INACTIVATION OF HEPATITIS A VIRUS AND OTHER MODEL VIRUSES BY FREE CHLORINE AND MONOCHLORAMINE

by

TAKASHI FUJI

A report submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in Public Health in the Department of Environmental Sciences and Engineering, School of Public Health.

Chapel Hill, 1988

Approved by:

Mark D. Sobsey, Advisor

Donald Johnson

Philip C. Singer

ABSTRACT:

TAKASHI FUJI. Inactivation of Hepatitis A Virus and Other Model Viruses by Free Chlorine and Monochloramine (Under the direction of Dr. Mark D. Sobsey).

The kinetics and extent of inactivation of two strains of Hepatitis A Virus (HAV HM175 and HAV MD1) as well as three other viruses, Coxsackievirus B5 (CB5) and Coliphage MS2 and ØX174, by 0.5 mg/l free chlorine at pH 6.0, 8.0 and 10.0 and by 10 mg/l monochloramine, pH 8.0, at 5°C in 0.01M phosphate buffer were determined. Both strains of HAV were relatively sensitive 0.5 mg/l free chlorine but relatively resistant to 10 mg/l monochloramine. Compared to the HAV strains, CB5 was quite resistant to inactivation by free chlorine but similar in resistance to inactivation by monochloramine. The coliphages were, in general, more sensitive to inactivation by free chlorine than the enteric viruses at pH 6.0 and 8.0. ØX174 was inactivated the most rapidly of all the viruses tested and showed a sensitivity to inactivation at pH 10.0 intermediate between that of HAV and CB5. MS2 was more sensitive to inactivation by free chlorine at pH 6.0 than the HAV strains, but at the higher pH's, was

inactivated at similar or slightly slower rates than that of the HAV. ØX174 was inactivated most rapidly of all viruses tested by 10 mg/l monochloramine, pH 8.0, while MS2 was the most resistant to inactivation under these conditions.

KEY WORDS:
Hepatitis A Virus, Coxsackievirus, Enterovirus, Coliphages, Disinfection, Inactivation, Free Chlorine, Monochloramine, Water.

TABLE OF CONTENTS

1. INTRODUCTION AND OBJECTIVES

In recent years, much attention has been focused on the viral contamination of drinking water and drinking water supplies. This has been the result of studies which have found pathogenic viruses present in supposedly pristine groundwater supplies and in finished drinking water. In addition, several waterborne outbreaks of viral disease, including infectious hepatitis, have emphasized the public health risks associated with such contamination. Of the waterborne enteric viruses, Hepatitis A Virus (HAV) is probably the most important due to the severity of the disease it causes, its persistence in the environment and the high levels with which it is excreted by infected individuals.

New reports have suggested that conventional water treatment practices, generally believed to be effective in producing microbiologically safe drinking water, may be allowing enteric viruses to pass through and that the traditional bacterial indicators of fecal pollution may be more sensitive than the enteric viruses to the same water treatment practices.

Thus there is a clear need for information on the kinetics and extent of pathogenic virus inactivation by water treatment processes to establish reliable water

treatment practices and water quality standards. Unfortunately, such information is unavailable for HAV due to the previous lack of sophisticated laboratory techniques for detecting and quantifying the virus. Recent advances in HAV technology have finally made such research possible.

This research is intended to provide a better understanding of the behavior of monodisperse preparations of HAV in buffered halogen-demand-free (HDF) water when exposed to known concentrations of free chlorine and monochloramine. The specific research objectives are as follows:

1. To determine the kinetics and extent of Hepatitis A virus (strain HM175) inactivation by 0.5 mg/l free chlorine at 5^{o}C over the pH range of pH 6.0 to pH 10.0.

2. To determine the kinetics and extent of HAV HM175 inactivation by 10 mg/l monochloramine at 5^{o}C and at pH 8.0.

3. To compare the inactivation kinetics of HAV HM175 with those of two bacteriophages, MS2 and ØX174, and Coxsackievirus B5.

4. To compare the inactivation kinetics to free chlorine and monochloramine between two different strains of HAV (HM175 and MD1).

2. REVIEW OF THE LITERATURE

The transmission of disease through the ingestion of water contaminated with pathogenic micro-organisms has been well documented throughout history. To prevent such outbreaks, drinking water has been subject to variety of treatment processes. In the United States, one of the earliest methods of treating water involved the addition of chlorine to drinking water to control the agents causing waterborne disease. This began in Jersey City, in 1908, and the practice of chlorine disinfection quickly became widespread (Akin & Hoff, 1984). The use of chlorine to disinfect public water systems had a significant public health impact, resulting in a dramatic reduction in the numbers of deaths reported from traditionally epidemic waterborne diseases in the first third of this century (Olivieri, 1984).

Over the years, new analytical techniques have improved our knowledge of drinking water contaminants and have brought to our attention pollutants and hazards unknown in earlier years. Water treatment technologies have had to advance to cope with these new "forms" of pollution, which have included the discovery of amoebic cysts resistant to chemical disinfection and water pollution by synthetic organic chemicals. Similarly, the recent developments of more sophisticated techniques of detecting and quantifying

enteric virus populations in water have focused attention on these agents as potential public health hazards in drinking water. Due to different methodologies involved in the attempts to isolate viruses from drinking water samples, there are contradictory results in the literature over the presence and significance of viruses in finished drinking water supplies.

In 1980, Sekla and co-workers found enteric viruses in finished drinking water samples in Manitoba, Canada (Sekla et. al., 1980). Payment, in 1985, undertook a larger survey and found enteroviruses in the finished water of five of seven drinking water treatment plants in Montreal, Canada (Payment et. al., 1985). Enteric viruses have also been found in water samples from South Africa (Nupen, 1976), Israel (Bitton et. al., 1986) and Great Britain (Bitton et. al., 1986) but there have been questions about the methodology used for virus isolation and whether the water treatment plants in these studies were operating correctly. In the United States, an EPA study of 54 community water systems across the country failed to find any virus in the finished drinking water (Bitton et. al., 1986) but viruses have been isolated from marginally treated drinking water (Hejkal et. al.,1982) and in fecally contaminated groundwater supplies (Sobsey et. al., 1985). In addition, several recent outbreaks of waterborne viral disease, including a 1980 epidemic in Georgetown Texas involving 36 cases of Hepatitis

A and 8000 cases of acute gastrointestinal illness of unknown etiology (Hejkal et. al., 1982), have been reported. These reports have emphasized both the risks associated with enteric virus contamination of drinking water and the need for further research on the behavior of these viruses in water and wastewater treatment processes.

2.1 ENTERIC VIRUSES

Enteric viruses are those that enter the body via the oral route and infect the alimentary tract. These viruses multiply in the gut and are shed in the feces in concentrations as high as 10^{10} infectious particles per gram. It is estimated that there are over 100 enteric viruses known to infect humans.

The enteric viruses encompass a large population of viruses with the most important groups being the Enteroviruses, Norwalk Virus, Rotavirus, Reovirus, Parvovirus, Adenovirus and other small, round "structured" gastrointestinal viruses. These viruses are responsible for a whole array of diseases in humans ranging from respiratory infections and nervous system infections to gastroenteritis disease.

The most studied group of enteric viruses are the enteroviruses belonging to the family Picornaviridae. These include Polioviruses, Coxsackieviruses A+B, Echoviruses and

Hepatitis A Virus. Enteroviruses are characteristically acid stable and are resistant to all known antibiotics, chemotheraupeutic agents and to organic solvents such as ether, chloroform and freon (Frosner, 1984; Melnick, 1985).

The mode of transmission of infections by enteroviruses is primarily through person-to-person contact via the fecal-oral route. The viruses can also be transmitted by ingesting fecally contaminated food or water. Waterborne transmission of viral disease have been documented for Norwalk virus, Norwalk-like viruses, Hepatitis A Virus Rotaviruses and possibly poliovirus.(Melnick, 1985).

2.2 Hepatitis A Virus

Hepatitis A or infectious hepatitis is one of several diseases grouped under the term viral hepatitis. Viral hepatitis can be caused by a variety of different agents with the only common feature being that the liver is the main target organ and the site of viral replication. Of the diseases linked with viral hepatitis, Hepatitis A is the most prevalent form, with approximately 30,000 cases per year reported in the U.S. (Hollinger & Melnick, 1985; Frosner, 1984).

Until recently, attempts to identify and characterize the causative agents of infectious hepatitis were hampered by a lack of sophisticated laboratory techniques to detect and

quantify the virus. In fact, early research on the causative agent of infectious hepatitis (Neefe et. al. 1945) depended upon the use of human volunteers to determine the presence or absence of the yet unknown agent.

Research into the physical and biological aspects of Hepatitis A Virus (HAV) became possible after Feinstone, in 1973, first visualized the viral particles in the stools of hepatitis A patients using immune electron microscopy (Feinstone et. al., 1973). Further information became available when HAV was first cultivated in cell culture in 1979 (Provost & Hilleman, 1979). The lack of any visible cytopathic effects and the late appearance of viral antigens after infection, by most strains of HAV, have still made detection and enumeration difficult (Frosner, 1984).

The recent work on HAV has led to the development of a number of serological and cell culture techniques to detect and enumerate HAV. These new techniques include the RadioImmunoFocus Assay (RIFA) which is the primary method used in this research to assay for HAV (Lemon et. al.,1983). In addition, a new rapidly replicating isolate of strain HM175 from persistently infected, serially passaged cell cultures has been found to produce cytopathic effects. This has lead to the development of a plaque assay for this strain of HAV and this new strain should allow for a more rapid assay procedure for HAV. Studies are currently underway in our lab to determine whether this new strain

behaves in a similar manner with established laboratory strains of HAV (Cromeans et. al., 1987).

Morphologically, HAV is a non-enveloped 27-28 nm icosahedral virion with 32 capsomers on its surface. HAV has now been classified as a member of the Genus Enterovirus within the family Picornaviridae and has been designated Enterovirus 72 (Frosner, 1984).

The HAV virion has a buoyant density in cesium chloride (CsCl) of 1.32 to 1.35 g/cm^3 and a sedimentation of 156 to 160S in a neutral sucrose solution. HAV isolated from human fecal specimens have shown both lighter and heavier populations with different sedimentation rates in addition to the mature virions (Bradley et. al., 1975; Feinstone et. al., 1974; Siegel & Fisher, 1978). The lighter portions band at a buoyant density of 1.29 to 1.31 g/cm^3 and are believed to represent empty, premature virions (Bradley et. al. 1975). The dense forms have buoyant densities of 1.40 to 1.48 g/cm^3 and these heavier virions have been shown to be morphologically similar and exhibit the same major surface antigens as the common virion (Dienstag et. al., 1976). It is thought that the higher density results from the penetration and binding of cesium cations to a more open conformation of the viral capsid (Hollinger, 1985).

The HAV genome contains single stranded RNA with 8,000 to 8,100 nucleotides and has positive polarity. The RNA codes for four major polypeptides: VP1 (30,000 - 33,000 daltons);

VP2 (24,000 -26,000 daltons); VP3 (21,000 - 23,000 daltons) and VP4 (7,000 - 14,000 daltons) and a minor polypeptide VPG which is attached to the 5' end of the viral RNA (Coulepis et. al., 1980).

HAV has been particularily singled out as a public health hazard due to the severity of its infection and its demonstrated persistence in the environment. Recent studies in our lab have shown that HAV can remain infectious for long periods of time in water and wastewater effluent. For example, studies have shown the T99% value (time necessary to achieve two log reduction in original virus concentration) for HAV to be : 8 weeks for groundwater, 12 weeks for primary effluent, 16 weeks for secondary effluent, and 3 to 4 weeks for seawater (Sobsey et. al., 1986).

2.3 Coxsackieviruses

Coxsackievirus are classified as enteroviruses and are non-enveloped, 28nm, icosahedral shaped virions. The viruses contain single-stranded, positive polarity RNA and the virions show a sedimentation of 153S in neutral sucrose. The Coxsackievirus are divided into two groups and are differentiated on the basis of the target tissue. Group A, with 23 serotypes, causes primarily striated muscle damage while Group B, with 6 serotypes, attacks primarily fatty tissue and central nervous tissue. Diseases attributed to Coxsackievirus group B include pleurodynia, aseptic meningitis, paralysis and severe systemic illness in newborns (Joklik, 1985). Coxsackie B5 was chosen as one of the model viruses in this study because disinfection experiments have shown that it is the most resistant of the enteric viruses to inactivation by chlorine (Engelbrecht et. al., 1980; Payment et. al., 1984).

2.4 Bacteriophage MS2

MS2 is an RNA bacteriophage and is classified within the family Leviviridae. Morphologically, MS2 is an icosahedral particle of 24nm diameter, lacking a tail or other surface features and has a sedimentation of 80 - 82S. The MS2

genome contains a single-stranded 3569-nucleotide long RNA molecule which codes for three proteins; the "A" protein or maturation protein, the coat protein and the viral replication protein (Fraenkel-Conrat, 1974). MS2, as are all RNA phages, are specific to male E. coli hosts because the phage receptor sites are on the f-pili of the E. coli (Joklik, 1985). MS2 was chosen as one of the test viruses because of its potential as a possible alternative indicator for enteric viruses.

2.5 Bacteriophage ØX 174

ØX174 is the most studied of the small isometric DNA phages. All these phages are quite similar and are serologically related. ØX174 differs from the other three viruses used in this study in that it contains single-stranded, circular DNA in its genome. The DNA molecule contains 5375 nucleotides which code for ten separate proteins; three are structural proteins, four are involved in DNA synthesis; one protein causes cell lysis while the function of the remaining protein remains unclear (Joklik, 1985).

Morphologically, ØX174 has an icosahedral shape with spikes protruding from the virion at the twelve vertices of the icosahedral. The particle has a diameter of 25nm if the

spikes are not included and has a buoyant density in CsCl of 1.43 g/ml (Fraenkel-Conrat, 1974; Denhardt, 1977).

2.6 Virus Contamination of the Environment

Viruses in the environment are a hazard due to the risk of human infection through close recreational contact with contaminated water, consumption of contaminated food and drinking tainted water. Throughout the world, viral contamination of water is very common. The contamination occurs when human fecal material, which may contain upwards of 10^{10} Infectious Units (IU) /g, are discharged directly or indirectly into the environment. In developing countries, the lack of adequate methods of disposing human wastes contributes greatly to the pollution of surface and groundwaters. The contamination may occur as a result of direct discharge of sewage into receiving waters and soils or from non-point sources such as run-off from inadequate land disposal of sewage.

Virus contamination of water is not only limited to developing countries. Surface water supplies in developed countries can become contaminated through improper or inadequate methods of disposing human wastes. Because in this country, the treatment given to wastewaters varies greatly from plant to plant, the quality of the effluents leaving these plants can also vary. Raw municipal

wastewater may contain virus concentrations ranging from 7 x 10^3 Plaque Forming Units (PFU)/L of sewage to 10^5/L (Bitton, 1980). Bitton in a review of over 150 studies, found that primary treatment of sewage does not provide effective removal of viruses and that the chlorination of sewage effluent does not guarantee a sewage-free effluent. It was also reported that biological treatment processes vary in their virus removal efficiencies with the activated sludge treatment showing the greatest reduction in viruses. The advanced treatment processes, however, were found to lead to significant reductions in virus concentrations (Bitton et. al., 1986). In another survey, Block found that 56 to 58% of chlorinated effluents, completing secondary treatment, contained viruses (Block, 1983).

In most cases effluents from wastewater plants are discharged directly into receiving waters, posing a potential threat to any downstream users of the water. Other sources of viral contamination of surface waters include the direct dumping of raw sewage into marine waters, run-off from land application of sludge, and leachate from septic tanks.

Recent studies have indicated that groundwater, generally considered well protected, has been subject to viral contamination from septic tank and cesspool overflows, groundwater recharge with wastewater effluent and from land application of sludge (Keswick & Gerba, 1980). The extent of viral contamination of groundwater is difficult to ascertain

due to the lack of sensitive detection measures. Viruses that have been isolated from groundwater include Echovirus, poliovirus, Rotavirus and Coxsackie B virus (Keswick & Gerba, 1980).

2.7 Indicators of Fecal Contamination

The detection of fecally contaminated water is of great public health concern due to the risks associated with consumption of and contact with polluted water. Because there are a large number of pathogenic micro-organisms potentially present in fecally polluted water, the specific identification of each type of bacteria or virus species would require a large number of samples and a wide variety of media and methods. Obviously, such an effort would be time consuming and prohibitively expensive. Therefore, regulatory agencies have adopted a program of using model micro-organisms to serve as indicators of fecal pollution. This has provided an inexpensive and rapid method for routinely testing drinking and recreational waters as well as wastewater.

In the United States, the traditional indicators of fecal pollution consist of a test for two groups of bacteria, the total coliforms and fecal coliforms. These groups are defined by the parameters of the methods used to isolate them. The first group, total coliforms, comprises all of

the aerobic and facultative anaerobic, gram negative, non-spore forming, rod shaped bacteria that ferment lactose with gas production within 48 hours at 35°C. The second group, which more accurately reflects the presence of fecal material from warm blooded animals are termed "fecal coliforms". The fecal coliforms are those coliforms that can ferment lactose at 44.5°C (Bitton, 1980).

An indicator can only perform its function accurately if it can satisfy certain criteria based upon the behavior and characteristics of the pathogen that it is supposed to model. Stetler proposed a set of eight criteria for an ideal microbial indicator of pathogenic organisms. These are: (1) An indicator should always be present when the pathogen is present and absent when the pathogen is absent. (2) The persistence and growth characteristics of both indicator and pathogen should be similar. (3) The pathogen and indicator should occur in a constant ratio so that counts of the indicator give a good estimate of the number of pathogens present. (4) Preferably, the indicator should be present in the source of pollution at levels far in excess of the pathogen concentration. (5) The indicator should be resistant to the environment and disinfectants at the same rate as pathogens. (6) The indicator should be non-pathogenic and easily quantifiable. (7) The test for the indicator should be applicable to all types of water. (8) The test should detect only the indicator organism and should not give false-positive reactions (Stetler, 1984).

Recent water borne outbreaks of viral disease and the reports of the isolation of viruses from finished drinking water samples have begun to cast doubt on the suitability of these bacterial indicators to accurately predict the behavior of enteric viruses in water and wastewater treatment processes. Studies have shown that coliform bacteria are more sensitive to inactivation by commonly used disinfectants and by water and wastewater treatment techniques than enteric viruses (Berg et. al., 1978; Grabow, 1983; Stetler, 1984; Havelaar & Nieuwstad, 1985). In addition, enteric viruses have been isolated from groundwater (Keswick & Gerba, 1980), sea water (Gerba et. al., 1979), ocean sludge dump sites (Goyall et. al., 1984), chlorinated primary wastewater effluent (Berg, 1978), and drinking water (Bitton et.al., 1986) which have been found to be free of total and fecal coliforms.

A possible alternative to the total and fecal coliforms which has shown promise in modeling the behavior of enteric viruses are the bacteriophages. There has been much discussion on the potential of using the phages as enteric virus indicators because they satisfy the following criteria: (1) The bacteriophages occur in high concentrations in fecally polluted water and wastewater and in higher concentrations than animal viruses (Bitton, 1980), (2) The bacteriophages are present whenever coliforms are present
(Kott et. al., 1974), (3) Human enteric viruses are absent

when phages are absent (4) The bacteriophages can be isolated from wastewater without concentration procedures and the assay for the phages are relatively inexpensive and rapid, with results becoming available within 24 hours. The bacteriophages are also biologically and morphologically similar to enteric viruses and should be expected to behave similarly in an aquatic environment (Funderberg & Sorber, 1985).

Research comparing the behavior of phages and viruses in water and wastewater treatment processes have produced favorable, though somewhat contradictory, results. Grabow (1984) found that the bacteriophage MS2 was more resistant than enteric viruses HAV and CB5 to exposure to chlorine which contained chlorine predominantly in the combined forms, while in studies conducted with free chlorine residuals, MS2 was alternatively more resistant and more sensitive than HAV depending on the pH of the test water.

Stetler (1984) monitored the behavior of coliphages, indicator bacteria, and enteroviruses in a drinking water plant. It was found that coliphages and enteroviruses behaved in similar patterns in the source water and in treated water before chlorination (which caused the total removal of all detectable viruses) and that enteroviruses could be more strongly correlated with coliphage counts than those of the traditional indicator bacteria.

A study testing the suitability of using coliphages as indicators of enteric viruses during activated sludge

treatment of domestic wastewater, done by Funderberg and Sorber (1985), revealed that counts of total endogenous coliphages could not be correlated with those of enteric viruses, however, a certain population (those forming plaques of > 3mm) were found to be highly correlated.

In this study, we will use two specific strains of phages in our controlled chlorine experiments to determine whether either strain can be correlated with the inactivation rates of the enteric viruses.

2.8 WATER AND WASTEWATER TREATMENT

To provide virus free drinking water, two seperate strategies must be pursued. Clearly, one would be to implement water treatment practices that can be expected to remove virus loads even under the worst case situations. The other is to protect the surface and groundwater sources of drinking water from becoming grossly contaminated with pathogenic viruses. Thus the treatment regimen given to both water and drinking water are vitally important to ensure biologically safe drinking water. Unfortunately, there is much evidence suggesting that conventional sewage treatment technologies are not completely effective at removing viruses from wastewater and that even conventional water treatment processes can allow viruses to pass into drinking water. In the following sections of this paper, I

will discuss the major treatment processes in use for water and wastewater and their respective abilities to reduce virus concentrations.

2.8.1 WASTEWATER TREATMENT

(1) Primary Treatment:

Primary treatment of wastewater, a process that includes screening, grit removal and primary sedimentation has been shown to be ineffective in removing viruses from wastewater with typical virus removal efficiencies of only 5 to 10% (Leong, 1983). In this process, the removal of viruses are largely dependant upon the efficient separation of solids as the viruses will readily adsorb to solids present in the raw sewage. With adequate sedimentation, virus removal may be increased up to 88% (Bitton, 1980).

It should be noted that solids associated viruses are still infectious and that the sludge removed from the sedimentaion tanks will contain 10 - 100 times the virus concentration of the raw sewage. Therefore the ultimate fate of these viruses will depend upon the methods used for sludge disposal.

(2) Secondary Treatment:

The two common forms of secondary treatment are activated sludge treatment and trickling filters. Virus removal by trickling filters is generally low and inconsistent. One study on the RNA phage f2 showed a removal rate of less than

20% by trickling filters and a cumulative viral reduction of less than 95% when trickling filters were used with clarification and chlorination (Bitton, 1980).

Activated sludge treatment is more effective at removing enteric viruses with removal efficiencies ranging from 0-99% with the median percent removal being 94%. In this process, virus removal can be attributed to both microbial mediated inactivation and adsorption. It has been suggested that longer contact times would result in a greater percentage of inactivation (Leong, 1983).

(3) Tertiary Treatment:

Tertiary treatment consists of a series of steps designed to further reduce pathogenic microbes, heavy metals, nutrients, trace organics, suspended solids and turbidity from wastewaters. Generally the techniques for advanced treatment are identical to those used in water treatment and will be discussed in greater detail in subsequent sections of this paper.

To summarize, coagulation-sedimentation with alum or lime and sand filtration can achieve from 2 to 4 log reduction in virus concentration if they are properly managed. The use of activated carbon has shown variable removal rates for viruses depending upon the length of time the carbon has been in service and the dissolved organic load of the water. Under optimal operating conditions, this process can remove an additional 90% of viruses (Leong, 1983).

(4) Sludge Treatment:

Due to the adsorption of viruses to sewage solids during primary and secondary treatment, the sludges produced by conventional wastewater treatment contains a very high level of viruses. Typically, sludges are digested and dewatered prior to disposal.

Sludges are digested to stabilize the organic matter prior to disposal. Unfortunately, there is very little information on the virus removal effectiveness of full-scale digesters. It appears that temperature and detention time are the most important factors affecting virus removal. In lab studies, 5 log reductions in viruses were obtained in 14 days at 32°C, while at 35°C, the same reduction took four days. However, full scale digesters rarely achieve the efficiency of lab scale models. In one study, viruses were found in 53% of samples of digested sludge (20 days at 35°C). Since most digesters work by continuous addition and removal of sludge, the retention time is variable and this increases the chance that the sludge will contain appreciable concentrations of viruses after digestion (Bitton, 1980).

Both raw and digested sludges are dewatered before ultimate disposal. The dewatering can be accomplished by open-air drying or by several mechanical methods. Of these methods, open air drying is the more effective method for reducing virus concentration. The rate of virus

inactivation in drying beds is directly related to drying time and the weather conditions. Inactivation can be as effective as 2 log reduction per week in Texas summer to as slow as 1 log reduction per month in Danish winters (Feachem et. al.,1983).

The mechanical methods of sludge dewatering are very ineffective at virus inactivation as the viruses are never exposed to conditions such as heat or dessication necessary to inactivate viruses.

(5) Disinfection:

The disinfection of secondary and tertiary effluents from wastewater plants is a common practice in some developed countries. The most widely used disinfectants for this purpose are free chlorine and combined chlorine. Unfortunately, the most effective viricidal properties of free chlorine are not utilized because the high nitrogenous content in the effluent results in its being quickly converted to combined chlorine, which is a much weaker viricide. In addition, the viruses may also be adsorbed to any solids still present in the effluent, which gives an added measure of protection.

It is clear that the effectiveness of the disinfection process is dependent upon the quality of the effluent prior to chlorination. It is possible to produce virus free effluent in well operated wastewater plants, but disinfection does not guarantee a virus free effluent.

Investigators have found from 10^1-10^3 enteroviruses/L in chlorinated effluents from plants around the country (Feachem et. al., 1983).

2.8.2 WATER TREATMENT:

The purpose of water treatment plants is to provide potable water that is both chemically and biologically safe for human consumption. The water is subject to a variety of treatments depending on the source and quality of the raw water. The treatments range from no treatment or just chlorination for groundwater sources, to coagulation sedimentation followed by filtration for shallow wells and surface water sources. Some processes include a water softening step when the source water contains high concentrations of Ca+ and Mg+.

For the most part, virus reduction in water treatment processes relies on two functions. The physical removal of the viruses by adsorption, coagulation, precipitation and filtration and the inactivation of the virus by the addition of disinfectant or exposure to high pH as in a water softening plant.

Water treatment by coagulation/flocculation followed by sedimentation has been shown to be very effective at removing viruses. Laboratory bench studies indicate that there is little difference among the coagulants used, with median virus removal efficiences of 99.5% for ferric, 98.8% for lime and 95% for alum. The proper design and operation

of the treatment facility is necessary to maintain optimum removal of viruses (Leong, 1983).

The use of rapid sand filters has a varied effectiveness in the case of virus reduction. Sand particles, in and of themselves, are poor adsorbants of viruses. Therefore, the removal of viruses by sand filtration alone is low and inconsistent (Bitton, 1980). But, if the water is subject to coagulation-flocculation, allowing the viruses to adsorb unto the flocs and the filter grains, the sand filtration is very effective and can achieve virus reductions of greater than 99%. Again proper maintenance and operation are necessary for effective virus reduction.

(1) WATER SOFTENING PROCESSES:

Water softening by the lime-soda ash process has been shown to be very effective in reducing and inactivating virus concentrations. The virus reduction is achieved by the physical removal of the virus as they are adsorbed to the $CaCO_3$ and $Mg(OH)_2$. In addition, the virus is inactivated by the high pH conditions generated by the process and virus reduction of greater than 99.9% have been achieved (Bitton, 1980).

2.9 DISINFECTION OF WATER BY CHLORINE

2.9.1 History:

Chlorine is one the most widely distributed elements on the earth. Due to its extremely reactive nature, it is not found in its free state in the environment, rather it exists mainly in combination with sodium, potassium, calcium and magnesium. Chlorine in its elemental state is a corrosive gas of yellow-green color (Dychdala, 1983).

The disinfectant properties of chlorine were not recognized nor used until the late 1700's. One of the earliest known uses of chlorine as a disinfectant was in 1791 when chlorine gas was used as a fumigant in hospitals (Sykes, 1965). It was not until a hundred years later that the use of chlorine in water treatment became common. The use of chlorine in water treatment was introduced here in the US in 1908 and the use increased tremendously over short period of time (Dychdala, 1983).

Chlorine is used in water and wastewater treatment as a disinfectant to destroy organisms present in the water that cause diseases in man. The disinfectant properties of chlorine result from its strong oxidizing powers and its high solubility in water. In addition, its relatively low cost, ease of application, and the availability of reliable detection methods have resulted in its almost universal use for potable water disinfection. In water and wastewater

treatment, it is most commonly applied as a gas, $Cl_2(g)$, generated from the vaporization of liquid chlorine under pressure (Snoeyink & Jenkins, 1980).

Chlorine gas is soluble in water and hydrolyzes rapidly to form hypochlorous acid:

$$Cl_2 + H_2O = HOCl + H^+ + Cl^-$$

At pH values and concentrations normally seen in water and wastewater treatment, this hydrolysis goes virtually to completion.

The hypochlorous acid can further disassociate following this reaction to generate hypochlorite ion:

$$HOCl = H^+ + OCl^-$$

At 25°C, this reaction has a pKa of 7.5, therefore at pH 7.5, the concentration of HOCl and OCL^- are equal. At pH levels below 7.5, HOCl predominates and at pH levels above 7.5 OCl^- is the dominant form.

Another common reaction of chlorine is with organic nitrogen and ammonia to form chloramines and includes these following stepwise reactions:

$$HOCl + NH3 \rightarrow H_2O + NH_2Cl \text{ (Monochloramine)}$$

$$HOCl + NH_2Cl \rightarrow H_2O + NHCl_2 \text{ (Dichloramine)}$$

$$HOCl + NHCl_2 \rightarrow H_2O + NCl_3 \text{ (Trichloramine)}$$

These reactions are governed primarily by pH and Chlorine-to-Nitrogen ratios. In general, as the pH of the water decreases and the Cl_2:N ratio increases, the ammonia molecules becomes successively more chlorinated.

Monochloramines are rapidly produced in the pH range pH 7.0-9.0 and most readily when the Cl_2:N is $\leq$ 5:1 by weight. Dichloramines are produced at lower pH's and higher Cl_2:N ratios than monochloramine, with optimum pH in the range of pH 4.4-6 and Cl_2:N ratios of 5:1 to 7.6:1 . Trichloramine formation occurs only at very low pH, pH < 4.4, or at very high Cl_2:N ratios, $\geq$7.6:1 (Wolfe et. al., 1984). Therefore under typical conditions found in water and wastewater treatment facilities, monochloramine is the predominant form of combined chlorine that is found.

In recent years, there has been a resurgance of interest in and the use of chloramines as an alternative disinfectant to free chlorine. This has been brought about by the discovery of high levels of trihalomethanes (THM), such as chloroform, in water disinfected by free chlorine. Chloramine disinfection has the advantage of producing insignificant quantities of THM's and has been shown to be more stable than free chlorine in the distribution system (Wolfe at. al., 1984; Stachia & Pontius, 1984). However, caution must be excercised in relying solely on chloramines as a disinfectant as they are known to be much less effective bactericides and viricides than free chlorine.

2.9.2 INACTIVATION OF VIRUSES:

The disinfectant properties of chlorine are based upon its strong oxidizing properties and it inactivates bacteria by attacking protein components of the bacteria and destroying the enzymatic processes necessary for life. In viruses, it is believed that the Cl_2 attacks either the protein coat or the nucleic acid components of viruses, rendering them non-infectious (Viessman & Hammer, 1985).

There are several environmental factors which have significant effects upon the disinfectant ability of chlorine. Of these, the factor of greatest influence is pH, which has a direct effect upon the speciation of chlorine. Other factors that are important are temperature, concentration of chlorine, and concentration of organic materials in the water.

The reason pH greatly infuences the effectiveness of chlorine as a disinfectant is because pH determines the amount of hypochlorous acid and hypochlorite ion in solution, with hypochlorous acid being a much stronger disinfectant than hypochlorite ion. As would be expected, an increase in pH substantially decreases the biocidal activity of chlorine in H_20. Research has shown that HOCl is 80 times more effective than OCl^- in the inactivation of E. Coli and about 150 times more effective for cysts of Entamoeba histolytica (Lippy, 1986).

Engelbrecht (1980) showed that the disinfectant effectiveness of the different chlorine species towards viruses were HOCL > OCl^- > Chloramines and that sensitivity of viruses to chlorine changed with the species of chlorine present.

The concentration of chlorine is important only as long as the other factors, such as pH, temperature and organic content are held constant. As would be expected, an increase in concentration will increase the disinfectant properties of chlorine.

The disinfection with chlorine results from a chemical reaction of chlorine with the protein components of micro-organisms. Therefore, an increase in temperature, which would increase the rate of reaction will also increase the rate of inactivation of micro-organisms.

Nitogenous materials in the water are important because they can react with the chlorine and consume the available chlorine or convert it into less effective species such as chloramines.

2.9.3 DISINFECTION OF HAV BY CHLORINE

One of the first studies on the disinfection ability of chlorine on HAV was conducted in 1945 by Neefe (1945). In this study, human volunteers were given water specimens containing dilute stool samples known to contain the agent of infectious hepatitis and the development of hepatitis A by the volunteers was used to determine the infectivity of

the specimens after chlorine treatment. Neefe found that the specimens treated with sufficient chlorine to provide a residual of 1 ppm after 30 minutes of contact time did not inactivate the virus, whereas the contaminated water treated with sufficient chlorine to provide a residual of 15 ppm after 30 minutes did inactivate the virus.

The development of tissue culture methods of maintaining HAV and new techniques for enumerating the virus has spurred new interest in disinfection experiments with HAV.

Peterson et. al. (1983) conducted a study in which partially purified preparations of HAV from the feces of prodomal chimpanzees were inoculated intramuscularly into adult marmosets to test for HAV infectivity. The preparations were treated for various time periods with 0.5, 1.0, 1.5, 2.0, and 2.5 mg/l of free residual chlorine (HOCl at pH 7.0). The results showed samples treated with 0.5, 1.0 and 1.5 mg/l free chlorine induced hepatitis in 14% (2 of 14), 8% (1 of 12), and 10% (1 of 10) of marmosets respectively, and induced seroconversions in 29%, 33%, and 10% of the animals, respectively. The preparation treated with 2.0 and 2.5 mg/l free chlorine did not induce hepatitis or seroconversions in any of the animals tested. It was therefore concluded that HAV is somewhat more resistant to free chlorine than other enteroviruses.

In contrast, Grabow (1983 and 1984) concluded from a series of experiments that HAV was very sensitive to disinfection by free chlorine. In 1983, Grabow, compared

the inactivation of HAV to various indicator organisms in water by free chlorine. The mixture of organisms were exposed to total chlorine concentrations of less than 1 mg/l and contained various concentrations of free vs. combined chlorine. The pH levels tested were 6, 8, 10.

The results show that HAV was more sensitive to a mixture of free and combined chlorine at all pH levels than Poliovirus 2, and MS2 coliphage but more resistant than Simian Rotavirus SA-11. In all cases, extrapolated 4 log reduction of HAV occurred before 6 minutes of contact time. It is interesting to note that in this study, the results did not show a great increase in resistance to chlorine as the pH was increased, for many of the viruses, and it also showed that the comparative sensitivity of viruses to chlorine changes with changing pH.

In a second study, Grabow (1984) compared the inactivation of HAV and other indicator organisms in autoclaved biofilter effluent and broth-enriched tap water by chloramines. The experiments were conducted at approximately pH 8.0 with combined chlorine at the concentrations of 11.8 mg/l and 27.0 mg/l for tap water and biofilter effluent respectively. This study found that chloramines were much weaker disinfectants than free chlorine and that HAV survived much longer than Poliovirus, E. coli and SA-11.

The inconsistent results between Grabow's and Peterson's work may be the result of the different methodologies used

in their disinfection experiment. Peterson's study used partially purified HAV preparations with no attempt to separate out the aggregated forms from the monodisperse virus. In addition, the virus preparations were never titered but were reported only as the number of 50% marmoset infectious doses per ml (MID_{50}/ml). Peterson's use of marmosets as indicators of infection must also be questioned, as the variable sensitivity among individual marmosets to infection, by HAV, is unknown.

Grabow is unclear in his papers as to the extent of virus purification of his samples. It appears that no effort was made to separate the aggregates from the monodisperse viruses. In addition, in the free chlorine experiments, the chlorine doses Grabow used, contained not only free chlorine, but significant amounts of monochloramine and dichloramine, with proportions of greater than 50% combined chlorine in some cases. Chlorine analysis after 15 minutes of contact time also revealed that, in almost all cases, whatever free chlorine that was initially added was quickly converted to the combined forms, making it uncertain which form of chlorine was responsible for the inactivation observed.

Because of the limited data on HAV in general and the conflicting findings of the few studies on HAV disinfection, no clear picture of the inactivation kinetics of HAV to disinfection by free and combined chlorine has emerged. The purpose of this present study is to evaluate monodisperse

HAV inactivation by free and combined chlorine under carefully controlled experimental conditions. Recently proposed drinking water regulations calling for mandatory disinfection of all municipal water supplies has made the need for definitive information on HAV disinfection all the more urgent. This particular study will be part of an overall project to provide this information.

3. MATERIALS AND METHODS:

3.1 Purification of Enteric Viruses

3.1.1 Hepatitis A Virus:

The HM-175 (NIH Prototype) strain of HAV, originally isolated from the feces of an infected human in Australia (Daemer et. al. 1981), is cultured and assayed in a continuous cell line derived from Primary African Green Monkey Kidney cells (BSC-1). The virus is harvested and passaged every 2-4 weeks in persistently infected BSC-1 cells grown in 850 cm^2 Roller Bottles or 6000 cm^2, ten-tiered cell factories (NUNC). At each harvest, two separate pools of HAV containing materials are collected: the cell culture medium and a crude virus stock containing persistently infected cells. The persistently infected cells are subject to low speed centrifugation (3,000 x g) and the pellet of infected cells is resuspended in equal volumes of Phosphate Buffered Saline (PBS) and chloroform. The mixture is homogenized for 1 to 2 minutes (Vertis Homogenizer, Sorvall Inc.) and another low speed centrifugation is done on the sample to pellet the cell debris and the chloroform and the resulting PBS supernatant containing HAV is recovered. The HAV in the cell culture medium is concentrated by precipitation with Polyethylene Glycol 6000 and is chloroform extracted. The crude HAV

stocks from the extracted cells and tissue culture medium are pooled and ultra-centrifuged at 70,500x g, for 4 hours, 5°C, using a type 35 fixed angle rotor in a Beckman Model LS-40 ultracentrifuge to pellet the virus.

In preparation for a Cesium-Chloride (CsCl) density gradient, the pellet of HAV is resuspended in 2ml of 0.01M Phosphate-Buffered Chlorine Demand-Free water. To the virus sample, 0.47 g/ml of CsCl is added to give a density of 1.33 g/ml, which is then ultracentrifuged at 25,000 RPM (90,000 x g), 5°C for three days using the SW27.1 swinging bucket rotor in the Beckman Model LS-40 ultracentrifuge. The gradient fractions (0.7 ml each) are collected from the bottom of the tube and assayed by RIFA for infectivity. The fractions containing the highest titer of HAV are pooled and desalted using the Centricon 30 Ultrafilter Tubes (Amicon, Inc.).

For rate zonal ultracentrifugation, the desalted fractions are layered upon a 10% - 30% sucrose gradient and spun at 25,000 RPM (90,000 x g) for 5 hrs and 25 min. The gradient is collected from the top in 0.7 ml fractions and the fractions are again assayed for infectivity by RIFA. The fractions containing single virions are pooled and stored at 4°C until use.

The MD1 strain of HAV, originally isolated from drinking wells of a small,rural community in Washington County, Maryland during an outbreak of Hepatitis A,

(Sobsey et. al.1985) was purified with the same process as that described for HM-175.

3.1.2 Coxsackievirus B5:

Coxsackievirus B5 (Faulkner Strain) is cultured and assayed by the plaque technique in the BGMK (African Green-monkey Kidney Derived) continuous cell line. The CB5 was initially plaque purified and then inoculated into 690 cm^2 Roller Bottles of BGMK cells to prepare a large stock of virus. The Roller Bottles were infected at a low multiplicity of infection, approximately 0.1 PFU/cell, and the viruses harvested when 75% - 100% of the cells showed virus induced cytopathic effects. The stock was then subjected to three freeze thaw cycles to liberate the virus and to disperse aggregates. The preparation was then spun at low speed (10,000 x g) for 30 min. to remove cellular debris. To the resulting supernatant, Sodium Dodecyl Sulfate (SDS) was added to make a 0.1% solution and the solution was then spun at 25,000 RPM (4 hours at $4^{o}C$) using a type 35 Rotor and Beckman LS-40 ultracentrifuge to pellet the virus.

The pellet was resuspended in 2ml of PBS, homogenized (Vertis Homogenizer, Sorvall Inc.) for 1 min., and the preparation was centrifuged at 5,000 RPM, $5^{o}C$, for 30 min. to precipitate and pellet the SDS. As with the HAV, CsCl was added to the preparation and the CB5 was concentrated in a density gradient as described in the previous section.

The fractions were collected and assayed and the high titer fractions were pooled and desalted with Centricon 30 Ultrafiltration tubes. Again, as for HAV, the desalted fractions were layered upon a 10 - 30% sucrose gradient and ultracentrifuged for 5 hours and 25 min. The gradient fractions were collected and assayed and the fractions containing single virions were pooled and stored at 4°C until use.

3.1.3 Bacteriophages:

Bacteriophages MS2 (ATCC 15597-B1) and ØX174 (ATCC 13706-B1) are grown and assayed by the top agar plaque technique (Adams, 1959) in E. coli C3000 (ATCC 15597) and E. coli (ATCC 13706) respectively. A large stock of each bacteriophage was collected by scraping the top agar off of plaque assay plates showing confluent lysis into small volumes of PBS. This crude stock was chloroform extracted and spun at low speed (5,000 x g) for 10 min. to remove cell debris, chloroform and agar. The resultant supernatant was then spun at 10,000 x g for 10 min. to remove additional cell debris and at 90,000 x g for 4 hours to pellet the phage. The pellets were resuspended in PBS and subjected to a CsCl density gradient having a density of 1.44 - 1.45 g/ml. As before, the fractions were collected and assayed for peak infectivity and the peak fractions were desalted with a Centricon 30 ultrafilter. To achieve a stock of single virions, the desalted fractions were

successively filtered through 0.2 and 0.08 um pore size polycarbonate filters (Nuclepore) pretreated with 0.1% Tween 80. The single virion stocks were not prepared by rate-zonal ultracentrifugation in sucrose gradients because the bacteriophages lost their infectivity in sucrose solutions. The filtered phage stocks were then stored at 4°C until use.

3.2 Preparation of Chlorine Demand Free Water, Glassware and Reagents

The chlorine demand free water used in these experiments is prepared from twice deionized, activated carbon-filtered water which is passed through a macroreticular scavenging resin bed (Dracor Co.). Studies done in our lab have shown that this water is of the same quality as or better than water prepared by the protocol described in Standard Methods (A.P.H.A., 1985).

All buffers used in the disinfection experiments were made up as 10X stocks (0.1M Phosphate Buffers) in CDF water, chlorinated by adding Clorox (Sodium Hypochlorite), then allowed to sit overnight and dechlorinated by exposure to U.V. light for at least twelve hours. The stock buffers were diluted ten-fold in CDF water to working concentration of 0.01M on the day of the experiment. All other reagents used in the experiments were also made up with CDF water.

The glassware used in the disinfection experiments was made CDF by soaking in a strong chlorine solution (10 - 50 mg/l) for at least 4 hours and then rinsing at least four times with CDF water. The rinsed glassware was then wrapped in aluminum foil and baked for at least two hours at 200°C to dry the glassware and volatilize any remaining chlorine.

3.3 Preparation of Chlorine Solutions

3.3.1 Free Chlorine:

A working stock of approximately 100 mg/l Cl_2 was prepared on the day of the experiments by diluting an appropriate amount of household bleach (5.25% Sodium Hypochlorite;Clorox) in CDF water. This working stock is then diluted to the concentration necessary to generate the standard curve for Cl_2 analysis and to give the target experimental concentration of 0.5 mg/l Cl_2.

3.3.2 Monochloramine

A working stock of 100 mg/l monochloramine was prepared by mixing ,on a weight to weight ratio, 4 mg of ammonia to 1 mg of chlorine (as HOCl) as described by Berman and Hoff (1984). In moles, this gives nearly a 10:1 ratio of NH_3 to Cl^-.

The stock, made the day of the experiment, was prepared by adding 0.37 ml Clorox to 100 ml 0.01M, pH 9.5 CDF buffer and 0.081g of NH_4Cl to 100 ml, 0.01M, pH 9.5 CDF buffer. These

two mixtures were combined slowly, while being mixed, to generate a 100 mg/l working stock of monochloramine.

3.4 Chemical Analysis:

In all experiments, the concentration of free chlorine and monochloramine was determine by the N,N,Diethyl-P-Phenylenediamine (DPD) colorimetric method described in Standard Methods. Before each experimental run, the spectrophotometer was calibrated for free chlorine or monochloramine analysis by generating a standard curve by the DPD Ferrous Titrimetric Method. The reliability of the chemical analysis for free chlorine was checked before each experimental run by analysis of chlorine standards prepared by the U.S. EPA.

3.5 Protocol for Disinfection Experiments

For the disinfection experiments, the purified virus stocks containing single virions of HAV HM175, CB5, MS2, and 0X174 are diluted and pooled in CDF PBS so that the titer of each virus in the mixture is approximately $1 - 5 \times 10^6$ infectious units/ml. A separate stock of HAV MD1 at the same titer is prepared for the HAV strain comparison experiments.

Prior to the start of each experiment, a stock solution of 0.01M phosphate buffer at the appropriate pH is prepared at the target chlorine concentration of 0.51 mg/l. In each experiment, three 16mm diameter x 150mm long glass test tubes are used: a reaction tube containing virus and disinfectant in buffer, a virus control tube containing virus in buffer and a chlorine control tube containing disinfectant in buffer.

11.76ml of chlorine in buffer and buffered water are added to the reaction and virus control tubes, respectively, and 12ml of chlorine in buffer is added to the chlorine control tube. These tubes are then placed in a circulating water bath set at $5^{o}C$ for 15 minutes prior to starting the experiment.

At the start of the experiment, 0.24ml of the virus mixture is added to the virus control and reaction tubes and each tube is quickly vortexed to mix. Samples of 0.7ml are taken from the reaction tube at 0.33, 1.0, 3.0, 10.0, 30.0, and 60.0 minutes and these samples are immediately neutralized by being added to 0.7ml of virus diluent (2X Eagle's MEM) containing 1% sterile Sodium Thiosulfate. 0.7ml samples from the virus control tube are withdrawn at time "0" and at time 60 min. and similarly added to 0.7ml, 1% sodium thiosulfate. At the end of the experiment, the remaining virus mixture in the reaction tube and the chlorine control sample are analyzed for the presence of free and combined chlorine.

To assay for the viruses, the experimental samples are diluted five-fold in appropriate virus diluent for HAV, CB5, and bacteriophages to give an initial ten-fold dilution, overall, from the reaction tube. Subsequent serial ten-fold dilutions are made and the dilutions are assayed in triplicate cultures of host cells. The assays were done usually within a week after the completion of the experiment. The samples were stored at $4^{o}C$ until assay.

3.6 Data Analysis

For each experiment, the results were compiled to represent the proportion of the initial virus remaining at each time point and the time necessary to achieve a 4 $\log_{10}$ reduction was calculated. The triplicate cell culture plates for each assay were averaged and taken as the concentration of each virus remaining at the time point (N_t). The virus concentration in the control tube at time "0" min. was taken as N_o. The proportion of the initial virus remaining at each time point was calculated by dividing the concentration of virus at each time point (N_t) by the initial virus concentration (N_o). The N_t/N_o values for each virus were averaged for the duplicate (in some cases triplicate) experiments at the same pH and chlorine concentration. These values were then transformed to log values ($\log_{10} N_t/N_o$) and then plotted versus time for each

pH condition. From these inactivation curves, the time necessary to achieve a two $\log_{10}$ reduction in original virus titer (T99%) was directly extrapolated.

To compare the inactivation curves of the different viruses the analysis of covariance methodology was used to fit separate regression lines for each virus at the different pH's and the time required to produce a four $\log_{10}$ reduction (T99.99%) in original virus titer was calculated using this parameter. On three occasions, ØX174 at pH 6.0 and pH 8.0 free chlorine and HAV MD1 at pH 8.0 Free chlorine, there were not enough data points to perform a linear regression. In these cases, a line was drawn from the origin (0,0) to the first data point and the T99.99% was directly extrapolated from this line. The T99.99% values are summarized in Table 2 and these values were used as a measure of the sensitivity of the virus to disinfection by free chlorine and monochloramine. The higher the T99.99% value, the more resistant the virus is to inactivation by the disinfectant.

To compare the sensitivity of the different viruses at each pH and disinfectant concentration and to compare the inactivation rates of each virus among the pH's and two types of disinfectants, the slopes and intercepts of the regression lines produced by the inactivation data were compared to test for statistically significant differences (p-value < 0.05) between the lines. Table 1 and 2, in Appendix II, gives a summary of the parameters of each

regression line used. The steeper the slope of the regression line, the more sensitive the virus is to disinfection by the particular disinfectant at that pH. The results of the statistical tests among the different viruses at each pH are presented in Table 3 and those tests comparing different pH's for each virus are presented in Table 4.

4. RESULTS

4.1 OVERVIEW

The results of the disinfection experiments using 0.5 mg/l free chlorine and 10 mg/l monochloramine, at 5°C, are presented in Tables 1 and 2 and Figures 1-8. In Table 1, I have presented the time necessary to achieve two log reduction in original virus titer (T99%) for all the viruses at the pH's tested. Table 2, contains the T99.99% values, or the time necessary to achieve a four log reduction in original virus titer. In Tables 1 and 2, the CT (concentration X time) values are presented in parentheses below the T99% and T99.99% values. As the starting concentrations of free chlorine and monochloramine were the same for all the experiments, the CT values are just multiples of the T99% and T99.99% values. The trends of virus inactivation among the different viruses and among the different pH's tested are the same for two log reduction and for four log reduction, therefore I will limit my discussion only to the four log reduction times. This will avoid repetition in the discussion section and as the new proposed EPA drinking water standards are for four log reduction of viruses, this is the more appropriate parameter to discuss under these conditions.

In Appendix I, I have presented the raw data from the experiments. The tables contain the titer of the virus remaining at each time point and in the two virus control samples. The titer of the viruses are given as the calculated Plaque Forming Units (PFU)/ml for CB5 and the bacteriophages and as Radio-Immunofocus Forming Units (RFU)/ml for the HAV strains. These titers are the average of triplicate experiments for the free chlorine studies and duplicate experiments for the monochloramine studies. The virus titer data was manipulated to give the form log Nt/No which is a measure of the percent of the original virus concentration remaing at each time point. In addition, the range of starting free chlorine and monochloramine concentrations and the range of starting pH's are presented in these tables.

The inactivation curves for the viruses (figures 1-8) were then drawn by plotting the log Nt/No versus contact time and are grouped by pH and disinfectant with separate graphs showing the comparison between the two diffrent strains of HAV. On many of the figures, the final point on the inactivation curves are marked as "limit of detection" points and are not true data points. The limit of detection point is an indication of the sensitivity of the virus assay based upon the least dilute sample (10^{-1}), the sample inoculum volume per dish (0.3 ml/dish) and the number of replicate dishes per samples (3 dishes) and the true data point is less than this calculated value. Due to the nature

of the "limit of detection" points, these values were not used in the statistical analysis of the inactivation curves described below. The error bars on the inactivation curves represent the range of log Nt/No values for the duplicate or triplicate experiments carried out at each pH and type of disinfectant.

4.2 pH 6.0

Figures 1 and 2 show the the response of the viruses tested to 0.5 mg/l free chlorine at 5°C pH 6.0. All of the viruses were inactivated very rapidly under these conditions with no virus being detected after the three minute time point. The two bacteriophages, ØX174 and MS2 were the most sensitive to disinfection with T99.99% values of 0.5 and 1.2 minutes respectively. Because the ØX174 bacteriophage was completely inactivated before the 1 minute time point, no statistical comparison can be made with the ØX174 data and the other viruses.

In comparison, the enteric viruses appeared somewhat more resistant than the phages with CB5 being the most resistant

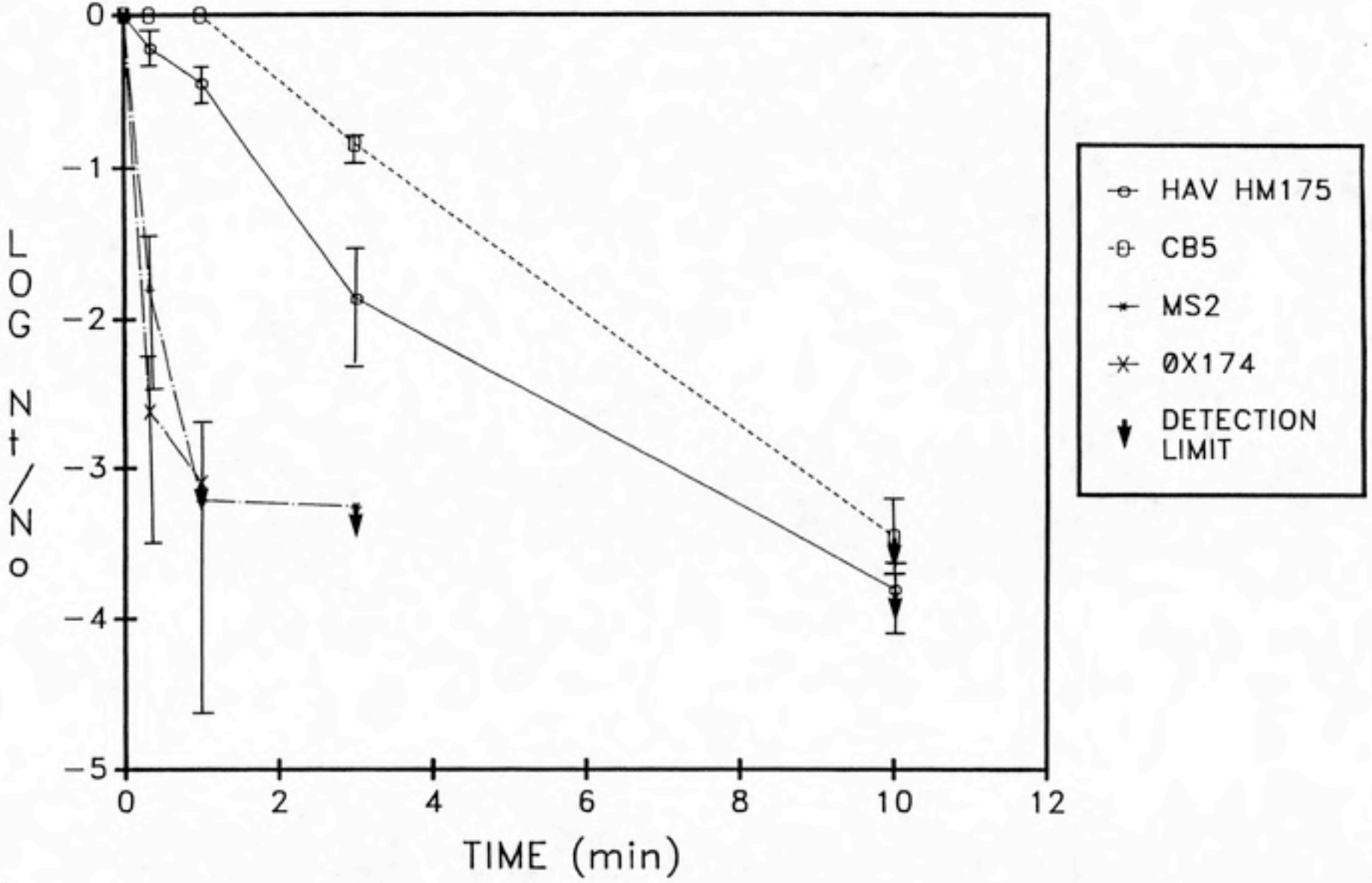

Fig. 1: INACTIVATION OF HAV HM175, CB5, MS2, AND ØX174 BY 0.5 mg/l FREE CHLORINE, pH 6.0, AND 5 C.

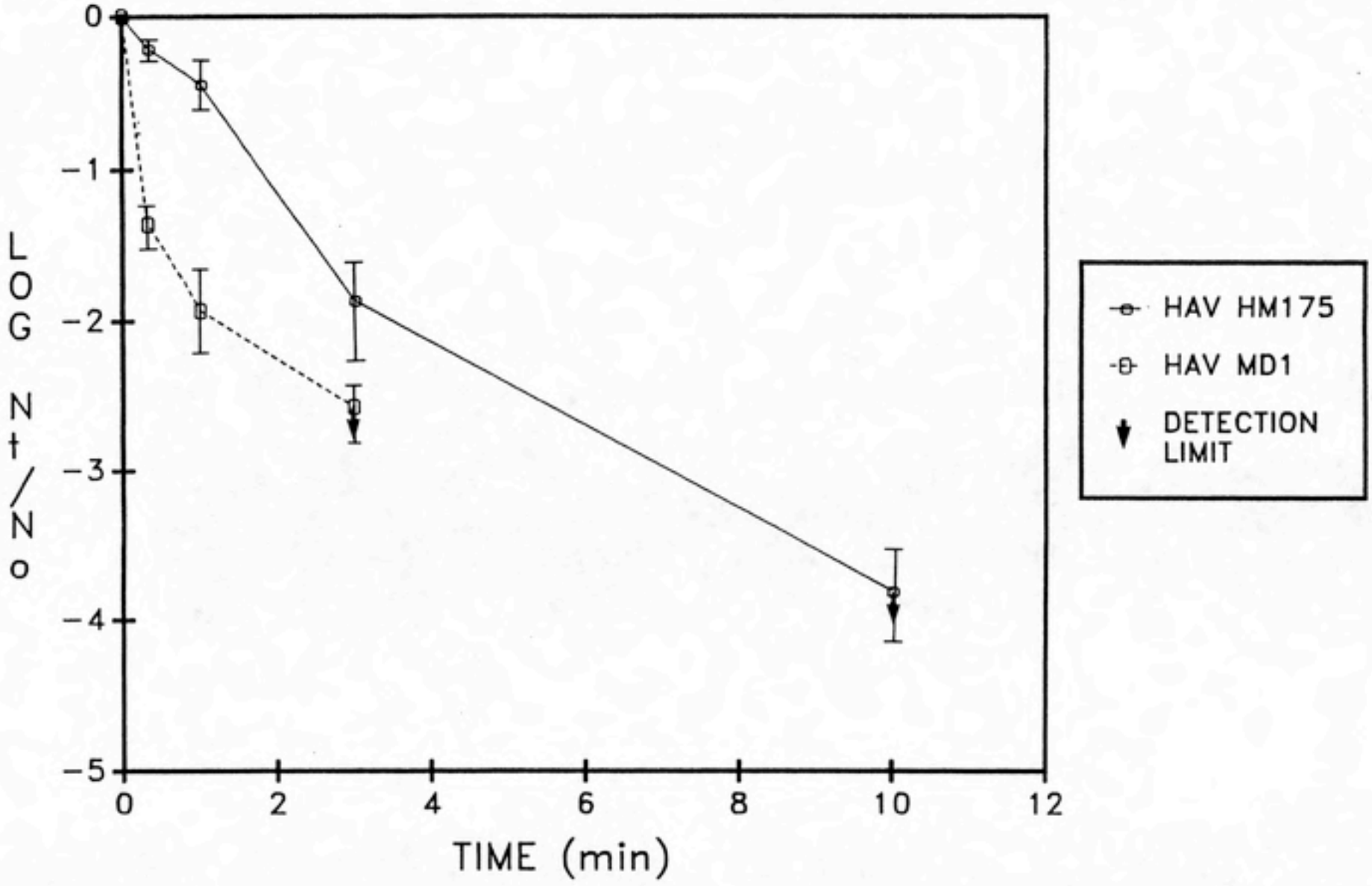

Fig. 2: INACTIVATION OF HAV HM175 AND HAV MD1 BY 0.5 mg/l FREE CHLORINE, pH 6.0, AND 5 C.

showing a T99.99% value of 13.18 minutes. The two strains of HAV were inactivated more quickly than CB5 with T99.99% values of 6.51 minutes for HAV HM175 and 2.08 minutes for HAV MD1. Statistical analysis showed no significant difference ($p=0.07$) between the responses of HAV HM175 and HAV MD1.

4.3 pH 8.0

Figures 3 and 4 show the response of the viruses tested to 0.5 mg/l free chlorine at 5°C pH 8.0. All of the virus types, except for CB5, were again inactivated relatively quickly with Bacteriophage ØX 174 and HAV MD1 being the most sensitive to disinfection with T99.99% values of 0.77 minutes and 0.68 minutes respectively. These viruses were followed in sensitivity by HAV HM 175 (T99.99%= 5.56 minutes) and MS2 (T99.99%= 16.66minutes). As was the case at pH 6.0, the enteric virus CB5 was the most resistant to disinfection by free chlorine with a T99.99% value of 57.50 minutes and was significantly more resitant than HAV HM175 ($p=0.03$) and bacteriophage MS2 ($p<0.01$). The inactivation of ØX174 and HAV MD1 occurred so rapidly that there was not sufficient data points to perform any statistical analysis.

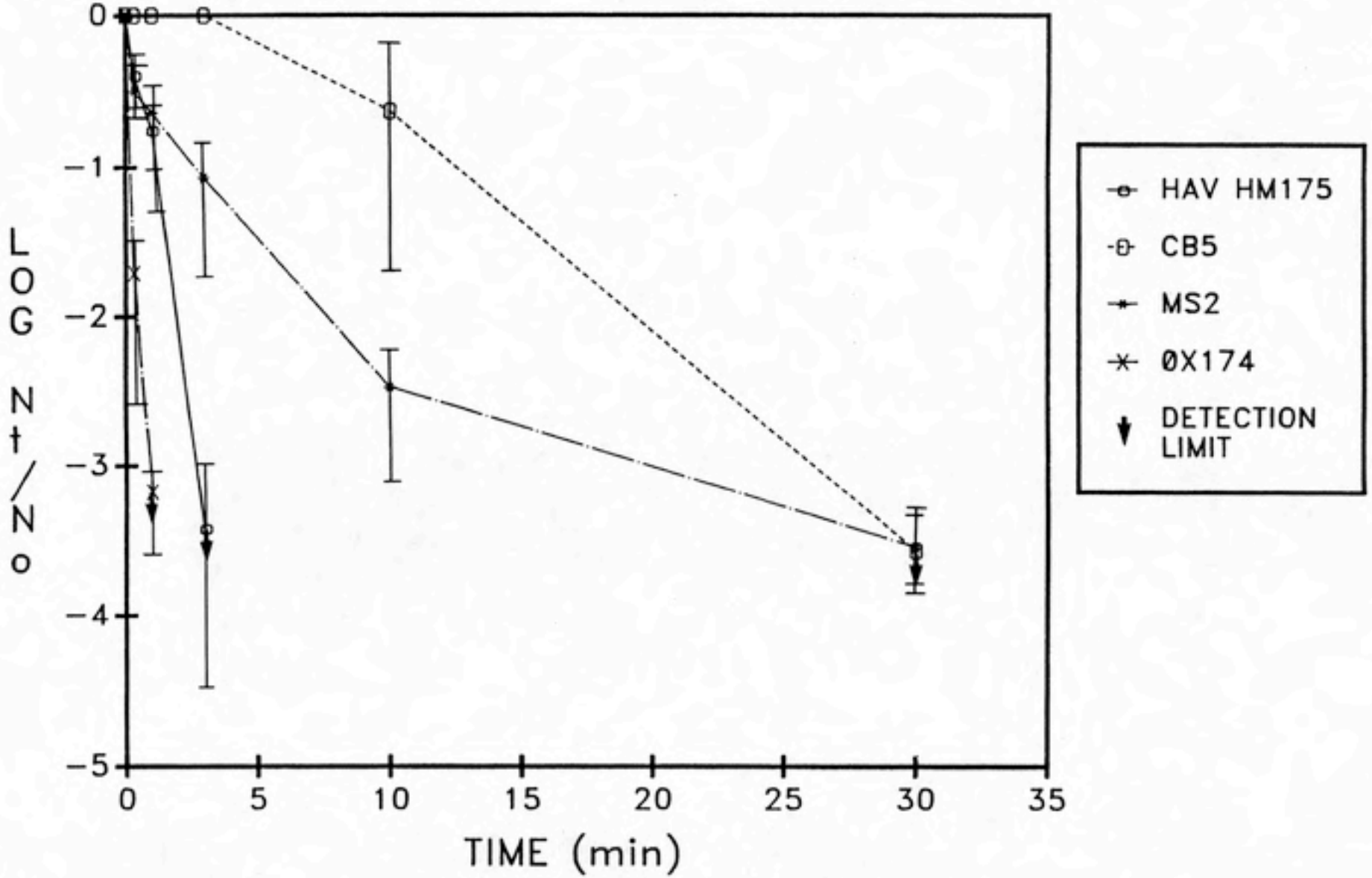

Fig. 3: INACTIVATION OF HAV HM175, CB5. MS2, AND ØX174 BY 0.5 mg/l FREE CHLORINE, pH 8.0, AND 5 C.

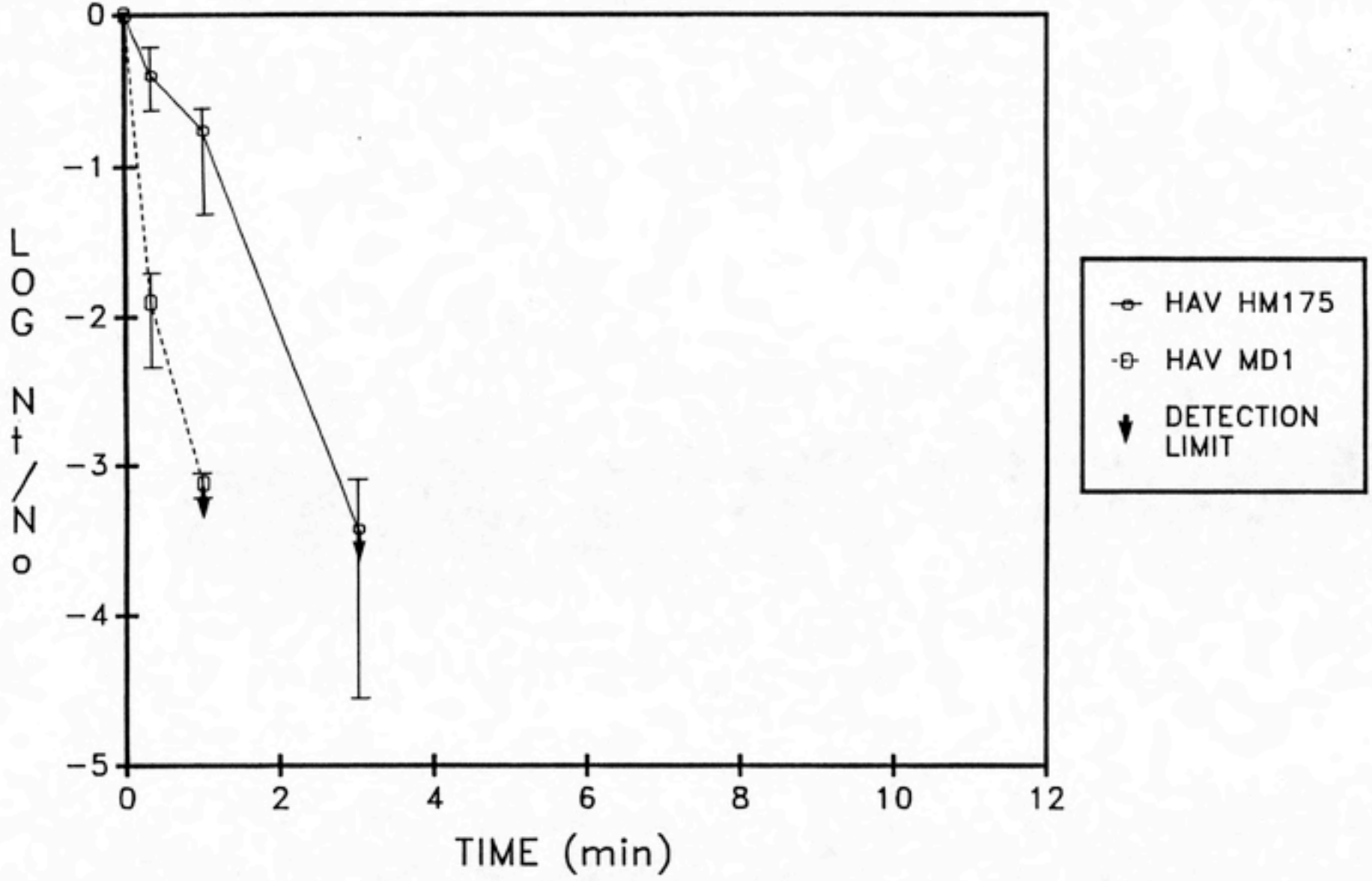

Fig. 4: INACTIVATION OF HAV HM175 AND HAV MD1 BY 0.5 mg/l FREE CHLORINE, pH 8.0, AND 5 C.

4.4 pH 10.0

Figures 5 and 6 show the response of the viruses tested to 0.5 mg/l free chlorine at 5°C, pH 10.0. All of the viruses were more resistant to disinfection at this pH than at pH 6.0 or pH 8.0. At this pH, the HAV strain MD1 and the bacteriophage MS2 were the most sensitive viruses to disinfection, showing very similar inactivation rates ($p=0.18$) and T99.99% values The calculated T99.99% values were 22.33 minutes for HAV MD1 and 26.54 minutes for Bacteriophage MS2. HAV strain HM175 was the next most sensitive virus (T99.99%=49.56 minutes) followed by bacteriophage ØX174 (T99.99%=118.18minutes). Again the enteric virus CB5 was the most resistant virus type, showing less than 1 log reduction after 60 minutes of contact time with free chlorine (T99.99%=825 minutes). A comparison of the inactivation rates of the two strains of HAV showed that there was a significant difference ($p=<0.01$) between the two. The two bacteriophages also showed a significant difference ($p<0.01$) betwwen their response to disinfection by free chlorine at pH 10.0.

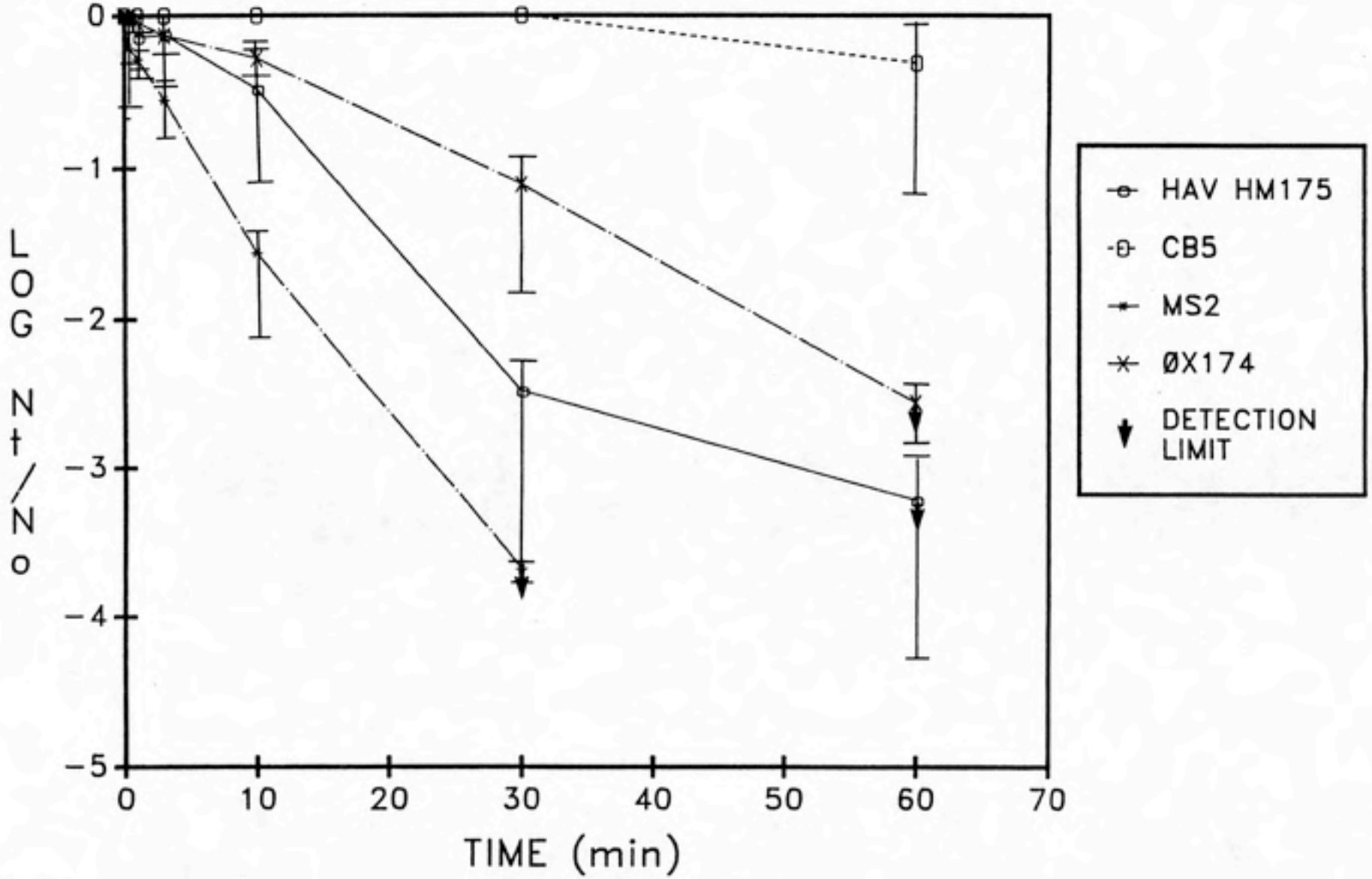

Fig. 5: INACTIVATION OF HAV HM175, CB5, MS2, AND ØX174 BY 0.5 mg/l FREE CHLORINE, pH 10.0, AND 5 C.

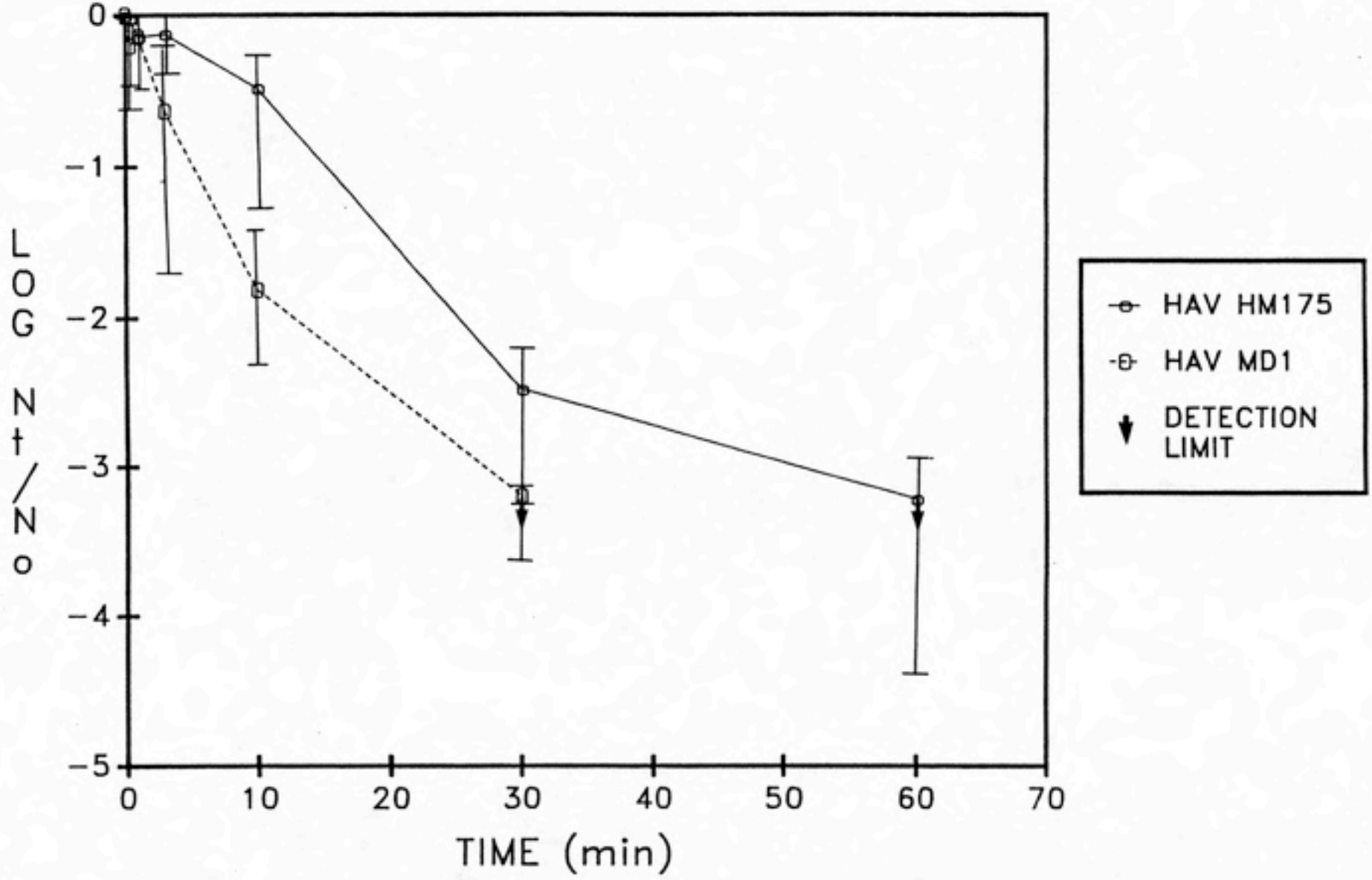

Fig. 6: INACTIVATION OF HAV HM175 AND HAV MD1 BY 0.5 mg/l FREE CHLORINE, pH 10.0, AND 5 C.

3.5 Monochloramine pH 8.0

The response of the viruses to 10 mg/l monochloramine at 5°C, pH 8.0 are presented in Figures 7 and 8. In general the viruses were very resistant to inactivation by 10 mg/l monochloramine, pH 8.0, and with the exception of Bacteriophage ØX174, were detected in the reaction tubes after 60 minutes of contact time. In contrast to the free chlorine experiments, CB5 was the most sensitive to inactivation of the enteric viruses, with a T99.99 value of 104 minutes, followed by HAV HM175 (T99.99%=117 minutes) and HAV MD1 (T99.99%=262.64 minutes). The bacteriophage MS2 was the most resistant to inactivation by monochloramine (T99.99%=420 minutes) showing less than 1 log reduction after 60 minutes of contact time while bacteriophage ØX174 was the most sensitive of the viruses tested (T99.99%=31.39 minutes). The inactivation rates of the two HAV strains were found to be similar ($p=0.11$) while those of the Bacteriophages were significantly different ($p<0.01$).

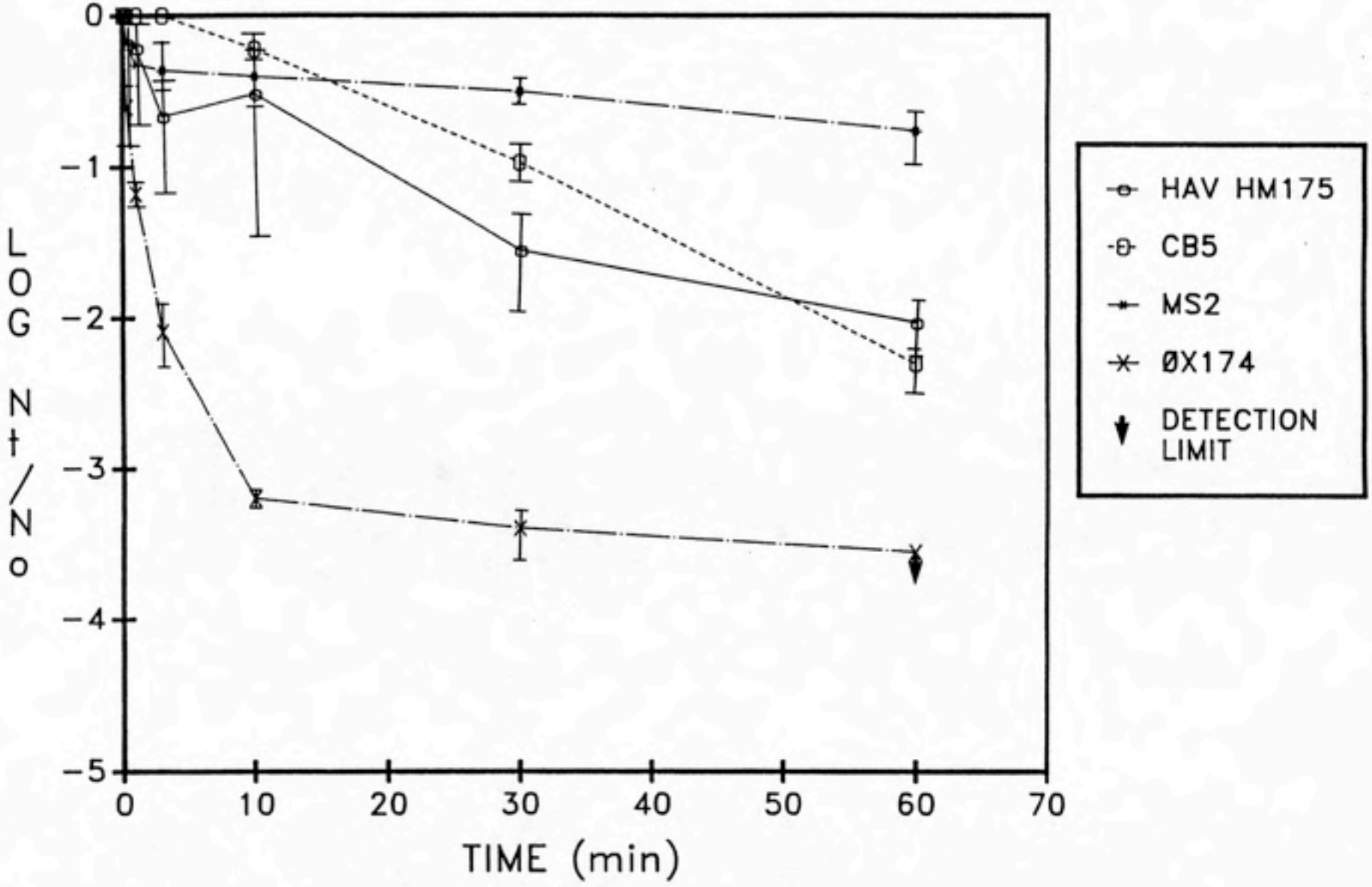

Fig. 7: INACTIVATION OF HAV HM175, CB5, MS2, AND ØX174 BY 10 mg/l MONOCHLORAMINE, pH 8.0, AND 5 C.

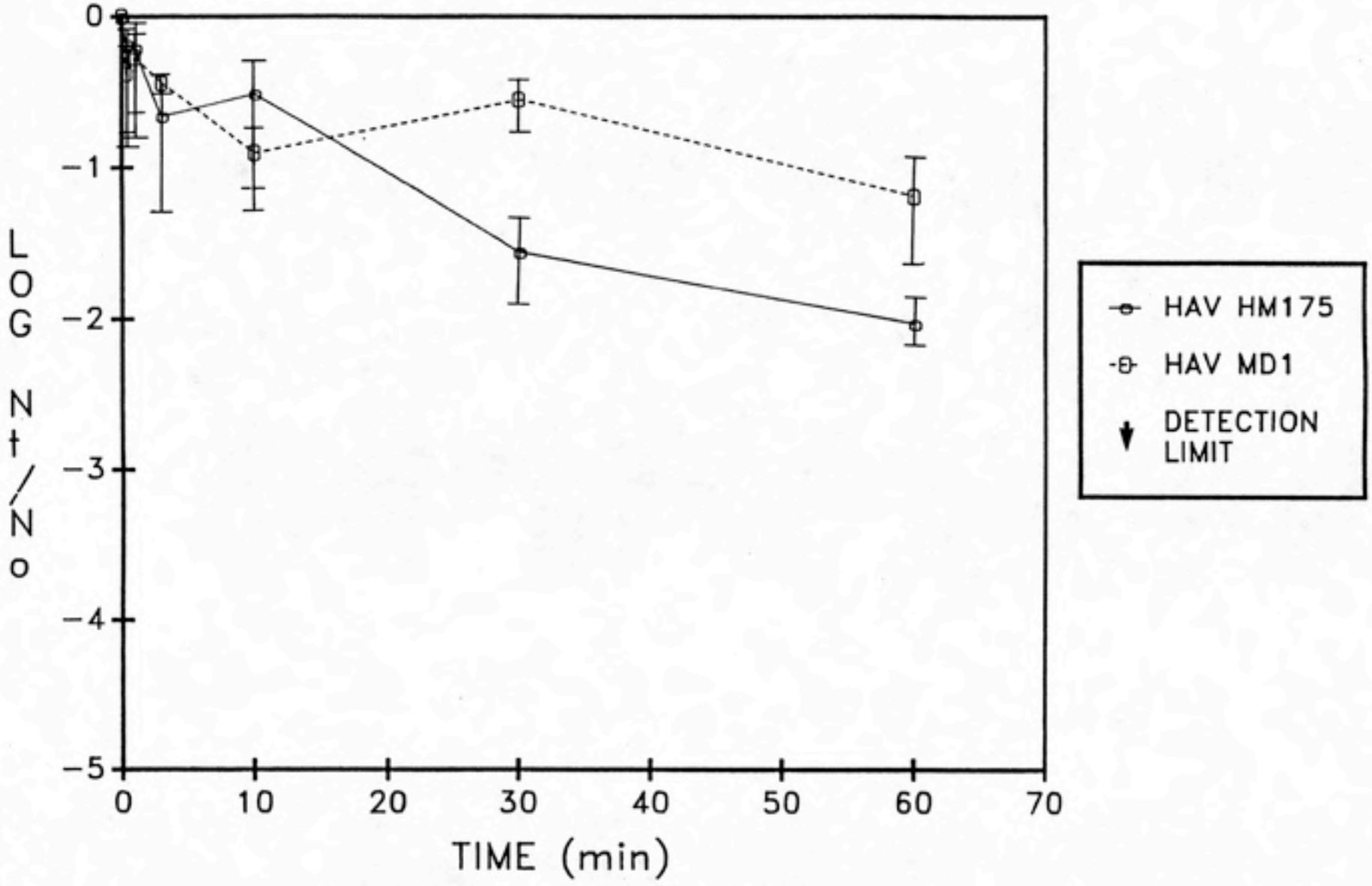

Fig. 8: INACTIVATION OF HAV HM175 AND HAV MD1 BY 10 mg/l MONOCHLORAMINE, pH 8.0, AND 5 C.

TABLE 1

INACTIVATION OF HAV HM175, HAV MD1, COXSACKIE B5, MS2, AND ØX174 BY 0.5 mg/l FREE CHLORINE AT pH 6.0, 8.0, 10.0 and 5 C AND BY 10 mg/l MONOCHLORAMINE AT pH 8.0 and 5 C IN BUFFERED, DEMAND FREE WATER. NUMBERS IN PARENTHESIS INDICATE CT VALUES.

	pH	MINUTES FOR 99% INACTIVATION HAV HM175	HAV MD1	CB5	ØX174	MS2
FREE CHLORINE	6.0	3.59 (1.79)	1.16 (0.58)	6.15 (3.07)	0.36 (0.18)	0.51 (0.25)
	8.0	2.13 (1.06)	0.52 (0.26)	19.04 (9.52)	0.42 (0.21)	7.55 (3.77)
	10.0	24.58 (12.29)	12.08 (6.04)	>>60	47.08 (23.54)	13.75 (6.87)
MONO-CHLORAMINE	8.0	59.17 (29.58)	>60	53.33 (26.66)	3.33 (1.66)	>>60

TABLE 2

INACTIVATION OF HAV HM175, HAV MD1, COXSACKIE B5, MS2, AND ØX174 BY 0.5 mg/l FREE CHLORINE AT pH 6.0, 8.0, 10.0 and 5 C AND BY 10 mg/l MONOCHLORAMINE AT pH 8.0 and 5 C IN BUFFERED, DEMAND FREE WATER. NUMBERS IN PARENTHESIS INDICATE THE CT VALUES.

	pH	MINUTES FOR 99.99% INACTIVATION				
		HAV HM175	HAV MD1	CB5	ØX174	MS2
FREE CHLORINE	6.0	6.50 (3.25)	2.08 (1.04)	13.20 (6.60)	0.50 (0.25)	1.20 (0.60)
	8.0	5.56 (2.78)	0.68 (0.34)	52.50 (26.25)	0.77 (0.38)	16.66 (8.33)
	10.0	49.56 (24.78)	22.33 (11.16)	825.60 (412.80)	111.28 (55.64)	26.54 (13.27)
MONO-CHLORAMINE	8.0	117.16 (58.58)	262.64 (131.32)	104.15 (52.07)	31.39 (15.69)	419.89 (209.94)

TABLE 3

COMPARISON OF THE INACTIVATION RATES AMONG THE DIFFERENT VIRUS TYPES AT EACH pH.

DISINFECTANT	pH	TEST	p-VALUE	
FREE CHLORINE 0.5 mg/l	6.0	HAV HM175 vs. HAV MD1	0.07	*
	6.0	HAV HM175 vs. CB5	0.22	*
	6.0	HAV MD1 vs. CB5	0.03	
	6.0	HAV HM175 vs. MS2	<0.01	
	6.0	HAV MD1 vs. MS2	0.12	*
	6.0	CB5 vs. MS2	<0.01	
FREE CHLORINE 0.5 mg/l	8.0	HAV HM175 vs. CB5	.03	
	8.0	HAV HM175 vs. MS2	.08	*
	8.0	CB5 vs. MS2	<0.01	
FREE CHLORINE 0.5 mg/l	10.0	HAV HM175 vs. HAV MD1	<.01	
	10.0	HAV HM175 vs. CB5	<.01	
	10.0	HAV MD1 vs. CB5	<.01	
	10.0	HAV HM175 vs. MS2	<.01	
	10.0	HAV MD1 vs. MS2	.18	*
	10.0	HAV HM175 vs. ØX174	<.01	
	10.0	HAV MD1 vs. ØX174	<.01	
	10.0	CB5 vs. MS2	<.01	
	10.0	CB5 vs. ØX174	<.01	
	10.0	MS2 vs. ØX174	<.01	
MONO-CHLORAMINE 10 mg/l	8.0	HAV HM175 vs. HAV MD1	.11	*
	8.0	HAV HM175 vs. CB5	.47	*
	8.0	HAV MD1 vs. CB5	.02	
	8.0	HAV HM175 vs. MS2	.05	*
	8.0	HAV MD1 vs. MS2	.67	*
	8.0	HAV HM175 vs. ØX174	<.01	
	8.0	HAV MD1 vs. ØX174	<.01	
	8.0	CB5 vs. MS2	.01	
	8.0	CB5 vs. ØX174	.01	
	8.0	MS2 vs. ØX174	<.01	

TABLE 4
COMPARISON OF THE INACTIVATION RATES BETWEEN THE pH's AND CHLORINE SPECIES FOR EACH VIRUS TYPE.

VIRUS	TEST	p-VALUE	
HAV HM175	pH 6.0 vs. pH 8.0	.74	*
HAV HM175	pH 6.0 vs. pH 10.0	<.01	
HAV HM175	pH 8.0 vs. pH 10.0	<.01	
HAV HM175	pH 6.0 vs. pH 8.0 NH_2Cl	.04	
HAV HM175	pH 8.0 vs. pH 8.0 NH_2Cl	.02	
HAV HM175	pH 10.0 vs. pH 8.0 NH_2Cl	<.01	
HAV MD1	pH 6.0 vs. pH 10.0	<.01	
HAV MD1	pH 6.0 vs. pH 8.0 NH_2Cl	<.01	
HAV MD1	pH 10.0 vs. pH 8.0 NH_2Cl	<.01	
CB5	pH 6.0 vs. pH 8.0	<.01	
CB5	pH 6.0 vs. pH 10.0	<.01	
CB5	pH 8.0 vs. pH 10.0	<.01	
CB5	pH 6.0 vs. pH 8.0 NH_2Cl	<.01	
CB5	pH 8.0 vs. pH 8.0 NH_2Cl	.05	*
CB5	pH 10.0 vs. pH 8.0 NH_2Cl	<.01	
MS2	pH 6.0 vs. pH 8.0	<.01	
MS2	pH 6.0 vs. pH 10.0	<.01	
MS2	pH 8.0 vs. pH 10.0	.07	*
MS2	pH 6.0 vs. pH 8.0 NH_2Cl	<.01	
MS2	pH 8.0 vs. pH 8.0 NH_2Cl	<.01	
MS2	pH 10.0 vs. pH 8.0 NH_2Cl	<.01	
ØX174	pH 10.0 vs. pH 8.0 NH_2Cl	.15	*

TABLE 2

PARAMETERS OF THE LINEAR REGRESSION LINES USED TO CALCULATE THE T99.99% VALUES.

pH	VIRUS TYPE	SLOPE	INT.	T99.99%
6.0	HAV HM175	-0.622	0.046	6.50
6.0	HAV MD1	-1.772	-0.311	2.08
6.0	CB5	-0.317	0.184	13.20
6.0	MS2	-3.059	-0.317	1.20
6.0	0X174	-7.939	0.000	0.50
8.0	HAV HM175	-0.719	0.000	5.56
8.0	HAV MD1	-5.756	-0.061	0.68
8.0	CB5	-0.080	0.200	52.50
8.0	MS2	-0.222	-0.301	16.66
8.0	0X174	-5.182	0.000	0.77
10.0	HAV HM175	-0.082	0.064	49.56
10.0	HAV MD1	-0.177	-0.048	22.33
10.0	CB5	-0.005	0.128	825.60
10.0	MS2	-0.147	-0.099	26.54
10.0	0X174	-0.036	0.006	111.28
8.0	HAV HM175	-0.032	-0.251	117.16
8.0	HAV MD1	-0.014	-0.323	262.64
8.0	CB5	-0.040	0.166	104.15
8.0	MS2	-0.009	-0.221	419.89
8.0	0X174	-0.094	-1.049	31.39

5. DISCUSSION

5.1 EFFECT OF pH:

Previous studies investigating the response of HAV to disinfection by chlorine have produced inconsistent results. However, these studies used different virus purification methods and different proportions of free and combined chlorine. In this present series of experiments, the inactivation kinetics of HAV were studied under carefully controlled conditions in the laboratory with purified monodisperse virus stocks. The results indicate that the pH, the virus type, and the form of chlorine used as a disinfectant are important variables affecting the inactivation kinetics of the viruses tested.

At pH's 6.0 and 8.0, HAV strains HM175 and MD1 were found to be very sensitive to inactivation by free chlorine. These results are in agreement with those of Grabow (1983). The inactivation rates between the two strains of HAV were found not to be statistically different at pH 6.0 ($p=0.07$) and, though no statistical comparison could be made at pH 8.0, the T99.99% values were relatively similar (5.56 minutes for HM175 and 0.68 minutes for MD1). The strain comparison for HAV were conducted to determine whether the established lab strain of HAV (HM175) would

react similarly to disinfection as a strain of HAV that has not been passaged in the lab for as long of a period of time and therefore would be expected to show more characteristics of the wild type virus. A previous study had reported (Payment et. al., 1985) that some strains of virus isolated from environmental sources were more resistant to chlorine disinfection than laboratory strains.

The results indicate that monodisperse HAV HM175 and MD1 would show 4 log inactivation under typical environmental conditions when exposed to a conventional chlorine disinfection regimen of 0.5 - 1.0 mg/l residual after 30 minutes contact time.

At pH 10.0, both strains of HAV were significantly more resistant to disinfection by free chlorine than at pH's 6.0 and 8.0 ($p < 0.01$). In contrast to pH's 6.0 and 8.0, the inactivation rates of the two strains of HAV were significantly different ($p < 0.01$) with T99.99% of 49.56 minutes for HM175 and 27.33 minutes for MD1. These results are somewhat puzzling as previous studies have shown that the relative resistance to chlorine disinfection among different types of viruses will change with pH (Engelbrecht, 1980; Grabow, 1983 and 1984), but to my knowledge, this phenomenon has never been demonstrated to occur within strains of the same virus type.

As mentioned above, the HAV inactivation rates for pH 10.0 were significantly lower than at pH 6.0 and 8.0, which is consistent with the observation that hypochlorous acid

(HOCl) is a much better viricide than hypochlorite ion (OCl^-) (Grabow, 1983; Engelbrecht, 1980; Berman & Hoff, 1984; Harekeh, 1984). It is interesting to note that both strains of HAV showed similar T99.99% values for pH 6.0 and for pH 8.0 (Table 1), with HM175 showing statistically similar inactivation rates ($p=0.74$) between pH 6.0 and pH 8.0. It would be expected that, because there is a higher proportion of hypochlorous acid at pH 6.0 than at pH 8.0, the inactivation rate would be greater at pH 6.0. Two possible theories have been proposed to explain this phenomenon. The first is that the HAV viruses are aggregating at the more acid pH, and that this process is giving a measure of protection against disinfection. Floyd and Sharp (1977) and Jenson et. al. (1980) have demonstrated that Poliovirus, Echovirus, Reovirus and Coxsackievirus B3 will aggregate at pH's 6.0 and below.

It is also possible that the HAV viruses are undergoing conformational changes which can result in substantial changes in resistance of viruses to disinfection by chlorine. Young and Sharp (1985) observed that Echovirus distributed into two pH-dependent and interconvertible isolelectric forms with differing inactivation rates and that these forms caused aberrant inactivation kinetics in monodisperse virus disinfection experiments with chlorine. At the present time, it is not known whether this phenomenon occurs with HAV. Clearly, this is an area which warrents further research.

The state of aggregation of the viruses can be measured by established electron microscopy techniques and the conformational changes may be studied using isolelectric focusing techniques.

The T99.99% values presented in Table 2, and the statistical analysis summaries in Table 3 and 4 suggest that the two bacteriophages and both strains of HAV have similar inactivation kinetics to chlorine disinfection at pH 6.0 and pH 8.0 and that MS2 shows similar response rates with HAV at pH 10.0. At pH 6.0, the response of the bacteriophage MS2 was similar to the response of HAV MD1 ($p=0.12$) but different from HAV HM175 ($p<0.01$) with T99.99% values of 1.20 minutes for MS2, 6.50 minutes for HAV HM175 and 2.08 minutes for HAV MD1. Bacteriophage ØX174 showed a calculated T99.99% of 0.50 minutes. At pH 8.0, the inactivation rates for MS2 and HAV HM175 were similar ($p=0.08$) with T99.99% values of 16.06 minutes for MS2 and 5.56 minutes for HAV HM175. The T99.99% values between HAV MD1 (0.68 minutes) and ØX174 (0.74 minutes) were also very close.

However at pH 10.0, ØX174 became much more resistant (T99.99%=11.28 minutes) than either strain of HAV (T99.99%=49.56 minutes for HM175, T99.99%=22.32 MD1) and the inactivation rates were significantly different from those of either strain of HAV ($p=0.01$). In contrast, MS2 (T99.99%=26.54 minutes) showed similar inactivation rates with HAV MD1 ($p=0.18$) but not with HAV HM175 ($p<0.01$).

These findings suggest that both of these bacteriophages have potential for modeling the behavior of HAV to disinfection by free chlorine, under pH's normally encountered in the environment, but that at high pH, MS2 is a better model than the more resistant ØX174.

The T99.99% values reported here are in partial agreement with those presented by Grabow (1983). Grabow reported that MS2 exposed to between 0.89 and 0.64 mg/l of total initial concentration of chlorine at 25°C showed T99.99% values of 6.2 minutes for both pH 6.0 and pH 8.0 and 3.1 minutes for pH 10.0. The T99.99% value at pH 10.0, reported by Grabow, was the most different from that reported here and may be due to the different buffer used (0.05 M Borate buffer vs. 0.01M Phosphate buffer) in his study and the different temperature used in the reaction tubes.

At all of the pH's tested for free chlorine, the enteric virus CB5 was the most resistant to inactivation, showing significantly different inactivation rates (p-values from 0.03 to <0.01) from the other viruses, with the exception of HM175 at pH 6.0 (p=0.72). The resistance of CB5 to inactivation by free chlorine has been widely reported (Engelbrecht 1980, Jensen and Sharp 1980, Payment and Trudell 1985, Grabow 1983) and the T99.99% values found here are in partial agreement with those of Engelbrecht, who reported T99% (2 log reduction time) of 3.4 minutes at pH 6.0, 4.5 minutes at pH 7.8 and 66.0 minutes at pH 10.0, 0.05 mg/l free chlorine, 5°C.

The two strains of HAV were very resistant to inactivation by monochloramine showing T99.99% values of 117.16 minutes for HM175 and 262 minutes for MD1. Analysis of the inactivation rates revealed that the two were statistically similar ($p=0.11$).

The bacteriophage MS2 was the most resistant of the viruses tested (T99.99%=419.89 minutes) and the inactivation rate were similar to HAV HM175 ($p=0.05$) and HAV MD1 ($p=0.67$), again showing its potential as a model for HAV disinfection. In contrast, the Bacteriophage ØX174 was the most sensitive virus to disinfection by monochloramine (T99.99%=31.39 minutes) and its inactivation rate was significantly different ($p<0.01$) than either strain of HAV.

CB5 was shown to be more sensitive than either HAV HM175 or MD1 (T99.99%=104.15 minutes). This is a very interesting observation, especially if we are considering the spontaneous aggregation of CB5 to be responsible for its resistance to free chlorine. The monochloramine experiments were conducted at pH 8.0, a pH at which CB5 is known to aggregate. However, the CB5 does not appear to be significantly more resistant than the other viruses, as was the case in the free chlorine experiments. Again, further research is necessary to determine the exact cause for CB5 resistance to inactivation by free chlorine.

The results of the monochloramine experiments show that at pH 8.0, 10 mg/l monochloramine would not achieve a 4 log reduction in any of the viruses tested and that caution should be excersized before setting up a water treatment regimen relying solely on disinfection by monochloramine.

As these experiments were conducted to establish "best case" baseline data on the disinfection of the monodisperse preparations of the viruses in clean water, there is a clear need for further research in this area. Similar experiments will soon be conducted in our lab using cell associated aggregates of the different viruses types as well as experiments with waters of varying quality. In addition, an attempt will be made to conduct these experiments using wild-type HAV. These later experiments will be able to provide a better understanding of how changes in water quality and virus aggregation will affect the kinetics and extent of virus inactivation by free chlorine and monochloramine.

6. CONCLUSIONS

1. HAV HM175 is sensitive to disinfection by 0.5 mg/l free chlorine, 5°C, at pH 6.0 and pH 8.0 . However, at pH 10.0, HAV HM175 is very resistant to inactivation.

2. HAV HM175 is very resistant to inactivation by 10 mg/l monochloramine, 5°C, pH 8.0 and monochloramine is a weaker viricide than free chlorine.

3. The bacteriophages, MS2 and ØX174, showed similar inactivation kinetics with HAV to disinfection by free chlorine at pH 6.0 and pH 8.0. At pH 10.0, ØX174 was more resistant than HAV while MS2, again, showed inactivation rates similar to HAV. CB5 was consistently more resistant than HAV to disinfection by free chlorine at all pH ranges tested.

4. The inactivation rate of MS2 by 10 mg/l monochloramine was comparable to that of HAV, while ØX174 was significantly more sensitive. The inactivation rates of CB5 and HAV were similar for disinfection by monochloramine.

5. There were no significant differences among the two strains of HAV to disinfection by 0.5 mg/l free chlorine at pH 6.0 and ph 8.0 and 10 mg/l monochloramine, pH 8.0. However, HAV HM175 was more resistant than HAV MD1 to inactivation by free chlorine at pH 10.0.

TEST CONDITIONS: pH = 6.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = CB5

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG. Nt/No	AVG. Log Nt/No
20 sec.	7.33E+04	1.47E+05	2.49E+04	1.10E+00	1.63E+00	1.37E+00	1.37E+00	0.14
1 min.	8.00E+04	1.08E+05	1.95E+04	1.20E+00	1.20E+00	1.07E+00	1.16E+00	0.06
3 min.	9.83E+03	1.53E+04	2.09E+03	1.47E-01	1.70E-01	1.15E-01	1.44E-01	-0.84
10 min.	1.67E+01	1.67E+01	1.10E+01	2.50E-04	1.86E-04	6.04E-04	3.47E-04	-3.46
30 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
60 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
VC 0	6.67E+04	9.00E+04	1.82E+04					
VC 60	7.17E+04	8.17E+04	1.94E+04					

TEST CONDITIONS: pH = 6.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = MS2

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG Nt/No	Log Nt/No
20 sec.	6.67E+02	1.93E+03	8.03E+03	2.62E-03	3.22E-02	1.33E-02	1.60E-02	-1.80
1 min.	3.33E+01	1.00E+02	1.10E+01	1.31E-04	1.67E-03	1.82E-05	6.05E-04	-3.22
3 min.		3.33E+01		0.00E+00	5.55E-04	0.00E+00	5.55E-04	-3.26
10 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
30 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
60 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
VC 0	2.55E+05	6.00E+04	6.05E+05					
VC 60	2.05E+05	5.43E+04	5.83E+05					

TEST CONDITIONS: pH = 6.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = HAV MD1

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG. Nt/No	Log Nt/No
20 sec.	1.21E+02	1.65E+02	3.19E+02	3.24E-02	5.25E-02	4.68E-02	4.39E-02	-1.36
1 min.	2.20E+01	7.70E+01	3.30E+01	5.88E-03	2.45E-02	4.84E-03	1.17E-02	-1.93
3 min.	1.10E+01	1.10E+01	1.10E+01	2.94E-03	3.50E-03	1.61E-03	2.69E-03	-2.57
10 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
30 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
60 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
VC 0	3.74E+03	3.14E+03	6.82E+03					
VC 60	8.47E+03	2.97E+03	8.58E+03					

TEST CONDITIONS: pH = 6.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = HAV HM175

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG. Nt/No	AVG. Log Nt/No
20 sec.	8.17E+04	3.33E+04	9.13E+04	6.28E-01	5.12E-01	7.30E-01	6.24E-01	-0.21
1 min.	4.50E+04	1.70E+04	6.16E+04	3.46E-01	2.62E-01	4.93E-01	3.67E-01	-0.44
3 min.	6.00E+02	5.00E+02	3.63E+03	4.62E-03	7.69E-03	2.90E-02	1.38E-02	-1.86
10 min.	1.67E+01	1.67E+01	1.10E+01	1.28E-04	2.57E-04	8.80E-05	1.58E-04	-3.80
30 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
60 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
VC 0	1.30E+05	6.50E+04	1.25E+05					
VC 60	1.83E+05	9.00E+04	1.52E+05					

TEST CONDITIONS: pH = 6.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = ØX174

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG. Nt/No	AVG. Log Nt/No
20 sec.	3.33E+01	3.33E+01	1.10E+01	3.08E-04	6.05E-03	7.91E-04	2.38E-03	-2.62
1 min.			1.10E+01	0.00E+00	0.00E+00	7.91E-04	7.91E-04	-3.10
3 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
10 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
30 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
60 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
VC 0	1.08E+05	5.50E+03	1.39E+04					
VC 60	9.30E+04	9.17E+03	1.33E+04					

STARTING C12 CONCENTRATION RANGE = 0.489 - 0.519 mg/l

ENDING C12 CONCENTRATION RANGE = 0.185 - 0.321 mg/l

pH RANGE = 5.87 - 6.10

TEST CONDITIONS: pH = 8.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = HAV HM175

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG. Nt/No	AVG. Log Nt/No
20 sec.	5.75E+04	9.67E+03	1.00E+05	3.48E-01	5.79E-01	2.85E-01	4.04E-01	-0.39
1 min.	6.00E+03	3.83E+03	9.35E+04	3.64E-02	2.29E-01	2.66E-01	1.77E-01	-0.75
3 min.	1.67E+01	1.67E+01	1.10E+01	1.01E-04	1.00E-03	3.13E-05	3.78E-04	-3.42
10 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
30 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
60 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
VC 0	1.65E+05	1.67E+04	3.51E+05					
VC 60	1.10E+01		2.78E+05					

TEST CONDITIONS: pH = 8.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = ØX174

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG. Nt/No	AVG. Log Nt/No
20 sec.	2.13E+03	3.33E+01	3.85E+02	1.97E-02	2.87E-03	3.60E-02	1.95E-02	-1.71
1 min.	3.33E+01		1.10E+01	3.08E-04	0.00E+00	1.03E-03	6.68E-04	-3.18
3 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
10 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
30 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
60 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
VC 0	1.08E+05	1.16E+04	1.07E+04					
VC 60	1.01E+05	1.28E+04	4.13E+03					

STARTING Cl2 CONCENTRATION RANGE = 0.494 - 0.511 mg/l

ENDING Cl2 CONCENTRATION RANGE = 0.230 - 0.358 mg/l

pH RANGE = 7.90 - 8.12

TEST CONDITIONS: pH = 8.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = CB5

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG. Nt/No	AVG. Log Nt/No
20 sec.	2.13E+05	1.78E+05	5.06E+04	2.03E+00	1.65E+00	2.28E+00	1.99E+00	0.30
1 min.	1.77E+05	1.53E+05	2.59E+04	1.69E+00	1.42E+00	1.17E+00	1.42E+00	0.15
3 min.	1.65E+05	1.05E+05	1.42E+04	1.57E+00	9.72E-01	6.40E-01	1.06E+00	0.03
10 min.	6.50E+04	2.20E+03	1.43E+03	6.19E-01	2.04E-02	6.44E-02	2.35E-01	-0.63
30 min.	1.67E+01	1.67E+01	1.10E+01	1.59E-04	1.55E-04	4.95E-04	2.70E-04	-3.57
60 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
VC 0	1.05E+05	1.08E+05	2.22E+04					
VC 60	1.03E+05	6.50E+04	1.89E+04					

TEST CONDITIONS: pH = 8.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = MS2

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG Nt/No	Log Nt/No
20 sec.	1.40E+05	1.87E+04	3.41E+03	4.91E-01	2.52E-01	2.16E-01	3.20E-01	-0.50
1 min.	9.67E+04	1.87E+04	1.57E+03	3.39E-01	2.52E-01	9.94E-02	2.30E-01	-0.64
3 min.	3.70E+04	8.00E+03	2.75E+02	1.30E-01	1.08E-01	1.74E-02	8.50E-02	-1.07
10 min.	1.40E+03	3.33E+02	1.10E+01	4.91E-03	4.48E-03	6.96E-04	3.36E-03	-2.47
30 min.	3.33E+01	3.33E+01		1.17E-04	4.48E-04	0.00E+00	2.83E-04	-3.55
60 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
VC 0	2.85E+05	7.43E+04	1.58E+04					
VC 60	3.13E+05	9.73E+04	2.20E+03					

TEST CONDITIONS: pH = 8.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = HAV MD1

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG. Nt/No	Log Nt/No
20 sec.	2.86E+02	6.60E+01		2.07E-02	4.23E-03	BBB	1.25E-02	-1.90
1 min.	1.10E+01	1.10E+01		7.97E-04	7.05E-04	BBB	7.51E-04	-3.12
3 min.				0.00E+00	0.00E+00	BBB	0.00E+00	
10 min.				0.00E+00	0.00E+00	BBB	0.00E+00	
30 min.				0.00E+00	0.00E+00	BBB	0.00E+00	
60 min.				0.00E+00	0.00E+00	BBB	0.00E+00	
VC 0	1.38E+04	1.56E+04						
VC 60	1.40E+04	6.71E+03						

TEST CONDITIONS: pH = 10.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = C85

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG. Nt/No	AVG. Log Nt/No
20 sec.	4.62E+04	5.06E+04	2.05E+04	5.68E-01	9.39E-01	1.11E+00	8.71E-01	-0.06
1 min.	8.36E+04	8.36E+04	2.54E+04	1.03E+00	1.55E+00	1.37E+00	1.32E+00	0.12
3 min.	8.80E+04	9.90E+04	3.04E+04	1.08E+00	1.84E+00	1.64E+00	1.52E+00	0.18
10 min.	1.55E+05	1.17E+05	2.32E+04	1.90E+00	2.17E+00	1.25E+00	1.78E+00	0.25
30 min.	1.43E+05	1.31E+05	1.14E+04	1.76E+00	2.43E+00	6.16E-01	1.60E+00	0.20
60 min.	7.81E+04	2.53E+04	1.05E+03	9.59E-01	4.69E-01	5.68E-02	4.95E-01	-0.31
VC 0	8.14E+04	5.39E+04	1.85E+04					
VC 60	7.04E+04	5.50E+04	1.70E+04					

TEST CONDITIONS: pH = 10.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = MS2

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG Nt/No	Log Nt/No
20 sec.	2.49E+04	4.68E+04	8.36E+02	5.39E-01	7.61E-01	5.69E-01	6.23E-01	-0.21
1 min.	2.52E+04	3.44E+04	7.15E+02	5.45E-01	5.59E-01	4.86E-01	5.30E-01	-0.28
3 min.	1.32E+04	2.29E+04	2.86E+02	2.86E-01	3.72E-01	1.95E-01	2.84E-01	-0.55
10 min.	1.98E+03	2.00E+03	1.10E+01	4.29E-02	3.25E-02	7.48E-03	2.76E-02	-1.56
30 min.	1.10E+01	1.10E+01		2.38E-04	1.79E-04	0.00E+00	2.08E-04	-3.68
60 min.				0.00E+00	0.00E+00	0.00E+00	0.00E+00	
VC 0	4.62E+04	6.15E+04	1.47E+03					
VC 60	1.37E+04	4.93E+04	1.09E+03					

TEST CONDITIONS: pH = 10.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = HAV MD1

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG. Nt/No	Log Nt/No
20 sec.	1.65E+04	5.72E+03		9.17E-01	3.45E-01	ERR	6.31E-01	-0.20
1 min.	1.99E+04	5.61E+03		1.11E+00	3.38E-01	ERR	7.22E-01	-0.14
3 min.	8.25E+03	1.30E+02		4.58E-01	7.83E-03	ERR	2.33E-01	-0.63
10 min.	4.84E+02	6.60E+01		2.69E-02	3.98E-03	ERR	1.54E-02	-1.81
30 min.	1.10E+01	1.10E+01		6.11E-04	6.63E-04	ERR	6.37E-04	-3.20
60 min.				0.00E+00	0.00E+00	ERR	0.00E+00	
VC 0	1.80E+04	1.66E+04						
VC 60	2.17E+04	1.93E+04						

TEST CONDITIONS: pH = 10.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = HAV HM175

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG. Nt/No	AVG. Log Nt/No
20 sec.	1.13E+04	1.61E+03	3.85E+05	1.21E+00	2.18E-01	1.42E+00	9.49E-01	-0.02
1 min.	6.71E+03	8.36E+03	9.24E+04	7.18E-01	1.13E+00	3.41E-01	7.31E-01	-0.14
3 min.	7.81E+03	8.14E+03	8.69E+04	8.35E-01	1.10E+00	3.21E-01	7.53E-01	-0.12
10 min.	4.95E+02	4.07E+03	1.05E+05	5.29E-02	5.52E-01	3.87E-01	3.31E-01	-0.48
30 min.	7.70E+01	1.10E+01	3.30E+01	8.24E-03	1.49E-03	1.22E-04	3.28E-03	-2.48
60 min.	1.10E+01		1.10E+01	1.18E-03	0.00E+00	4.06E-05	6.09E-04	-3.22
VC 0	9.35E+03	7.37E+03	2.71E+05					
VC 60	8.03E+03	3.30E+03	1.57E+05					

TEST CONDITIONS: pH = 10.0
TEMPERATURE= 5 C
CL2 CONC.= 0.5 mg/l
VIRUS = 0X174

SAMPLE	PFU/ml	PFU/ml	PFU/ml	Nt/No	Nt/No	Nt/No	AVG. Nt/No	AVG. Log Nt/No
20 sec.	2.50E+03	4.03E+03	6.93E+03	9.26E-01	9.98E-01	9.55E-01	9.59E-01	-0.02
1 min.	2.52E+03	4.16E+03	5.72E+03	9.33E-01	1.03E+00	7.88E-01	9.17E-01	-0.04
3 min.	2.11E+03	3.89E+03	3.63E+03	7.81E-01	9.63E-01	5.00E-01	7.48E-01	-0.13
10 min.	1.58E+03	2.54E+03	2.65E+03	5.85E-01	6.29E-01	3.65E-01	5.26E-01	-0.28
30 min.	2.97E+02	4.29E+02	1.10E+02	1.10E-01	1.06E-01	1.52E-02	7.71E-02	-1.11
60 min.	1.10E+01	1.10E+01	1.10E+01	4.07E-03	2.72E-03	1.52E-03	2.77E-03	-2.56
VC 0	2.70E+03	4.04E+03	7.26E+03					
VC 60	3.27E+03	3.74E+03	7.59E+03					

STARTING Cl2 CONCENTRATION RANGE = 0.500 - 0.517 mg/l

ENDING Cl2 CONCENTRATION RANGE = 0.360 - 0.414 mg/l

pH RANGE = 9.87 - 10.17

TEST CONDITIONS: pH = 8.0
TEMPERATURE= 5 C
NH2Cl CONC. = 10 mg/l
VIRUS = HAV HM175

SAMPLE	PFU/ml	PFU/ml	Nt/No	Nt/No	AVG. Nt/No	AVG. Log Nt/No
20 sec.	1.38E+05	3.30E+04	1.19E+00	1.20E-01	6.55E-01	-0.18
1 min.	1.25E+05	3.96E+04	1.08E+00	1.44E-01	6.11E-01	-0.21
3 min.	4.51E+04	1.43E+04	3.89E-01	5.20E-02	2.20E-01	-0.66
10 min.	6.93E+04	5.39E+03	5.97E-01	1.96E-02	3.09E-01	-0.51
30 min.	5.06E+03	3.52E+03	4.36E-02	1.28E-02	2.82E-02	-1.55
60 min.	1.55E+03	1.49E+03	1.34E-02	5.42E-03	9.39E-03	-2.03
VC 0	1.16E+05	2.75E+05				
VC 60	1.41E+05	1.40E+05				

TEST CONDITIONS: pH = 8.0
TEMPERATURE= 5 C
NH2Cl CONC. = 10 mg/l
VIRUS = ΦX174

SAMPLE	PFU/ml	PFU/ml	Nt/No	Nt/No	AVG. Nt/No	AVG. Log Nt/No
20 sec.	8.58E+03	1.24E+04	1.86E-01	3.13E-01	2.49E-01	-0.60
1 min.	2.63E+03	2.97E+03	5.69E-02	7.50E-02	6.60E-02	-1.18
3 min.	1.98E+02	4.73E+02	4.29E-03	1.19E-02	8.12E-03	-2.09
10 min.	3.30E+01	2.20E+01	7.14E-04	5.56E-04	6.35E-04	-3.20
30 min.	1.10E+01	2.20E+01	2.38E-04	5.56E-04	3.97E-04	-3.40
60 min.		1.10E+01	0.00E+00	2.78E-04	2.78E-04	-3.56
VC 0	4.62E+04	3.96E+04				
VC 60	4.95E+04	3.96E+04				

STARTING NH2Cl CONCENTRATION RANGE = 9.96 - 10.20 mg/l

ENDING NH2Cl CONCENTRATION RANGE = 8.25 - 9.90 mg/l

pH RANGE = 7.81 - 7.97

TEST CONDITIONS: pH = 8.0
TEMPERATURE= 5 C
NH2Cl CONC. = 10 mg/l
VIRUS = CB5

SAMPLE	PFU/ml	PFU/ml	Nt/No	Nt/No	AVG. Nt/No	AVG. Log Nt/No
20 sec.	4.73E+04	2.41E+04	1.95E+00	9.92E-01	1.47E+00	0.17
1 min.	4.84E+04	3.00E+04	2.00E+00	1.23E+00	1.62E+00	0.21
3 min.	3.52E+04	2.06E+04	1.45E+00	8.48E-01	1.15E+00	0.06
10 min.	1.77E+04	1.13E+04	7.31E-01	4.65E-01	5.98E-01	-0.22
30 min.	2.97E+03	2.21E+03	1.23E-01	9.09E-02	1.07E-01	-0.97
60 min.	7.70E+01	1.65E+02	3.18E-03	6.79E-03	4.99E-03	-2.30
VC 0	2.42E+04	2.43E+04				
VC 60	2.40E+04	1.73E+04				

TEST CONDITIONS: pH = 8.0
TEMPERATURE= 5 C
NH2Cl CONC. = 10 mg/l
VIRUS = MS2

SAMPLE	PFU/ml	PFU/ml	Nt/No	Nt/No	AVG Nt/No	Log Nt/No
20 sec.	5.50E+05	3.27E+05	8.77E-01	4.37E-01	6.57E-01	-0.18
1 min.	4.73E+05	1.53E+05	7.54E-01	2.05E-01	4.79E-01	-0.32
3 min.	3.96E+05	1.86E+05	6.32E-01	2.49E-01	4.40E-01	-0.36
10 min.	3.30E+05	2.01E+05	5.26E-01	2.69E-01	3.98E-01	-0.40
30 min.	1.84E+05	2.51E+05	2.93E-01	3.36E-01	3.15E-01	-0.50
60 min.	1.57E+05	7.37E+04	2.50E-01	9.85E-02	1.74E-01	-0.76
VC 0	6.27E+05	7.48E+05				
VC 60	3.08E+05	7.26E+05				

TEST CONDITIONS: pH = 8.0
TEMPERATURE= 5 C
NH2Cl CONC. = 10 mg/l
VIRUS = HAV MD1

SAMPLE	PFU/ml	PFU/ml	Nt/No	Nt/No	AVG. Nt/No	Log Nt/No
20 sec.	2.42E+03	8.14E+02	6.42E-01	1.85E-01	4.13E-01	-0.38
1 min.	3.08E+03	1.10E+03	8.17E-01	2.50E-01	5.33E-01	-0.27
3 min.	1.21E+03	1.75E+03	3.21E-01	3.98E-01	3.59E-01	-0.44
10 min.	7.70E+02	2.09E+02	2.04E-01	4.75E-02	1.26E-01	-0.90
30 min.	1.46E+03	7.48E+02	3.87E-01	1.70E-01	2.79E-01	-0.55
60 min.	7.70E+01	4.73E+02	2.04E-02	1.08E-01	6.40E-02	-1.19
VC 0	3.77E+03	4.40E+03				
VC 60	5.50E+03	1.63E+03				

BIBLIOGRAPHY:

Adams, M.H. 1959. Bacteriophages. Interscience, N.Y.

Akin, E.W., and J.C. Hoff. 1984. Microbiological Risks Associated with Changes in Drinking Water Disinfection Practices. in R.L. Jolley et. al.(eds) Water Chlorination: Chemistry, Environmental Impacts and Health Effects. Vol. 5. Lewis Pub. Inc.

American Public Health Association, 1985. Standard Methods for the Examination of Water and Wastewater, 16th Edition. American Public Health Association, Washington, D.C.

Berg, G., D.R. Dahling, G.A. Brown, and D. Berman. 1978. Validity of Fecal Coliforms, Total Coliforms, and Fecal Streptococci as Indicators of Viruses in Chlorinated Primary Sewage Effluents. Appl. Env. Micro. 36:880-884.

Berman, D. and J.C. Hoff. 1984. Inactivation of Simian Rotavirus SA11 by Chlorine, Chlorine Dioxide and Monochloramine. Appl. Env. Micro. 48:317-323.17...Bitton,G. 1980. Introduction to

Bitton, G. 1980. Introduction to Environmental Virology. John Wiley and Sons, Inc., N.Y.

Bitton,G.,S.R. Farrah, C.L. Montague, E.W. Akin. 1986. Viruses in Drinking Water. Env. Sci. Tech. 20:216-222.

Block, J. 1983. Viruses in Environmental Waters. pp. 117-146. In: G. Berg (ed) Viral Pollution of the Environment. CRC Press, Inc., Boca Raton, FL.

Bradley, D.W., C.L. Hornbuck, C.R. Gravelle, E.H. Cook, J.E. Maynard. 1975. CsCl Banding of Hepatitis A-Associated Virus Like Particles. J. Infect. Dis. 131:304-306.

Coulepis, A.G., S.A. Locarnini, I.D. Gust. 1980. Iodination of Hepatitis A Virus Reveals a Fourth Structural Polypeptide. J. Virol. 35:572-574.

Cromeans, T., M.D. Sobsey, and H.A. Fields. 1987. Development of a Plaque Assay for a Cytopathic, Rapidly Replicating Isolate of Hepatitia A Virus. J. of Med. Vir. 22:45-56.

Daemer, R.J., S.M. Feinstone, I.D. Gust, R.H. Purcell. 1981. Propogation of Human Hepatitis A virus in African Green Monkey Kidney Cell Culture: Primary Isolation and Serial Passage. Infect. Immun. 32:384-393.

Denhardt, D.T. 1977. The Isometric Single-Stranded DNA Phages. pp.1-89. In: H. Fraenkel-Conrat and R. Wagner (eds) Comprehensive Virology, Volume VII. Plenum Press. N.Y.

Dienstag, J.L., A.N. Schulman, R.J. Gerety, J.H. Hoofnagle, D.E.Lorenz, R.H. Purcell, L.F. Barker. 1976. Hepatitis A Antigen Isolated From Liver and Stool: Immunologic Comparison of Antisera Prepared in Guinea Pigs. J. Immunol. 117:876-881.

Dychdala, G.R. 1983. Chlorine and Chlorine Compounds. pp. 157-182. In: S.S. Block (ed) Disinfection Sterilization and Preservation. Lea & Febiger. Philadelphia.

Engelbrecht, R.S., M.J. Weber, B.L. Salter, C.A. Schmidt. 1980. Comparative Inactivation of Viruses by Chlorine. Appl. Env. Micro.40:249-256.

Feachem, R.G., D.J. Bradley, H. Garelick, D.D. Mara. 1983. Sanitation and Disease: Health Aspects of Excreta and Wastewater Management. John Wiley and Sons, Inc., N.Y.

Federal Register, Vol.52, No. 212. November 3, 1987. p 42178-42222.

Feinstone, S.M., A.Z. Kapikian, J.L. Genn, R.H Purcell. 1974. Buoyant Density of the Hepatitis A Virus-Like Particle in Cesium Chloride. J. Virol. 13.1412-1414.

Feinstone, S.M., A.Z. Kapikian, R.H. Purcell. 1973. Hepatitis A Detection by Immune Electron Microscopy of a Virus Like Antigen Associated with Acute Illness. Sci. 182:11026-1028.

Fraenkel-Conrat, H. 1974. Descriptive Catalogue of Viruses. In: H. Fraenkel-Conrat and R. Wagner (eds) Comprehensive Virology, Volume I. Plenum Press. N.Y.

Frosner, G. 1984. Hepatitis A Virus. pp. 707-727. In Belshe, R.B. (ed), Textbook of Human Virology. PSG Publishing co., Inc., Littleton, MA.

Funderberg, S.W. and C.A. Sorber. 1985. Coliphages as Indicators of Enteric Viruses in Activated Sludge. Water Research. 19:547-555.

Gerba, C.P., S.M. Goyal, R.L. LaBelle, I. Cech, G.F. Bogdan. 1979. Failure of Indicator Bacteria to Reflect the Occurence of Enteroviruses in Marine Waters. Am. J. Pub. Health. 69:1116-1119.

Grabow, W.O.K., V. Gauss-Miller, O.W. Prozesky, F. Dienhardt. 1983. Inactivation of Hepatitis A Virus and indicator Organisms in Water by Free Chlorine Residuals. Appl. Env. Micro.46:616-624.

Grabow, W.O.K., P. Coubrough, C. Hilner, B.W. Bateman. 1984. Inactivation of Hepatitis A Virus, Other Enteric Viruses and Indicator Organisms in Water by Chlorination. Wat. Sci. Tech. 17:657-664.

Goyall, S.M.,W.N. Adams, M.L. O'Malley, D.W. Lear. 1984. Human Pathogenic Viruses at Sewage Dump Sites in the Middle Atlantic Region. Appl. Env. Micro. 40:758-763.

Harakeh, M.S. 1984. Factors Influencing Chlorine Disinfection of Wastewater Effluent Contaminated by Rotavirus, Enterovirus and Bacteriophages. in R.L. Jolley et. el. (eds). Water Chlorination: Chemistry, Environmental Impacts and Health Effects. Vol. 5. Lewis Pub. Inc.

Havelaar, A.H. and T.J. Nieuwstad. 1985. Bacteriophages and Fecal Bacteria as Indicators of Chlorination Efficiency of Biologically Treated Wastewater. J.W.P.C.F. 57:1084-1088.

Hejkal, T. B. Keswick, R. LaBelle, C. Gerba, Y. Sanchez, G. Dreesman, B. Hafkin and J.L. Melnick. 1982. Viruses in a Community Water Supply Associated with an Outbreak of Gastroenteritis and Infectious Hepatitis. J.A.W.W.A. 74:318-321.

Hollinger, F.B., 1985. Hepatitis A Virus. pp.1379-1383. In. Fields, B.N. et.al. (eds.), Virology. Raven Press, NY.

Hollinger, F.B., and J.L.Melnick. 1985. Viral Hepatitis Epidemiology. pp.1434-1460. In: B.N. Fields, et.al.(eds). Virology. Raven Press, N.Y.

Jensen, H., K. Thomas, and D.G. Sharp. 1980. Inactivation of Coxsackieviruses B3 and B5 in Water by Chlorine. Appl. Env. Micro. 40:633-640.

Joklik, W.K. 1985. The Structure, Components, and Classification of Viruses. pp.15-53. In. Joklik, W.K. (ed), Virology. Appleton-Century-Crofts. Connecticut.

Keswick, B.H. and C.P. Gerba. 1980. Viruses in Groundwater. Env. Sci. Tech. 14:1290-1297.

Kott, Y., N. Roze, S. Sperber, N. Betzer. 1974. Bacteriophages as Viral Pollution Indicators. Water Research. 8:165-171.

Lemon, S.M., L.N. Binn, R.H. Marchwicki. 1983. Radioimmunofocus Assay for Quantification of Hepatitis A Virus in Cell Culture. J. Clin. Microbiol. 17:834-839.

Leong, L.Y.C. 1983. Removal and Inactivation of Viruses by Treatment Processes for Potable Water and Wastewater-A Review. Wat. Sci. Tech. 15:91-114.

Lippy, E.C., and S.C. Waltrip. 1984. Waterborne Disease Outbreaks, 1946-1980: A Thirty-Five Year Perspective. J.A.W.W.A. 76:60-67.

Lippy, E.C. 1986. Chlorination to Prevent and Control Waterborne Disease. J.A.W.W.A. 80:49-52.

Melnick, J.L. 1985. Enterovirus: Polioviorus, Coxsackieviruses, Echoviruses and Newer Enteroviruses. pp.739-794. In: B.N. Fields, et.al.(eds) Virology. Raven Press, N.Y.

Neefe, J.R., J.R. Stokes, jr., J.B. Baty, J.G. Reinhold. 1945. Disinfection of Water Containing Causative Agent of Infectious (Epidemic) Hepatitis. J. Am. Med. Assoc. 128:1076-1080.

Nupen, E.M. 1976. Viruses in Renovated Waters. In. Viruses in Water G. Berg et. al. (eds) APHA., Washington, D.C.

Olivieri, V. P. 1984. Human Pathogens, Disinfection and Chlorine. in R.L. Jolley et. al. (eds). Water Chlorination: Chemistry, Environmental Impacts and Health Effects. Vol. 5. Lewis Pub. Inc.

Payment, P., M. Trudel, and M. Trembley. 1985. Elimination of Viruses and Indicator Bacteria at Each Step of Treatment During Preparation of Drinking Water at Seven Water Treatment Plants. Appl. Env. Micro. 49:1418-1428.

Payment, P., M. Trembley and M. Trudel. 1985. Relative Resistance to Chlorine of Poliovirus and Coxsackievirus Isolates from Environmental Sources and Drinking Water. Appl. Env. Micro. 49:981-983.

Peterson, D.A., T.A. Hurley, J.C. Hoff, L.G. Wolfe. 1984. Effect of Chlorine Treatment on Infectivity of Hepatitis A Virus. Appl. Env. Micro. 45:223-227.

Provost, P.J., and M.R. Hilleman. 1979. Propagation of Human Hepatitis A Virus in Cell Culture in Vitro. Proc. Soc. Exp. Biol. Med. 160:213-221.

Sekla, L.W., W. Stackin and L. VanBuckenhout. 1980. Enteric Virus in Renovated Water in Manitoba. Can. J. Micro. 26:518-523.

Siegel, G. and G.G. Fisher. 1978. Characterization and Classification of Virus Particles Associated with Hepatitis A. I. Size, Density and Sedimentation. J. Virol. 26:40-47.

Sobsey, M.D., S.E. Oglesbee, D.A. Wait and A.I. Cuenca. 1985. Detection of Hepatitis A Virus (HAV) in Drinking Water. Wat. Sci. Tech. 17:23-38.

Sobsey, M.D., P.A. Shields, F.H. Hauchman, R.L. Hazard., and L.W. Caton, III. 1986. Survival and Transport of Hepatitis A Virus in Soils, Groundwater and Wastewater, Wat. Sci. Tech.

Stetler, R.E., R.L. Ward, and S.C. Waltrip. 1984. Enteric Viruses and Indicator Bacteria Levels in a Water Treatment System Modified to Reduce Trihalomethane Production. Appl. Env. Micro. 47:319-324.

Snoeyink, V.L. and D. Jenkins. 1980. Water Chemistry. John Wiley and Sons, N.Y.

Stacha, J. H., and F.W. Pontius. 1984. An Overview of Water Treatment Practices in the United States. J.A.W.W.A. 76:73-83.

Sykes, G. 1965. Disinfection and Sterilization. E. and F. N. Spon, Ltd. London.

Viessman, W. and M.J. Hammer. 1985. Water Supply and Pollution Control. Harper and Row, Pub. N.Y.

Wolfe, R.L., N.R. Ward and B.H. Olson. 1984. Inorganic Chloramines as Drinking Water Disinfectant: A Review. A.W.W.A. 76:74-84.

Young, D. and D.G. Sharp. 1985. Virion Conformational Forms and the Complex Inactivation Kinetics of Echovirus by Chlorine in Water. Appl. Env. Micro. 49:359-362.

APPENDIX I

RAW DATA FROM DISINFECTION EXPERIMENTS

APPENDIX II

PARAMETERS OF THE LINEAR REGRESSION LINES USED TO CALCULATE THE T99.99% VALUES

TABLE 1

PARAMETERS OF THE LINEAR REGRESSION LINES USED TO CALCULATE THE T99.99% VALUES.

DISINFECTANT	pH	VIRUS TYPE	STANDARD ERROR	R-SQUARE
FREE CHLORINE 0.5 mg/l	6.0	HAV HM175	0.049	0.988
	6.0	HAV MD1	0.804	0.829
	6.0	CB5	0.086	0.872
	6.0	MS2	0.820	0.933
	6.0	0X174		-1.000
FREE CHLORINE 0.5 mg/l	8.0	HAV HM175	0.158	0.954
	8.0	HAV MD1		-1.000
	8.0	CB5	0.017	0.876
	8.0	MS2	0.025	0.962
	8.0	0X174		-1.000
FREE CHLORINE 0.5 mg/l	10.0	HAV HM175	0.006	0.977
	10.0	HAV MD1	0.010	0.990
	10.0	CB5	0.003	0.328
	10.0	MS2	0.008	0.990
	10.0	0X174	0.002	0.992
MONO-CHLORAMINE 10 mg/l	8.0	HAV HM175	0.004	0.912
	8.0	HAV MD1	0.005	0.636
	8.0	CB5	0.002	0.991
	8.0	MS2	0.002	0.771
	8.0	0X174	0.036	0.627