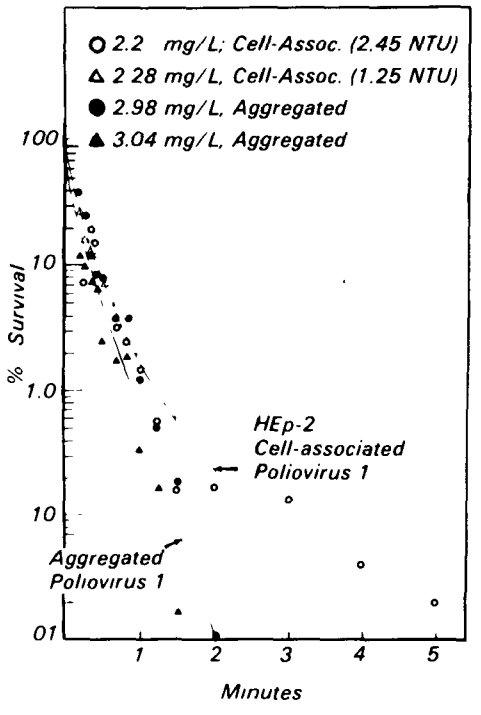


Figure 5. *Inactivation of aggregated and HEp-2 cell-associated poliovirus 1 with hypochlorous acid at pH 6 and 5 C*

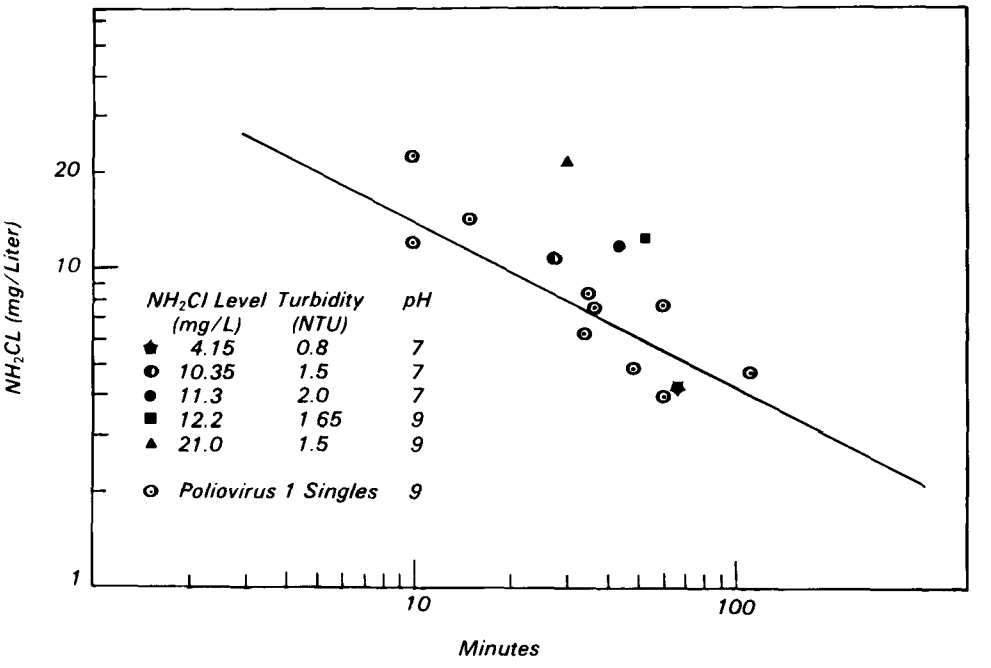


Figure 6. *Concentration-time relationship for 90% inactivation of poliovirus 1 singles and HEp-2 cell-associated poliovirus 1 at different turbidity levels and concentrations of monochloramine at 5 C and pH 7 and 9*

Disinfection of BGM Cell-Associated Poliovirus 1 With Hypochlorous Acid, Monochloramine, and Dichloramine

The inactivation of BGM-cell-associated poliovirus 1 was studied using three disinfectants — hypochlorous acid at 15 C and pH 6.0, monochloramine at 15 C and pH 9, and dichloramine at 5 C and pH 4.5. All the survival curves showed extended tailings caused by the association of the poliovirus 1 to the cells and to themselves (aggregation) during the disinfection process. Disinfection rates of BGM-cell-associated poliovirus 1 by monochloramine and hypochlorous acid at similar turbidities and concentrations were compared. Whereas only 15 sec was needed for 90% of the cell-associated viruses to be inactivated by the hypochlorous acid, 95 min was required to reach 90% inactivation with the monochloramine. Even under these difficult conditions for disinfecting viruses, hypochlorous acid was about 380 times as effective as the monochloramine. Figure 7 summarizes the monochloramine concen-

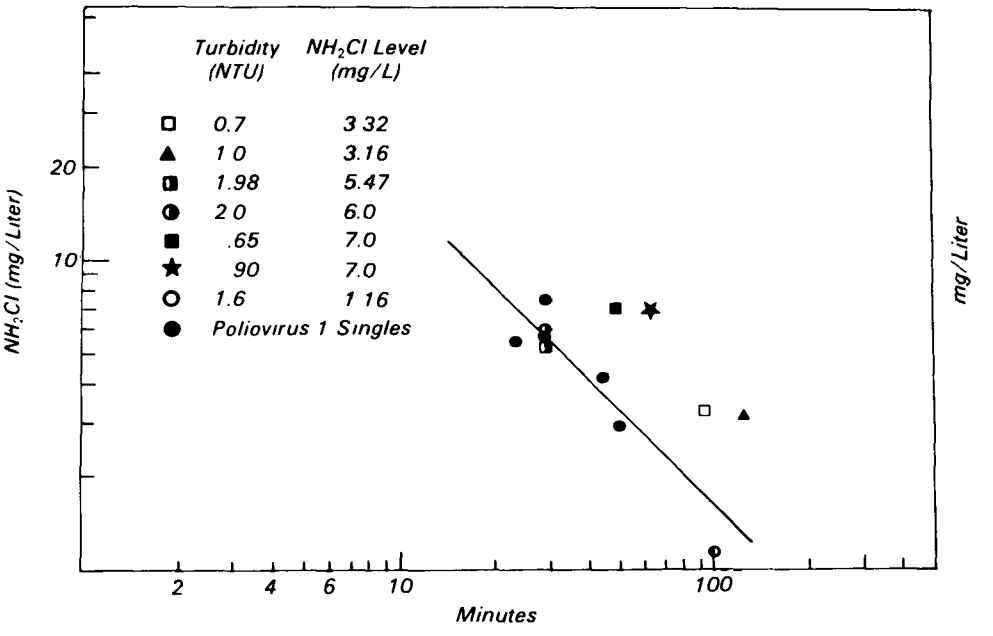


Figure 7. *Concentration-time relationship for 90% inactivation of BGM-cell-associated and unassociated poliovirus 1 by various concentrations of monochloramine at 15 C and pH 9.*

tration-time plot for the 90% inactivation of BGM-cell-associated and unassociated viruses. The BGM-cell-associated poliovirus 1 points are represented by bold symbols. Most of these associated points were above the poliovirus 1 unassociated singles curve, indicating that the cell-associated viruses were being protected from inactivation.

A final study was done comparing the inactivation of BGM cell-associated poliovirus with the survival of unassociated poliovirus 1 singles using dichloramine as the disinfectant at 5 C and pH 4.5. No differences were observed in the disinfection rates of the two poliovirus preparations, though the dichloramine concentrations were similar (17.0 mg/L for the unassociated versus 17.35 mg/L for the associated poliovirus). The lack of protection could be due to rapid penetration of the cell mass by the dichloramine.

Coliform Disinfection Studies

Disinfection studies using coliforms were divided into two groups: (a) disinfection of naturally occurring coliforms from fecal suspensions, and (b) disinfection of fecal coliforms associated with primary effluent solids. The turbidity associated with either system was found to interfere with disinfection efficiency.

Conclusions

Virus aggregates are, along with organic particulates, a major part of the mechanism for the maintenance of virus infectivity in water. In these studies, aggregated poliovirus 1 (at the 99% inactivation point) at 15 C and pH 9 was about 1.7 times more resistant to disinfection by monochloramine than unassociated virus singles. The singles virus preparation disinfected by dichloramine at 15 C and pH 4.5 was inactivated (at the 90% inactivation point) about 8 times as rapidly as the aggregated virus.

The average temperature coefficient for a 10-degree change (Q_{10}) was 2.75 in monochloramine-temperature reactivity studies with poliovirus 1 singles at pH 9 and temperatures of 5, 15, and 25 C. For dichloramine, a 10-degree change in temperature gave a Q_{10} of 2.5 for poliovirus 1 singles. Both Q_{10} values are within the 2 to 3 factor increase used as the rule of thumb for each 10-degree rise in temperature.

Monochloramine formed at pH 9 and then adjusted to pH 7 was a better disinfectant for bacteria but not for the test virus. Lowering the pH from 9 to 7 increased monochloramine disinfection efficiency about 10 times for the bacteria.

Forming monochloramine was about 1.2 times more effective than the preformed monochloramine for disinfecting *E. coli*. The faster disinfection rate could be due to the initial presence of hypochlorous acid before the monochloramine was completely formed.

Resistance to monochloramine developed gradually in single viruses prepared by one of the two methods used. Poliovirus 1 survivors exposed eight times to monochloramine and then disinfected with 8.95 mg/L monochloramine were 2.3 times more resistant to monochloramine than either the virus never exposed to monochloramine or the virus previously exposed seven times to monochloramine.

The presence of HEp-2 and BGM cell-associated turbidity interfered with the cell-associated virus by hypochlorous acid and monochloramine but not by dichloramine.

The solids in human feces and primary effluents protect naturally occurring coliforms from disinfection.

Recommendations

A dramatic increase in monochloramine disinfection efficiency was produced for *E. coli* by lowering the pH of monochloramine from 9 to 7. Since most finished drinking waters are maintained in the United States at a pH below 9, the mechanism of action should be further investigated. In addition, other animal viruses besides poliovirus should be studied to determine whether they are affected by the pH change.

Additional studies are required with different cell lines to determine whether they have similar viral protective effects during disinfection.

Studies should be conducted to determine the cost effectiveness of reducing the turbidity levels from 5 to 1 NTU in drinking water treatment. A reduced turbidity level of 1 NTU is recommended because of our studies and those of others on coliforms associated with primary effluent solids, fecal solids, and cell-associated viruses.

In future turbidity studies, methods for determining the organic or inorganic nature of the particulates must be developed to ascertain their potential for inhibiting disinfection.

Methods should be established for standardizing the apparatus and the methods for disinfection research and for reporting the physical state of the test organisms — that is, viral-associated (aggregates or cell-associated) or unassociated (singles) preparations.

The usefulness of chloramines, especially the monochloramines, in field situations should be more carefully evaluated. Under certain conditions such as forming situations, they may be useful.

Dichloramine's ability to penetrate organic masses such as cells should be more thoroughly investigated.

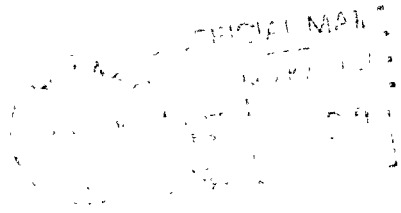
The development of resistant strains of viruses in nature should be thoroughly studied. Laboratory studies can only point out a potential problem; field studies are required to pinpoint possible health risks that might exist in the natural environment.

Pasquale Scarpino is with University of Cincinnati, Cincinnati, OH 45221.
***John C. Hoff** is the EPA Project Officer (see below).*
The complete report, entitled "Effect of Particulates on Disinfection of Enteroviruses in Water by Chloramines," (Order No. PB 84-190 693; Cost: $11.50, subject to change) will be available only from:
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650
The EPA Project Officer can be contacted at:
Municipal Environmental Research Laboratory
U.S. Environmental Protection Agency
Cincinnati, OH 45268

*USGPO: 1984-759-102-10681

United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268



Official Business
Penalty for Private Use $300