ABSTRACT

In order to ensure virologically acceptable drinking water, the US EPA promulgated the Surface Water Treatment Rule and is preparing the groundwater disinfection rule (as well as amendments to the SWTR) to define requirements for disinfection to achieve specified degrees of virus inactivation. While free chlorine disinfection has been widely used since the early 20th century, the recent evidence that THMs and other chlorine by-products are carcinogens and cause other adverse health effects has focused attention on alternate disinfectants, including monochloramine and chlorine dioxide. Although previous studies have examined both disinfectants at high doses on inactivation of some important waterborne viruses, little information is available at realistic concentrations used in water treatment plants and at a range of pH levels.

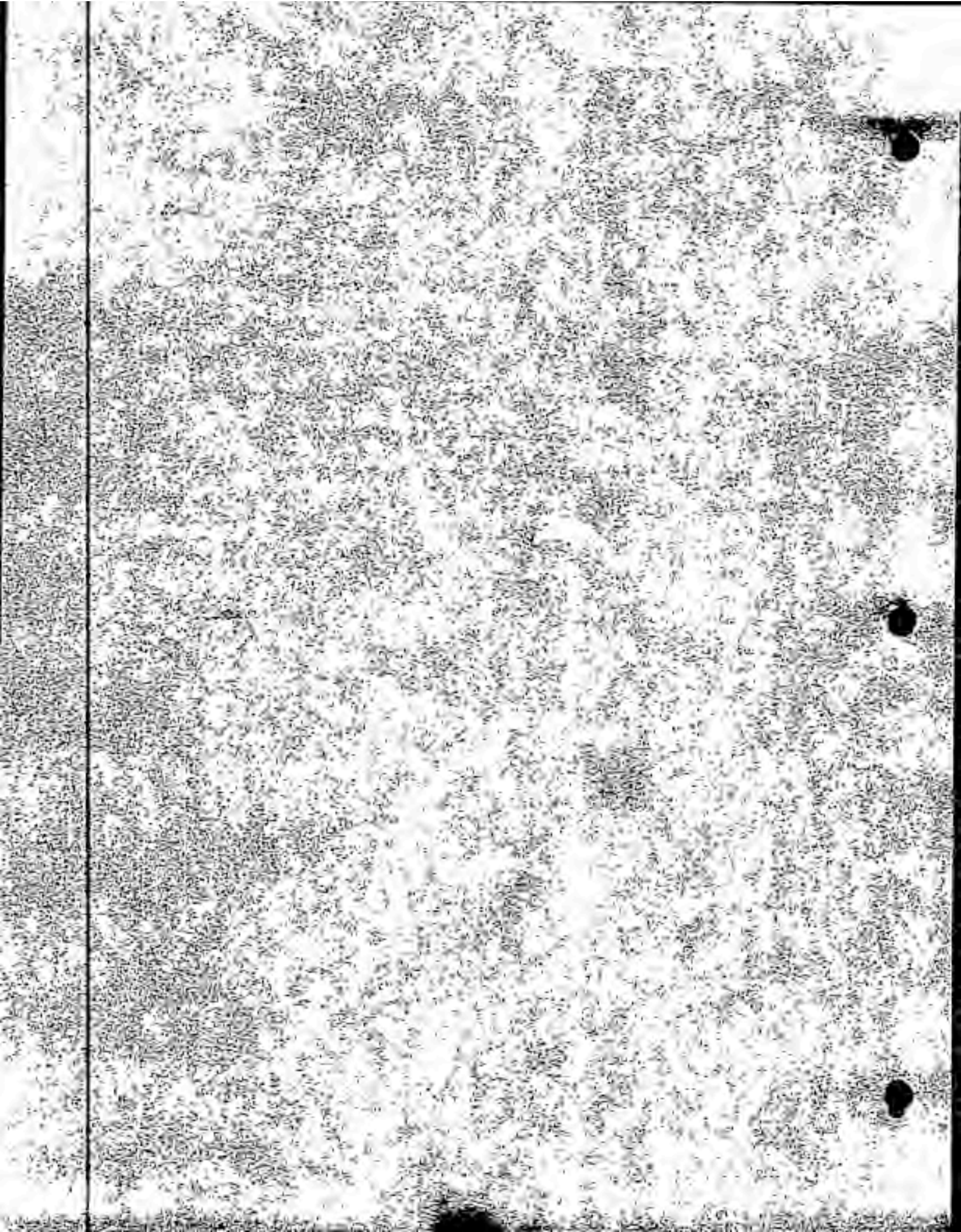
Therefore, in order to further characterize NH2Cl and ClO2 disinfection, inactivation kinetics were examined for two viruses: (1) HAV, a major waterborne pathogen, and (2) MS2, an indicator virus. Experiments were conducted using purified, monodispersed virus stocks in 0.01 M phosphate buffers at pH 6, 8, and 10. Disinfectant concentrations were at the realistic levels of 2.0 and 0.5 mg/l, respectively, for NH2Cl and ClO2. Inactivation kinetics were determined by

computing the proportions of surviving viruses at carefully measured time intervals. Viruses were assayed by plaque techniques and both disinfectants were measured using the DPD colorimetric method.

In order to compare inactivation data for the two viruses and the different test conditions, times to achieve a specified percent of virus inactivation as well as values for disinfectant concentration (C) x time for specified percent inactivation (T), or CT values were computed. In In previous studies inactivation data were treated as first-order in extrapolating to the times for 99.99% (4 $\log_{10}$) virus reduction. From examination of the experimental data from our experiments, it was evident that HAV and MS2 inactivation kinetics did not conform to the first-order model and were instead of the retardant die-off type. Subsequently, five alternative mathematical models were constructed and used to predict the kinetics of HAV and MS2 inactivation based in the experimental data. These models included: (a) a one-population model which assumes a decreasing disinfectant concentration over time, (b) a one hit, two-populations model assuming two subpopulations with different rate constants of inactivation, (c) a third model similar to (b) with the exception that the concentration of the disinfectant decreases over time, (d) a multistate model in which various stages of sublethal injury are assumed prior to inactivation, and finally (e) the distributive rate constant model, which is based upon a spectrum of

inactivation rate constants for the viruses. The measure of fit was determined for each model using the least-squares method and the results for 2, 3, and 4 $\log_{10}$ inactivation times were compared to the standard first-order regression model.

The results indicate that a large discrepancy in the predicted times is found both between the various models and within the models when experiments of different sampling time points are used. Consequently, these data suggest that the assumption of first-order disinfection kinetics underestimate the time necessary for adequate reduction of viruses in drinking water.



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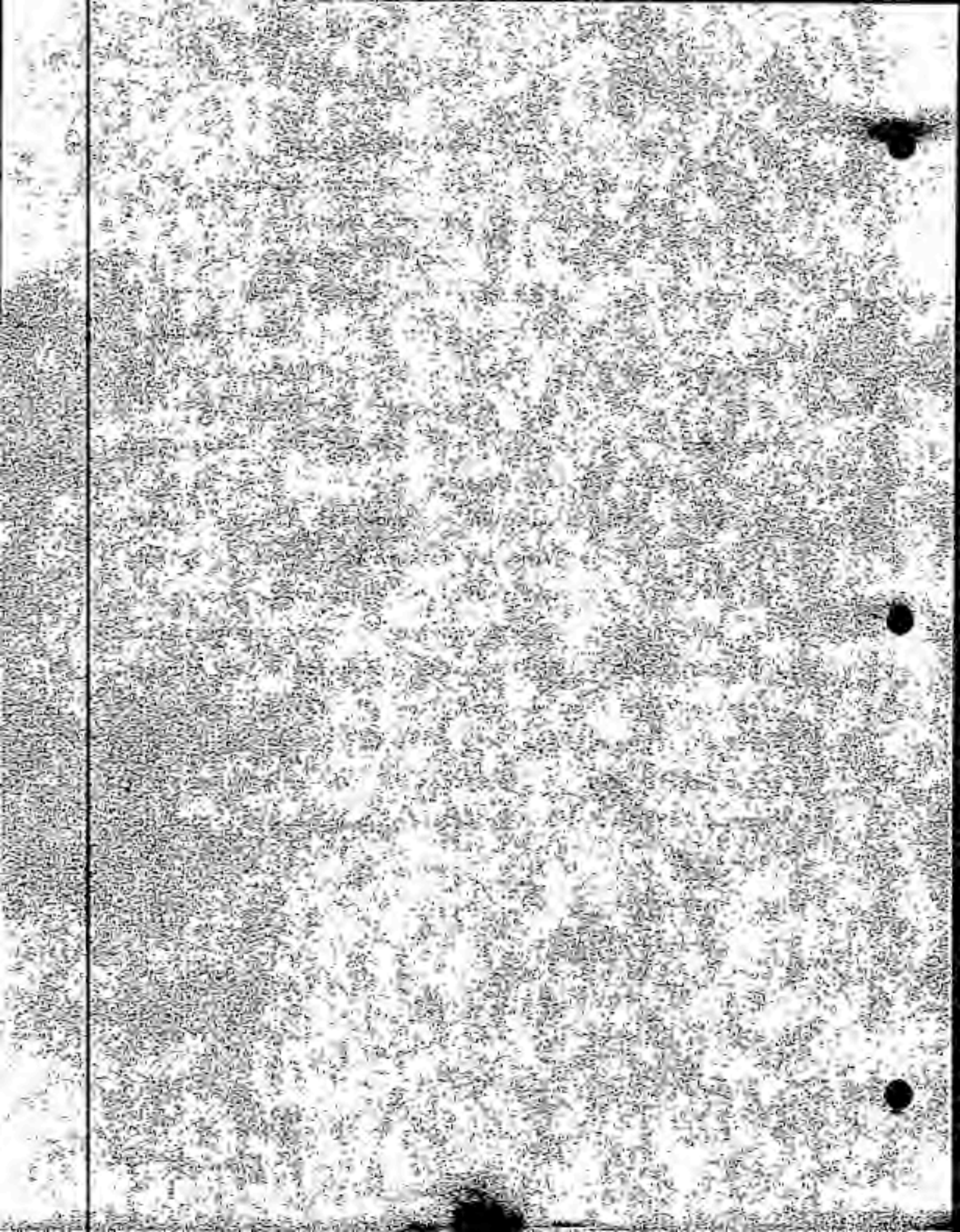


INTRODUCTION

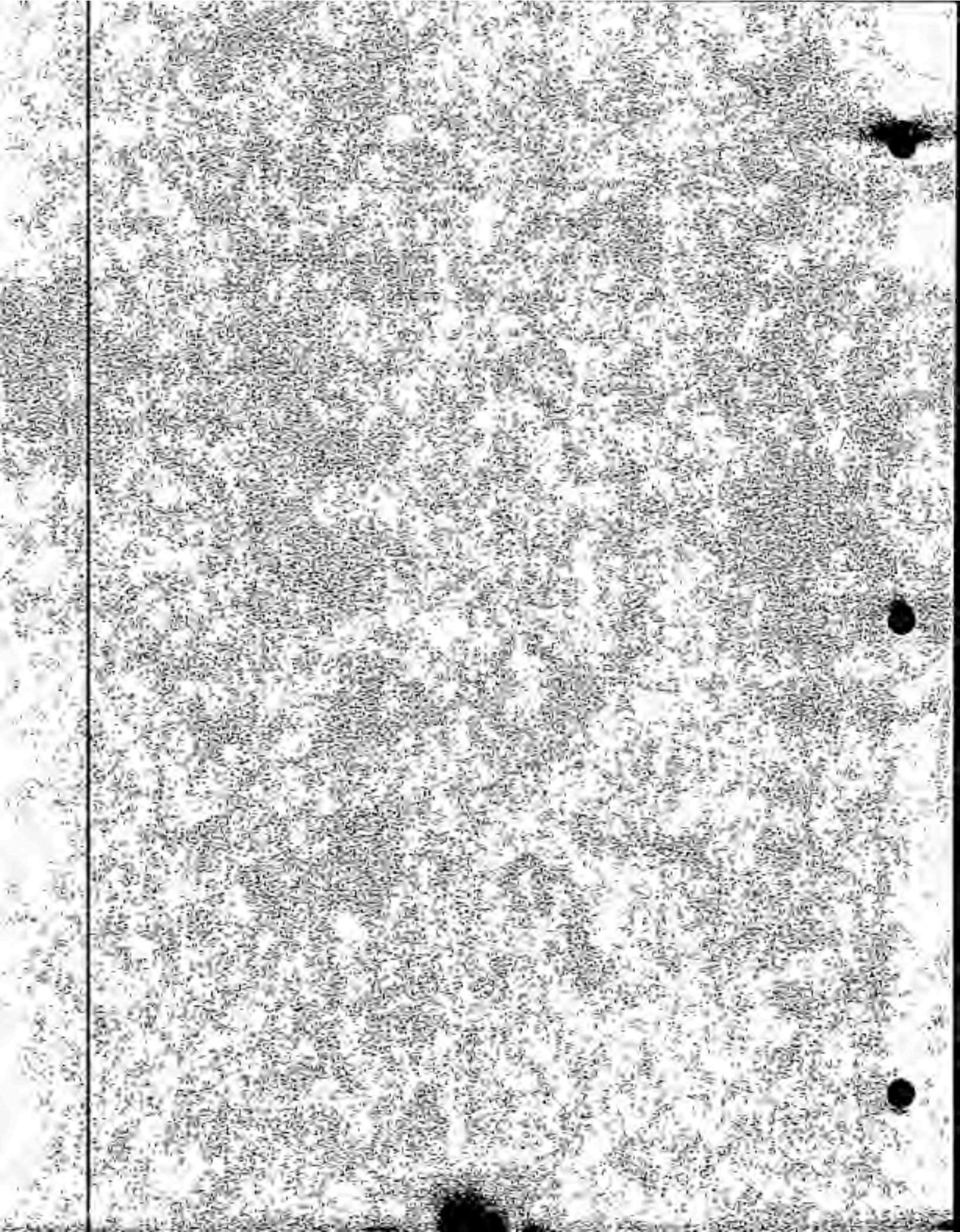
In recent years, the responsibility of water treatment plants to provide adequate removal or disinfection of pathogens has been complicated by the negative impact of disinfection by-products. With the discovery that free chlorine combines with natural organic matter to form trihalomethanes (THMs) and other by-products that are implicated as carcinogens and toxicants, the Environmental Protection Agency (EPA) and others responsible for drinking water quality are considering more seriously the use of alternative disinfectants. A drinking water disinfectant must meet the Surface Water Treatment Rule's maximum contaminant level for THMs while effectively destroying pathogens. Monochloramine has become a more attractive disinfectant due to its low THM-forming potential as well as stability in the distribution system. However, the efficacy with which microbes are inactivated by monochloramine is generally lower when compared to other agents such as free chlorine, chlorine dioxide and ozone. Chlorine dioxide use in the U.S. has been primarily limited to the control of taste and odors. It is a strong oxidant and has not been shown to produce THMs. However, other by-products are produced by chlorine dioxide such as the chlorite ion which has been shown to cause hemolytic anemia (Couri et al.,

1982) when administered to rodents via drinking water.

In 1980, the Safe Water Drinking Committee (1980) selected the CT concept (concentration of a disinfectant in milligrams per liter multiplied by the time in minutes for a specified percent inactivation) to allow comparison of various disinfectants. According to the Surface Water Treatment Rule, it is assumed that a 99.9% reduction in Giardia cysts by monochloramine will result in a 99.99% reduction of viruses, if chlorine is applied prior to ammonia. These reductions are based upon data assuming first-order reaction kinetics. Previous extrapolation studies have questioned the validity of the log-linear model (Young and Sharp, 1985; Haas and Karra, 1984). Deviations from a simple first-order relationship may be due to a number of factors, including aggregation of microorganisms, variations of susceptibility within the microbial population, and changes in disinfectant species and concentration (Chang, 1971; Hoff, 1986).

The purpose of this study was to further characterize the disinfection capabilities of preformed monochloramine and chlorine dioxide using hepatitis A virus and the model coliphage MS2 in a demand-free system. The EPA has identified the latter virus as a model organism in developing CT values and conducting pilot plant studies. The following experiments were performed: 1) One hour-long experiments to determine the role of pH as a factor in

disinfection 2) Three day-long experiments with monochloramine to document the inactivation kinetics of these organisms over longer time periods 3) and three day-long studies using monochloramine in which additional viruses or monochloramine were added to test alternative disinfection kinetic models, such as a state-vector model and a distributive rate constant model, both based on virus population heterogeneity.



LITERATURE REVIEW

2.0 DRINKING WATER AND PATHOGENS

2.0.1 Historical Perspective Prior to the development of sanitary water systems, epidemics of cholera and other waterborne infections claimed thousands of lives in urban areas such as New Delhi, New York City and London (Melnick 1971, Keswick 1984). Typhoid fever, hepatitis, and dysentery were regularly transmitted by drinking water contaminated with human waste. Today, strict measures in the U.S. ensure, at least theoretically, that human sewage does not contaminate drinking-water sources, and in most developed countries many of these diseases have been controlled or eliminated. Drinking water in most municipal water systems using surface sources is filtered and chlorinated to eliminate contaminants. However, pathogens still find their way into the water supply. Water filtration and disinfection systems break down or are poorly maintained and operated. Faulty distribution systems and cross-connections re-contaminate water delivered to the consumer. Faulty septic tanks and sewer line breaks flood or otherwise reach nearby wells.

2.0.2 Outbreaks Overall, approximately half of all waterborne outbreaks of gastrointestinal illness are

transmitted by groundwater that is inadequately treated or untreated, and nearly 25% are related to contaminated surface water. The remainder are attributed to post-treatment (distribution system) problems (Craun, 1988).

Approximately half of all waterborne outbreaks are designated as "acute gastrointestinal illness of unknown etiology". It is suspected that much of this G.I. is due to enteric viruses. Furthermore, it has been estimated that viral gastroenteritis produces 30 to 40 percent of the documented cases of infectious diarrhea in the U.S., outnumbering the documented cases of both bacterial and parasitic diarrhea (Dupont, H. L. and Pickering, LK, 1980). Acute gastroenteritis is the second most common infectious disease in the U.S. (to respiratory infections), with about 1-1.5 episodes per person per year. Much G.I. illness occurrs as individual endemic cases and "household epidemics", but other sources and settings of epidemic G.I. are well documented, including outbreaks in camps, hospitals, day care facilities and nursing homes as well as contaminated water or food.

2.0.3 Enteric Viruses Several characteristics of enteric viruses and that influence their risk of infection through contaminated water include: 1) their general persistance in water, in which they can survive for weeks or months, 2) their inability to multiply in water because they are obligate parasites and the need for a suitable host to

initiate a new multiplication cycle, and 3) their low infective dose, with as few as one infectious virus units capable of initiating infection in a susceptible host. Several studies have therefore been conducted to determine whether drinking water provided by municipalities is free of viral contamination.

Coin was the first to isolate viruses from drinking water in France, with the cause attributed to inadequate treatment (settling and marginal chlorination). Currently, Rose (1990) claims that " viruses can be recovered from treated drinking water because approximately 53 percent of the reported isolations came from water with complete treatment, while 26 percent came from water which was only disinfected and 15 percent came from untreated water." According to one study by Payment et al. (1989), " Virus were detected in seven percent of the finished water samples... and the water met the current standards of water quality".

Of the many viruses which can be found in potential drinking water sources, hepatitis A virus has been studied recently and is used in disinfection experiments which serve as the basis for U.S. EPA drinking water regulations.

2.0.4 Hepatitis A Virus The main characteristics of Hepatitis A virus (HAV) are listed in Table 2.1. HAV was the first viral disease to be conclusively shown to be transmitted by water (Gerba, et al., 1985). Waterborne

disease outbreaks of HAV continue to pose a public health threat in both the U.S. and developing countries. According to Mosley (1967) and Goldfield (1976), more than 80 outbreaks of HAV traced to contaminated drinking water have been documented between the years 1967-1972.

Hepatitis A is also transmitted by person to person contact and ingestion of contaminated food, such as bivalve molluscan shellfish. According to the Viral Hepatitis Surveillance Program (VHSP), contact with a person infected with hepatitis A, association with a day care center, and international travel were factors strongly linked to acquiring the disease (MMWR,Vol. 34, No. 1SS,1990) Overall, the rates of infection by hepatitis A have remained fairly constant in the years 1981-1990 (see Figure 2.0). The reported number of cases for the year 1990 is 31,441 (MMWR, Vol. 39, No. SS-1, 1990).

Hepatitis A was identified by radioimmunoassay as the causative agent of a waterborne outbreak involving 36 cases of illness in Georgetown, Texas. The outbreak was traced to pathogens in the drinking water from city wells (Hejkal, et al., 1982). Since then, more efficient methods of HAV concentration and detection have been developed which have associated hepatitis A with more outbreaks and cases of waterborne illness (Sobsey et al., 1985; Bosch, et al., 1991): Due to the severity with which HAV manifests itself in the host, the high levels of its excretion by infected

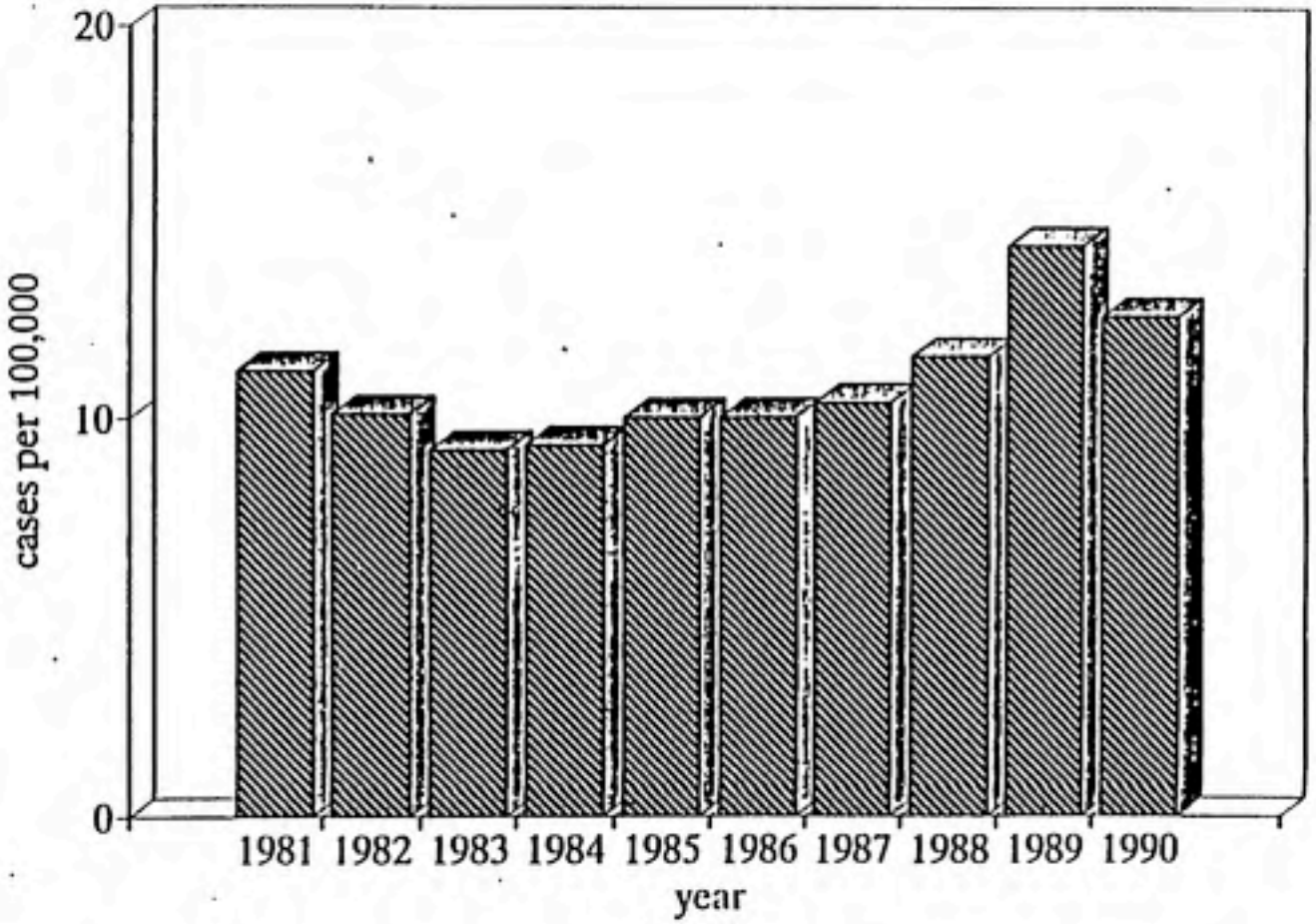
individuals, its persistence in the environment and in treatment processes and the documented evidence that it causes waterborne disease (Sobsey, 1988; Rao, 1988), this virus has been chosen by the U.S.EPA as the target virus for which disinfection criteria are to be established.

Table 2.0 : Characteristics of Hepatitis A Virus

Feature	Description
Family/Genus	Picornaviridae/Enterovirus 72
Size/Genome	27-30 nm/ ssRNA
Incubation period	15-40 days
Route	fecal-oral
Host range	humans and possibly other primates
Seasonality	higher in fall and winter
Age group	much higher incidence in children
Virus in blood and feces	2-3 wks prior to illness, 1-2 wks after recovery
Effective vaccine	currently being developed

Adapted from Volk et al. , 1991

TABLE 2.0: CASES OF HEPATITIS A VIRUS
OF INDIVIDUALS REPORTED IN THE US



2.0.5 Bacteriophages: MS2

As mentioned previously, the difficulty in isolating human enteric viruses from environmental samples, as well as the hazards involved in their use in pilot plant studies, have led to the use of surrogate model of fecal contamination indicators. A list of attributes for an ideal indicator were described by Bonde (1966). They include:

1) presence when pathogen is present
2) presence only when the presence of pathogens is an imminent danger (no proliferation to any greater extent in the environment)
3) occurrence in much greater numbers than pathogen
4) greater resistance to the environment and disinfectants than pathogens
5) grow readily on relatively simple media
6) yield characteristic and simple reactions enabling an unambiguous i.d. of the group
7) random distribution in the test sample, or ability to obtain random distribution by simple homogenization
8) growth independent of other organisms present when inoculated in artificial media

Bacteriophages are very similar to enteric viruses both physically and in their relative resistance to chemical disinfectants (see Table 2.1). The coliphage group are candidates as potential indicators of fecal pollution because they infect E. coli (which are found in the gut of warm-blooded mammals). They are found in high numbers in sewage and polluted water sources, and are not known to multiply in the environment. Male-specific coliphages, such

as MS2 and fd, are a subgroup which, by virtue of their ability to attach to their bacterial hosts only at elevated temperatures (the bacteria's receptor sites are only produced at or near body temperature) have been promoted as a model indicator organism. MS2 has been studied previously as to its resistance to disinfectants relative to other pathogens (Fuji,1988; Grabow, 1983; Kruse, 1968). It was therefore included in this project to furnish more complete information about its inactivation kinetics by realistic doses of both monochloramine and chlorine dioxide.

Table 2.1: Characteristics of Bacteriophage MS2

Feature	Description
Family	Leviviridae
Size/genome	24 nm/ ssRNA
Morphology	icosohedral/no tail
Host	male *E. coli*
Attachment site	F-pili of *E. coli*
Inactivation kinetics compared to HAV by 10 mg/L NH_2Cl;pH 8 and 0.5 mg/L free chlorine, pH 6, 8, and 10	relatively similar*

* See technical report by Fuji (1988)

2.0.6 Detection Methods Frequently, common source outbreaks due to water and food vehicles are over before the public health officials can collect and analyze samples in a timely fashion. Even more germane to the difficulties involved in identifying the infectious agents that cause epidemics are the paucity of methods for detecting viral pathogens in both clinical and environmental samples.

In the case of environmental samples, where concentrations of the causitive agent are likely to be relatively low, the media itself may contain interfering parameters such as organic material, turbidity, and high salinity (Safe Drinking Water Committee, 1977) that bias testing. This is especially pertinent for assays designed to detect viruses. Current virological techniques involving cell culture and radioimmunofocus assays require expensive materials and are cumbersome, or are not possible with several as yet non-culturable viruses such as the Norwalk virus. Methods presently being developed include polymerase chain reaction and gene probes. A caveat exists, being that " a disadvantage of utilizing a new method is the lack of rigorous testing and confirmation by other investigators that conventional methods often have undergone" (DeLeon and Sobsey, 1991). If these novel methods can be validated, their rapidity and high sensitivity will ameliorate the ability to detect viruses in environmental samples.

2.0.7 Drinking Water Regulations In order to regulate the quality of drinking water, the U.S. EPA has embarked on the establishment of a series of drinking water regulations that will require states to assure disinfection of all ground water sources and filtration and disinfection of all surface water. The Surface Water Treatment Rule (SWTR) requiring mandatory filtration and disinfection has already been promulgated. Microbial contaminants to be regulated included enteric viruses, Giardia lamblia, Legionella and heterotrophic plate count bacteria (Federal Register, 1989). The 1986 ammendments to the SDWA require all public water suppliers, including those from both ground and surface waters, to disinfect drinking water prior to distribution. Specific objectives of disinfection include:
1) A 3 log and 4 log inactivation of Giardia lamblia cysts and enteric viruses, respectively 2) assure control of other harmful microorganisms 3) not impart toxicity to the disinfected water 4) minimize the formation of undesirable disinfection by-products and 5) meet the Maximum Contaminant Levels (MCLs) for the disinfectants used and by-products that may form. According to the goals defined by the American Water Works Association (AWWA) Disinfection Committee,"a functionally ideal water should contain no pathogenic organisms and be free from biological forms that may be harmful to human health or aesthetically objectionable" (Disinfection Committee Report, 1982).

Despite these goals and regulations, the task of regulating the more than 59,000 community water systems (public or investor-owned water companies that serve 25 or more year-round residents) and 140,000 noncommunity systems (such as those found in institutions and parks) is infeasible (Regli, personal communication). In 1985, 60 percent of all waterborne outbreaks occurred in noncommunity water systems (Craun, 1986). Another 40 million people get their drinking water from private wells and other individual systems (Gerba et al., 1985).

2.1 Treatment of Drinking Water

2.1.1 Historical Background The lethal cholera epidemic of 1854 in London ironically provided an opportunity for a milestone in public health to be established. Dr. John Snow suggested water as being the means of transmission of the disease, and as an experiment removed the handle from the pump which delivered the suspected water. He subsequently eliminated the outbreak, and the link between contaminated water and illness was made (Cohen and Snow, 1969). The rapid development of the discipline of microbiology during the second portion of the 19th century clarified the role of bacteria and later viruses as the agents responsible for many waterborne diseases.

The removal and destruction of disease-producing

contaminants in polluted drinking water has been studied and methods of achieving this goal have been developed. One of the first attempts to counter an epidemic of typhoid fever by disinfection was at the Austro-Hungary naval base of Pola in 1896 by using bleaching powder. In the U.S. the first full-scale application of gaseous chlorine to a public water supply took place at Wilmington, DE, in 1913 (Houston, 1913).

2.1.2 Current Practice Contemporary methods of water treatment in the U.S. consist of 1)screening 2) coagulation/flocculation 3) sedimentation 4) filtration and 5) disinfection. The multiple barrier concept .....The predicted efficiency of these processes with respect to removal of viruses and Giardia is listed in Table 2.2. It is clear that although processes prior to disinfection are effective to a limited extent, it is this step which provides the highest degree of elimination of pathogens.

Table 2.2: Expected Virus and Giardia Reductions in a Water Treatment System

Treatment Process	Log 10 Reduction	
	Viruses	Giardia
Coagulation/flocculation and sedimentation	2	2
Direct filtration	1	2
Slow sand filtration	2	2
disinfection (free chlorine)	3+	4

Adapted from Federal Register, Vol. 54, No. 124

The most recently established guidelines pertaining to water treatment and microbial contaminants were promulgated in 1989 as the Surface Water Treatment Rule. Regulations require finished water to have a 99.9% removal of Giardia cysts and a 99.99% removal of viruses. A system is considered in compliance with this requirement if it utilizes the treatment technology requirements specified in the rule. Groundwater regulations currently being developed will require mandatory disinfection of all drinking water supplies derived from ground water sources. The target organisms to be controlled are enteric viruses, with HAV again cited as a main concern.

2.2 Disinfection

All of the standard methods of water treatment in the U.S. involving physico-chemical methods (e.g. coagulation-flocculation, filtration, adsorption) remove potentially harmful microorganisms to some extent, thus reducing their numbers prior to the final or terminal disinfection stage. However, it is this latter process which functions specifically to destroy infectious agents in the water.

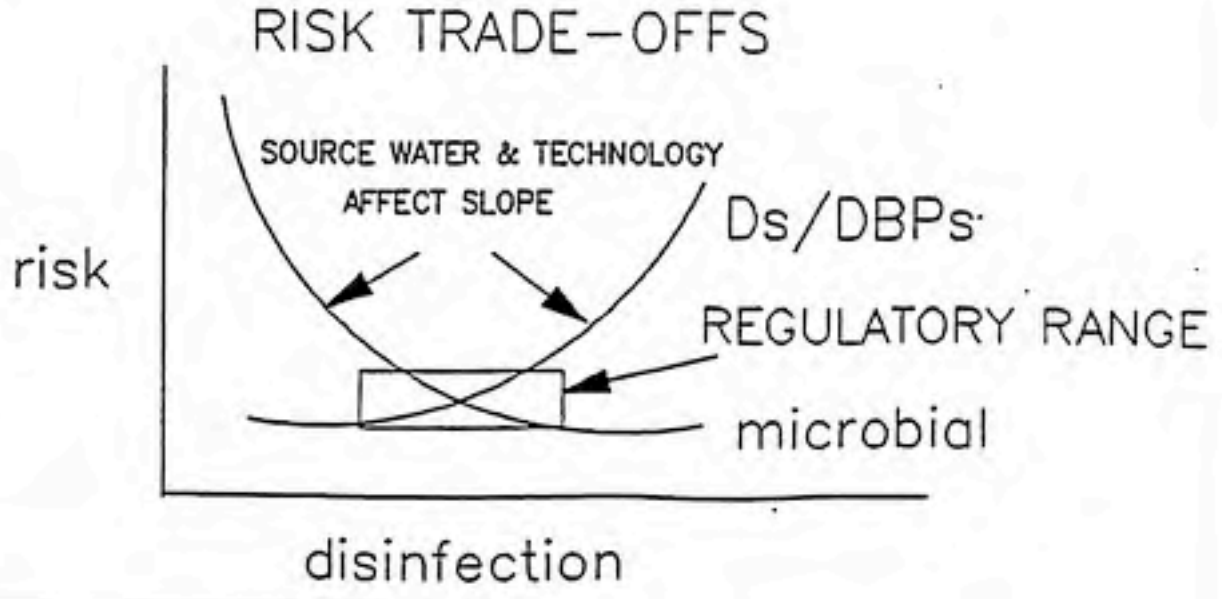
Disinfecting agents can be classified into the following general groups: (1) physical agents such as heat and ionizing and UV radiation (2) oxidizing agents (3) cations of heavy metals (4) quaternary ammonium and pyridine compounds and (5) others. Ideally, a disinfectant used in water treatment should eradicate the causative microbes of waterborne infectious diseases at concentrations which are harmless to the public, while not producing any offensive odors or tastes. Additionally, the disinfectant should be economical and provide residual protection in the distribution system. Realistically, no one disinfectant has all of these qualities. In the past, the method of choice was chlorination. However, studies by Rook (1974) and Beller (1974) suggested possible health risks associated with the formation of haloforms during chlorination. Haloforms are produced by the reaction of free chlorine with naturally occurring humic and fulvic substances. Subsequent nationwide

investigations were conducted by the EPA which identified trihalomethanes as a major organic constituent produced in drinking water during chlorination. A significant association was confirmed between bladder cancer and the levels of haloforms in drinking water by Cantor (1977) using information gathered by the National Organics Reconnaissance Survey (NOMS).

Although further research is necessary in the field of disinfection by-products, one result of the preceding studies was the set of regulations created by the 1979 treatment rules aimed at reducing the levels of trihalomethanes to 100 ug/l in finished waters (Federal Register 44, No. 231).

Consequentially, a search for alternatives to chlorine are being explored. The risk trade-offs must be balanced between the original goal of disinfection in reducing the chances of waterborne illness and the long-term cancer risks of disinfection by-products (see figure 2.1).

FIGURE 2.1



Proposed alternatives to chlorine include ozone, chlorine dioxide, monochloramine, and UV light. A comparison of their advantages and disadvantages are made in Table 2.3. Monochloramine and chlorine dioxide, used in this study, are discussed in further detail in the following sections.

Table 2.3: Comparison of Disinfectants

Disinfectant	Advantages	Disadvantages
Chlorine	Effective;widely used; Variety of application points.	Halogenated by-products
Ozone	Very effective; few harmful byproducts	Requires secondary disinfectant; stimulates microbial growth in water.
Chlorine dioxide	Effective;low cost;low THM production	Some harmful byproducts; generated on-site;may not persist in the distr. system.
Monochloramine	Long-lasting residual low THM production;low cost	Some harmful byproducts; poor biocide

2.3 Monochloramine History

Chloramines were first applied to water treatment in Ottawa, Canada in 1917 by Race who was seeking an alternative to sodium hypochlorite (Race, 1918). Since that time, monochloramine usage in the U.S. became popular in the 1930s as a technique to control taste and odor problems and regrowth of bacteria in distribution systems (Ruth, 1931; Skinner, 1932). However, the understanding of breakpoint chlorination (Griffin and Chamberlain, 1941) and the shortage of ammonia during WWII led to a decrease in the use of chloramines in general.

A resurgence of interest in monochloramine was driven by the discovery that chlorination produced by-products harmful to human health. Monochloramine is not as strong an oxidizer as chlorine and is less reactive in water. Recent toxological studies by Moore (1982) have suggested that monochloramine is not absorbed into the blood stream and that the liver and kidneys are able to detoxify and excrete any harmful products. Nevertheless, other studies have shown that kidney dialysis patients were at risk of complications due to the inability of facilities to remove chloramines from the water used to make their dialysate solutions (Krasner et al., 1986). As of now, no reference dose has been devised based on either noncancer health effects or cancer assessment for monochloramine.

2.4 Monochloramine chemistry

Monochloramine can be produced in three ways: (1) adding chlorine to water containing ammonia (as is often the situation in wastewater treatment) (2) adding ammonia to water containing chlorine, and (3) mixing the two compounds together prior to application. When water, chlorine and ammonia are combined, the following reactions are observed:

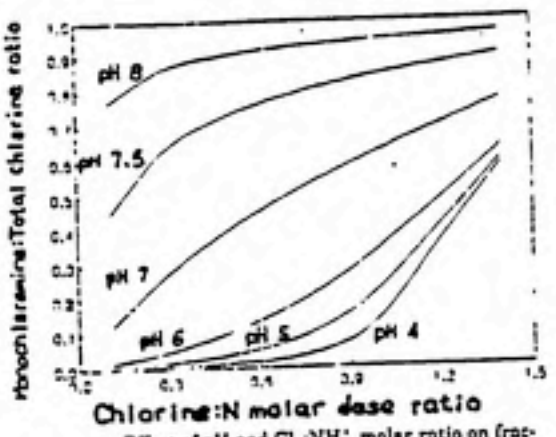
$NH_3 + HOCl = H_2O + NH_2Cl$ **(monochloramine)**

$NH_2Cl + HOCl = H_2O + NHCl_2$ **(dichloramine)**

$NHCl_2 + HOCl = H_2O + NCl_3$ **(nitrogen trichloride)**

The species of chloramines produced depends upon several factors, with the ratio of ammonia to chlorine and the pH values having a strong influence on the resulting dominant species (see Figure 2.2). The product at a pH of 8 and higher is predominantly monochloramine when a > 3:1 ratio of ammonia:chlorine is used. In the pH range of 5-8, a mixture of mono- and dichloramines are produced. Dichloramine is formed readily between pH 4-5, and is catalyzed by the presence of H_3O^+. Excess ammonia will retard the reaction rate (White, 1972).

Figure 2.2:Effect of pH and Cl_2:NH4+ Ratio on Monochloramine Formation



Effect of pH and Cl_2:NH_4^+ molar ratio on fractional amount of combined chlorine existing as monochloramine at equilibrium at 20°C.

2.5 Monochloramine: Mode of Inactivation

Several studies have been performed to elucidate the mechanisms involved in inactivation of pathogens by monochloramine. This subject is important because if a disinfectant is only capable of destroying the protein structure and not the infectious genetic material, it may still allow the pathogen to cause disease in humans. According to a study by Fujioka et al. (1981) monochloramine inactivates poliovirus by attacking the protein coat, while

the extracted RNA was shown to still be viable. More detailed research using the bacteria E. coli has indicated that "monochloramine reacts with the whole nucleic acid and with free purine and pyrimidine bases rather than with nucleotides or nucleosides...and reacts more readily with amino acids than with nucleic acids." (Jacangelo and Olivieri, 1986). In light of these somewhat conflicting results, caution should be exercised when considering the use of monochloramine. Further work should examine whether microorganisms exposed to monochloramine are indeed still infectious in humans and other hosts.

2.6 Previous Virus Disinfection Studies Using Monochloramine

Several experiments have been conducted which used combined chlorine, but did not distinguish the exact proportions of mono- di- or trichloramines in the reaction. For example, Kelley and Sanderson (1960) examined the inactivation kinetics of coxsackie and polio viruses using chloramine concentrations of 1 mg/L. At pH 10, where monochloramine can be assumed to be the dominant species, 3 $\log_{10}$ inactivation was not reached for both viruses after more than five hours.

Additional work by Shah and McCamish (1972) using coliphages T_2 and f_2 and poliovirus I also indicates the high resistance of pathogens to monochloramine. A 99% reduction in poliovirus titer took 45 minutes, while the f_2

phage was markedly more resistant, requiring over 190 minutes. Unfortunately, although the concentration of combined chlorine was reported as 4 mg/L, neither the pH used nor the speciation of chloramines was reported.

The inactivation kinetics of f_2 coliphage upon exposure to monochloramine was further studied by Michael Snead (1974). Using a demand-free system and a range of disinfectant concentrations, he demonstrated that the rate of inactivation was dependent on monochloramine concentrations only if they exceeded 4 mg/L. The bacteriophage also followed a biphasic pattern of inactivation, with scarcely more than a 99% reduction in titer after 3 hours.

In a recent review article (Sobsey,1989) it was summarized that "studies on indicator bacteria such as E. coli, coliforms and HPC bacteria and pathogens such as Salmonella and Shigella show that chloramine residuals of 1-2 mg/L and contact times of up to hours are needed to produce appreciable inactivation. Furthermore, compared to these bacteria, some other health-related bacteria such as mycobacteria as well as viruses (e.g. HAV and rotaviruses) and Giardia cysts are extremely resistant to chloramines."

Cell-associated and dispersed HAV were studied under demand-free conditions using monochloramine concentrations of 10 mg/L (Sobsey et al., 1988). While the cell-associated form was about 40% more resistant than dispersed virus, both

forms of HAV indicated a strong resistance to monochloramine, with CT values for 4 $\log_{10}$ (99.99%) inactivation estimated at 1,740 and 1225 mg-min/L.

While a considerable amount of research has been performed regarding monochloramine inactivation of viruses, a significant lack of quantitative information is available concerning the doses and times necessary to achieve specific levels of inactivation of some important pathogens, including HAV at realistic doses and over a range of typical pH levels in drinking water. This is especially pertinent to the Safe Water Drinking Water Act, the SWTR and its future amendments and the forthcoming groundwater disinfection rule. The disinfection requirements of these rules will have profound effects on drinking water treatment practices in the U.S.

2.7 History of chlorine dioxide

Chlorine dioxide was initially produced by Davy from the reaction of potassium chlorate and hydrochloric acid in 1811 (Miller, et al., 1978). It has been used extensively as an industrial bleaching agent (White, 1972) but only recently has it been employed by the water industry, mainly to control taste and odor problems. From a recent of survey of large water utilities serving more than 50,000 people (of which there were 438), 45 used chlorine dioxide in their pre-oxidation step and only 9 used it as a post-disinfectant

for surface water (Water Information Database, 1991). Recent interest in chlorine dioxide is largely a result of the upcoming Federal regulations on disinfection by-products, and the current concern over THMs.

Similarly to monochloramine, chlorine dioxide has also been stringently tested for potential toxicity or carcinogenicity for humans. The principal concern with the use of chlorine dioxide is the potential toxicity of the chlorite and chlorate ions produced. White (1972) substantiated the general acceptance of the production of chlorite being the end-product of ClO_2 reactions in water. Hefferman et al. claim that chlorite " carries the oxidation of hemoglobin to methoglobin in vivo" and recommend the concentration of chlorite to be zero due to the adverse effect upon nursing babies when the oxidation reaction takes place. (Hefferman, 1979). In 1987, the Subcommittee on Disinfectants and Disinfectant By-products concluded that thyroid and neurological disorders observed in laboratory animals could be due to the oxidation of dietary iodine by chlorate in the intestinal tract (Lykins et al., 1990). In contrast, in a prospective epidemiological study, Michael et al. (1981) compared 197 people exposed to water treated with chlorine dioxide (avg. conc. of 0.7, 5.1 and 0.7 mg/L of ClO_2, chlorite and chlorite, respectively) to 112 unexposed subjects and found no increased risk of adverse health effects associated with the use of ClO_2. Due to the

conflicting reports, the USEPA currently recommends a maximum combined concentration of chlorine dioxide and its by-products of no more than 0.5 mg/L. Analyses are now in progress to better define the health effects criteria for chlorine dioxide and its by-products in drinking water.

2.8 Chlorine dioxide chemistry

At room temperature, chlorine dioxide exists as a yellow-greenish gas. It has an irritating odor above concentrations of 45 ppm, and due to its instability must be generated on-site (White, 1972). One of its most significant properties is its solubility in water, which is five times that of chlorine. The solubility of chlorine dioxide is 2.9 g/L at room temperature (Gordon, 1972).

The most routine method of generating chlorine dioxide by the water industry is to react a strong chlorine solution having a minimum concentration of 500 mg/L with a concentrated stream of sodium chlorite (minimum concentration of 300 mg/L). Other researchers have found that using an excess of chlorine both prevents the potentially toxic salt from getting into the water supply and creates the optimal conditions for rapid conversion and nearly 100% yield of the chlorine dioxide. The reaction is as follows :

$$2\ NaClO_2 + Cl_{2\ gas} = 2\ ClO_2\ gas + 2\ NaCl$$

The formation of the undesirable by-products, chlorite and chlorate, occur mainly at pH values greater than 11 and even then is a slow kinetic process (Masschelein, 1979). However, even at neutral pH chlorine dioxide has been shown to disproportionate upon reacting with organics in the water with the main by-product being the chlorate ion (Rav-Acha, et al., 1983):

$$2\ ClO_2 + 2OH^- = ClO_3^-\ \text{(chlorate)} + ClO_2^-\ \text{(chlorite)} + H_2O$$

Several methods have been developed to reduce these unwanted by-products including: (1) passage through a granular activated carbon (GAC) column (2) reduction by sulfur dioxide, and (3) reduction by ferrous chloride. Though no process is 100% successful, progress has been made in significantly reducing the levels of chlorate and chlorite in drinking water.

2.9 Chlorine dioxide: Mode of Inactivation

Several studies have been performed with both bacteria and viruses to determine the mechanism of attack of chlorine dioxide. Roller (1980) stated that "ClO_2 does not appear to inactivate bacteria by altering the DNA and oxidizes the thiol group to the sulfoxide or sulphone stage, which is biologically irreversible." Alverez and O'Brien (1982) used

poliovirus 1 in their study and found that ClO_2 inactivates the virus by reacting with the viral RNA and impairing the ability of the viral genome to act as a template for RNA synthesis. They also examined the hypothesis that the disassociation products of chlorine dioxide were responsible for the observed inactivation, especially at higher pH. However, the measured amount of chlorate and chlorite present was less than 5% of the total chlorine dioxide species and therefore could not account for the virucidal action. They concluded the increased inactivation at elevated pH was due to the elevated sensitivity of viruses under alkaline conditions.

Since chlorine dioxide appears to act upon the viral RNA and irreversibly damages bacteria, it may be capable of completely destroying these pathogens. Further research with other microorganisms needs to be conducted to determine if this assumption is justified. If it holds true, chlorine dioxide could be used with confidence, at least with respect to its biocidal ability.

2.10 Previous disinfection studies using chlorine dioxide

Several investigations have reported that chlorine dioxide disinfection capabilities are enhanced at higher pH values using *E. coli*, bacteriophages, and enteroviruses (Brett and Ridgeway, 1981; Noss and Olivieri, 1985; Scarpino *et al.*,1979). In a recent report by Chen and Vaughn (1990),

rapid inactivation of rotavirus was observed at pH 8. A 99.9% reduction in virus titer was achieved after 10 seconds versus >600 seconds at pH 6 and 8 respectively, with a ClO_2 residual of 0.05 mg/L. In the same study, under identical conditions it was concluded that under acid or neutral pH, ClO_2 was inferior to ozone and chlorine while above pH 7 ozone was still the most effective virucide, followed by ClO_2 and lastly chlorine. Unpublished data by Sobsey and Battigelli again suggest enhanced inactivation of HAV and coliphage MS2 at pH 9 versus pH 6 in a buffered demand-free system. Similar results were reported by Bedulvich et al. (1953), in which ClO_2 possessed a higher bacteriocidal efficiency than chlorine against E. coli, Salmonella typhi, and Salmonella paratyphi.

Giardia has also been targeted by the Surface Water Treatment Rule, in which a 99.9% removal is required by drinking water utilities. The effect of pH on ClO_2 inactivation of Giardia muris is analogous to that of other pathogens, with the protozoan being slightly more resistant to ClO_2 than viruses or bacteria.

No information at incremental pH values between 6-10 and otherwise constant conditions are available concerning inactivation kinetics of viruses by ClO_2. Such data would be useful in establishing regulations for disinfection of drinking water supplies derived from ground or surface sources.

2.11 Disinfection as a kinetic process

The first documented study of the process of disinfection was realized by Kronig and Paul (1897). Samples of surviving microorganisms taken at precise intervals during the disinfection experiment were quantified . Analysis of their data indicated that the disinfection process occurred in an orderly manner and that the rate of inactivation decreased as the number of survivors diminished. A decade later, Madsen and Nyman and Harriet Chick independently concluded that during the process of chemical disinfection of anthrax spores (using phenol and mercuric chloride), the surviving fraction over time followed a logarithmic pattern. In essence, the number of spores destroyed per unit time was proportional to the number present in a unit volume of the medium at that moment. These early observations became the foundation for the exponential law describing the action of disinfectants over time in their ability to destroy various microorganisms.

Chick's Law (Chick, 1908), widely accepted due to its simplicity and convenience, considers the rate of inactivation of microorganisms to follow a first-order relationship dependent on the number of organisms present at any instant:

$$N/No = e^{-kt}$$

where N = number of organisms at time t

No = number of organisms at time 0

k = proportionality constant

t = time

Ideally, the plot of the log of N/No against time should produce a straight line. Although this is often the case with chemicals, this is seldom the result with microbial suspensions. Several proposed reasons for this deviation from first-order kinetics include: (1) resistant subspecies (2) aggregated organisms (3) the presence of several inactivation sites or "targets" on the organism (4) changes in the properties of the disinfectant, and (5) interfering agents in the suspension media, such as particulates.

With the development by Salk of the formaldehyde-inactivated polio vaccine (Salk, 1954), extrapolation from experimental data became necessary in determining inactivation kinetics below the level of detection. Accuracy became an issue which had direct ramifications on human health. Since then, the use of kinetic models to describe and compare disinfectants used in water treatment has functioned as the basis for current regulations.

The currently accepted method of analyzing disinfection data in the U.S. has been termed the "CT concept". It is

based on the empirical relationship $k = C^n t$ known as Watson's Law, where :

k = rate constant of inactivation

C = concentration of disinfectant

t = time

n = coefficient of dilution

Baumann and Ludwig (1962) proposed the use of this relationship in predicting the time and concentration of disinfectant necessary to achieve a certain reduction in microorganisms, given a specific pH and temperature. This idea was disregarded until 1980 when the Safe Drinking Water Committee selected CT values as the method for comparing biocidal efficiency. As mentioned earlier, deviations from first-order kinetics limit the definitude of this method. Table 2.3 lists the CT values for several microorganisms when the disinfectants monochloramine and chlorine dioxide are used.

Table 2.3: CT Values of Various Microorganisms

Organism	Disinfectant	Temp. (°C)	pH	CT value* (mg*min/L)
E. coli	ClO_2	5	6.5	0.60
E. coli	ClO_2	20	6.5	0.29
poliovirus I	ClO_2	5	7.0	3.60
poliovirus I	ClO_2	25	7.0	0.90
simian rotavirus	ClO_2	5	6.0	0.22
simian rotavirus	ClO_2	5	10.0	0.18
giardia muris	ClO_2	5	7.0	11.2
giardia muris	ClO_2	25	7.0	5.30
E. coli	NH_2Cl	5	7.0	22.0
E. coli	NH_2Cl	25	9.0	37.0
poliovirus I	NH_2Cl	5	9.0	1420
poliovirus I	NH_2Cl	25	9.0	216
simian rotavirus	NH_2Cl	5	8.0	4034
giardia muris	NH_2Cl	3	7.0	496
giardia muris	NH_2Cl	15	7.0	848

*CT value for 99% inactivation

Adapted from Hoff, 1986.

The USEPA has stated that "the CT values for ClO_2, O_3, and NH_2Cl are based on limited data compared to the more extensive data that provide the basis for the Cl_2 CT values, and that, for these disinfectants, new data are more likely to become available in the near future that may support different CT values or other means of determining what percent inactivation of Giardia cysts and viruses a disinfectant achieves."

A variety of different curves generated from disinfection experiments are depicted in Figure 2.3:

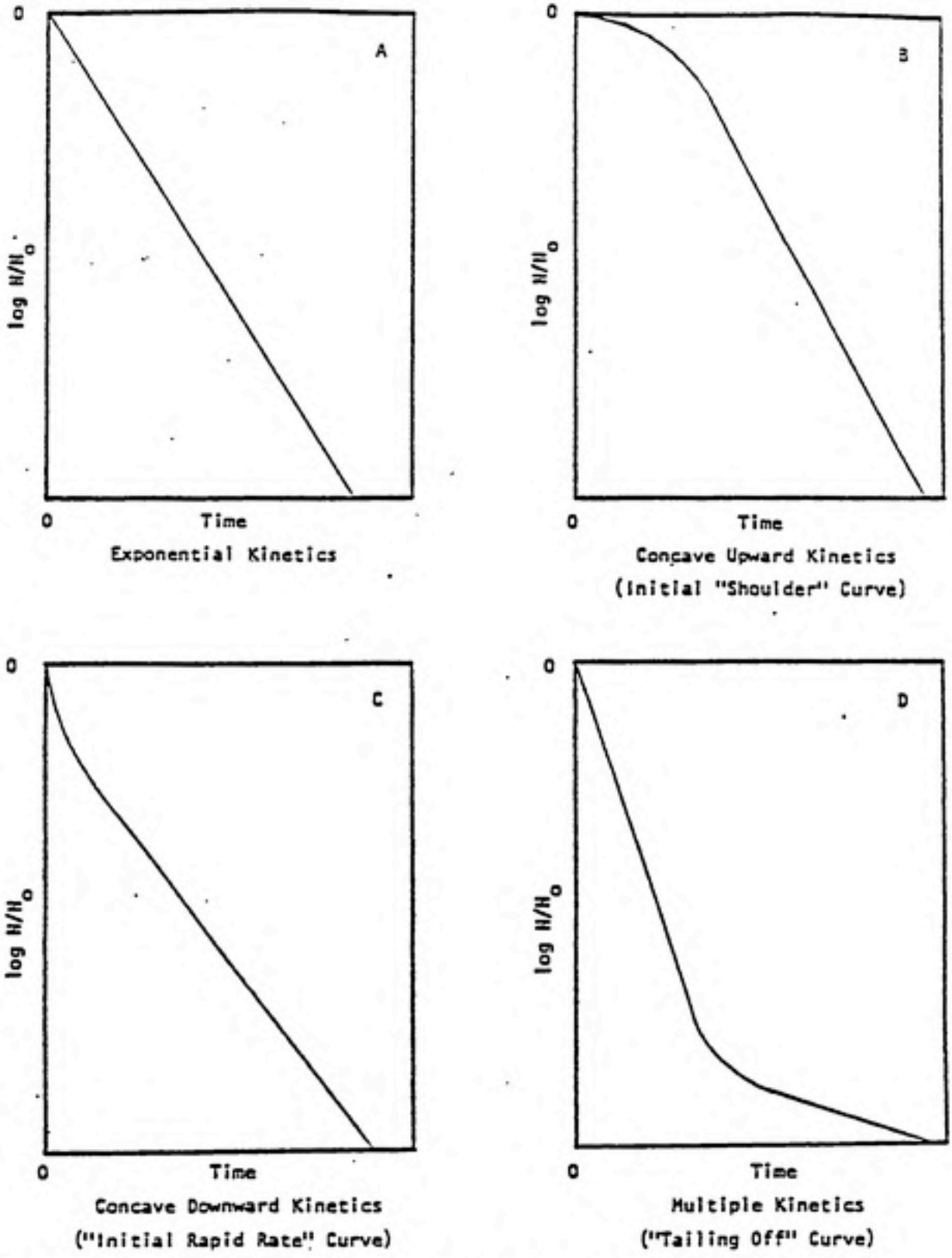


Figure Typical survival curves for disinfection experiments.
After: Prokop and Humphrey (1970)

Several attempts have been made to construct mathematical models which better comply with the disinfection curves displayed previously. The "shoulder curve" has been hypothesized by numerous studies as being the result of aggregation (Floyd and Sharp, 1977; Chen et al., 1985; Hoff and Akin, 1986; Hom, 1972.)

The multi-hit theory, in which a single target must be hit "n" number of times before it is destroyed, was also proposed as being a model for shoulder curves. Atwood and Norman (1949) developed the following mathematical relationship:

$N/N_o = 1- (1-e^{-aD})^n$ where: N/N_o = surviving fraction

n = # hits to kill
a = sensitivity volume
D = dose

Mechanistic effects have also been proposed as being responsible for the deviations from first-order, log-linear kinetics in disinfection experiments. For example, an infectious particle could develop a resistance to the inactivating process as the reaction proceeds. Taking data from experiments in which poliovirus 1 was exposed to formaldehyde, Gard (1957) developed the following formula:

$\log N/N_o = -a \log (1-bt)$ where N/N_o = surviving fraction

a = sensitivity
b = potency of disinf.
t = time

Heterogeneity of organisms, where a distribution of susceptibility to a disinfectant is inherent to the population, has also been the subject of several modeling approaches. Hiatt (1964) claimed that a suspension of viruses may become increasingly heterogeneous as the disinfection process continues, and constructed the following model:

$$N/N_o = (1- P)e^{-(k1 + k2)t} + Pe^{-k2t}$$ where:

N/N_o = surviving fraction
P = probability of infection
k_1 = inactivation constant 1
k_2 = inactivation constant 2
t = time

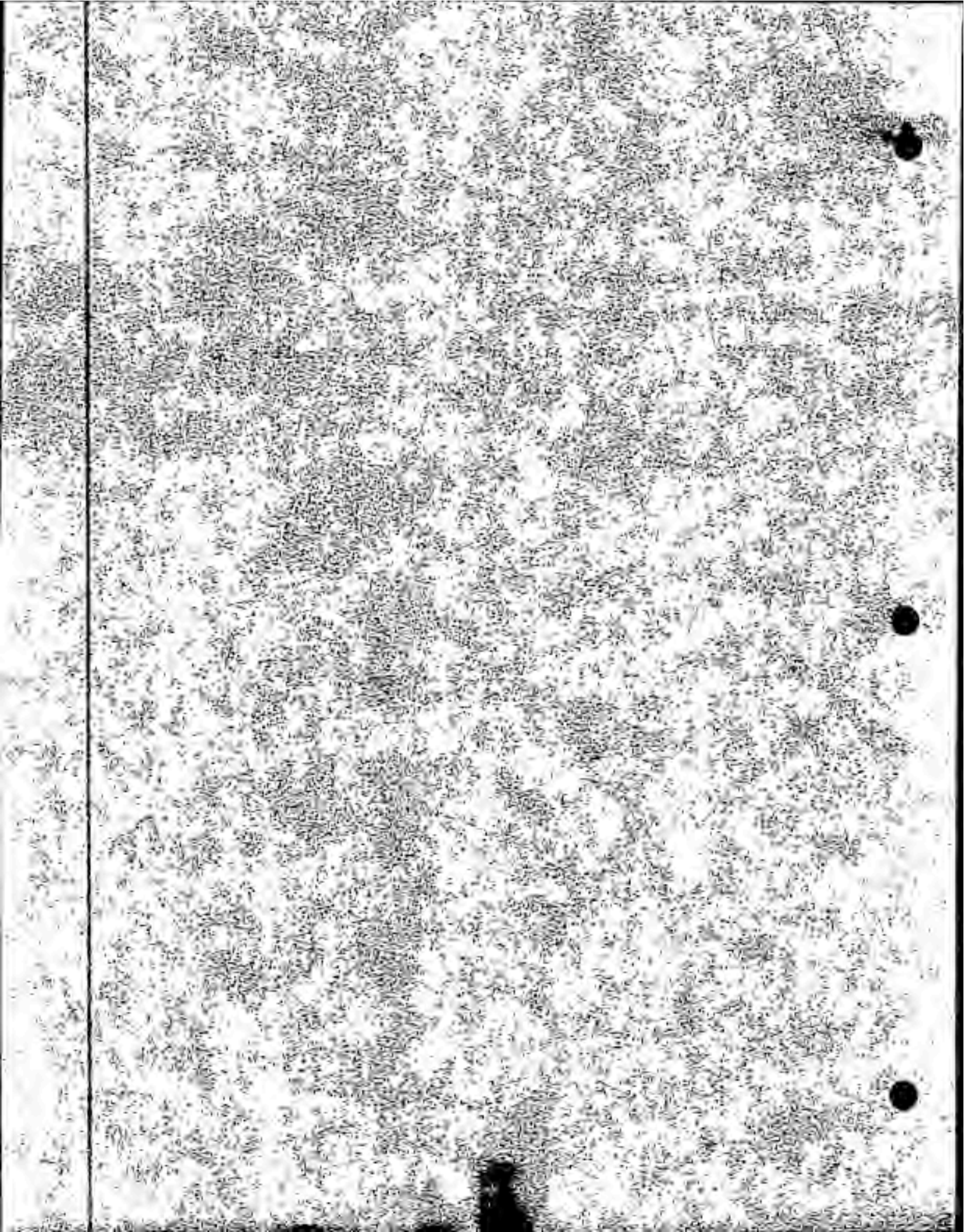
Haas (1984) adopted a kinetic model which accounted for changes in disinfectant species in a given system; For example, the dissociation of monochloramine to hypochlorous acid:

$$C_c = C_o[x \exp(-k_1t) + (1-x)\exp(-k_2t)-\exp(-k_3t)$$ where:

C_c = combined chlorine
C_o = chlorine dose
x = fraction of combined chlorine decaying by rapid pathway
k_1 = fast rate constant of decay
k_2 = slow rate constant of decay
k_3 = pseudo-first order rate

constant of conversion of free to combined chlorine

So far, no attempt at modeling the kinetics of disinfection data has encompassed the wide range of not only disinfectants, but also the microorganism's resistance under different environmental conditions. Nevertheless, the pursuit of more reliable models has produced information which can be used in devising regulations aimed at protecting public health.



METHODS AND MATERIALS

Preparation of Glassware and Halogen-Demand-Free Water and Buffers

Experimental water was prepared by passing twice-deionized, activated carbon-filtered water through a macroreticular scavenging resin bed to produce water of the quality described in Standard Methods (A.P.H.A., 1989). Glassware was rendered demand-free by soaking in a tank containing 25-50 mg/L chlorine solution for a minimum of 6 hours. The glassware was rinsed in halogen-demand-free (HDF) water four times, wrapped in aluminum foil and baked at 200 degrees Celsius for 6 hours. All buffers for the experiments were made demand-free according to the protocol in Standard Methods (A.P.H.A., 1989).

Monochloramine Reagents and Monochloramine Analysis

Stock solutions of monochloramine were prepared the day of the experiment to produce an approximate concentration of 100 mg/L by modification of the method established by Berman and Hoff (1984). Household bleach (5.25 % sodium hypochlorite) was diluted in HDF 0.01 M phosphate buffer, pH 9.5, to a concentration of 200 mg/L free chlorine. Concurrently, ammonium chloride was diluted in identical buffer to achieve an 800 mg/L solution. Equal volumes of

each of these solutions were combined in HDF glassware and mixed thoroughly to ensure formation of monochloramine. These stock solutions were diluted in phosphate-buffered, HDF water at the desired pH to a final concentration of 2.0 mg/L.

Concentrations of monochloramine in stock solutions and samples taken during the course of the experiments were measured by the N,N,Diethyl-P-Phenylenediamine (DPD) colorimetric method as described in Standard Methods (A.P.H.A, 1989).

Chlorine Dioxide Reagents and Chlorine Dioxide Analysis

Chlorine dioxide was generated according to the technique described in Standard Methods (A.P.H.A., 1989) with slight modifications as described in the technical report of Karen Werdehoff (Werdehoff, 1986), and shown diagramatically in Appendix IV, Figure 1. A solution consisting of 750 mls HDF water supplemented with 10 g sodium chlorite was placed in the reaction vessel. The proceeding tower contained sodium chlorite flakes moistened with 4-5 mls HDF water. Glass wool was placed on top to prevent any flakes from being carried out of the tower. The chlorine dioxide collection bottle consisted of 1500 mls HDF water. This bottle was wrapped in aluminum foil and the entire system was connected with teflon tubing.

A steady stream of N_2 gas was initially passed through

a gas scrubber consisting of a 5% KI solution. The following sequence was performed at five-minute intervals for twenty minutes: 1) 5 mls 1.8 N H_2SO_4 were added to the generating bottle 2) The gas flow was augmented to the point that mild bubbling was observed in the collection bottle 3) After five minutes the gas flow was interrupted and the system disconnected at point W 4) 5 mls of acid were again added and the system reconnected, 5) After twenty minutes 5 mls of acid were again added and the gas flow allowed to continue for an additional 30 minutes.

The resulting stock solution ranged in concentration from 500-800 mg/L ClO_2. Yields were analyzed using the DPD method as described in Standard Methods (A.P.H.A., 1989).

A standard curve was developed the same day using the DPD colorimetric method described in Standard Methods (A.P.H.A., 1989). Experiments were performed within 48 hours. Stock solutions of ClO_2 were diluted in phosphate-buffered HDF water to a target concentration of 0.5 mg/L. Disinfectant residuals were measured as described for the standard curve.

Preparation of Monodispersed Hepatitis A Viruses

HAV, a cytopathic strain HM175, was grown and assayed enumeratively by the plaque technique in confluent layers of FRhK-4 (fetal rhesus kidney-derived) cells as

previously described (Cromeans et al., 1987; Sobsey et al., 1991).

Viruses were propagated, purified and concentrated by the method described previously (Sobsey et al., 1991). Confluent layers of host cells were infected at multiplicities of 0.01-0.1 infectious units/cell. After an incubation period of one week, HAV was harvested from the infected cells by freezing and thawing and then centrifuging at 3,000 x g for 20 minutes. Viruses in the resulting supernatant were precipitated with 12% polyethylene glycol (PEG) and extracted to separate free viruses from cell debris using chloroform. HAV in resuspended PEG precipitates and cell extracts was pelleted at 105,000 x g for 3 hours at 5 degrees Celsius. The pellets were pooled and resuspended in HDF phosphate buffer. Cesium chloride was added to achieve a density of 1.33 g/ml, and then ultracentrifuged to equilibrium for three days at 90,000 x g and 5° C in a self-generated gradient. Fractions of the gradient were assayed to determine the location of the virus peak and those portions were desalted using centrifugal ultrafilters (100,000 molecular weight cut-off). The desalted fractions were then layered onto 10 to 30 % sucrose gradients in phosphate-buffered HDF water and ultracentrifuged under conditions such that the peak of single virions migrated approximately 2/3 down the tube (Sobsey et al., 1988). Those gradient fractions found to contain the peak of single

virions were collected and stored at 4 degrees Celsius for use in experiments.

Preparation of Monodispersed MS2 Bacteriophages

Coliphage MS2 (ATCC 15597-B1) was grown and assayed by the top agar plaque method (Adams, 1959) in *E. coli* C3000 (ATCC 15597). The top agar of plaque assay plates having confluent lysis was scraped into 3-5 ml/ plate of phosphate buffered saline, extracted with equal volumes of chloroform, and centrifuged at 10,000 x g for 15 minutes. The supernatant was collected and ultracentrifuged at 90,000 x g and 5^0 C for 4 hours to pellet the phage. The pellets were pooled in phosphate-buffered HDF water, supplemented with CsCl at a final density of 1.44 g/ml and ultracentrifuged to equilibrium in self-generated gradients for three days at 5° C to concentrate the phages. The gradient fractions containing the virus peak were pooled and desalted as described for HAV. To ensure that the phages were monodispersed, the desalted portions were filtered successively through Tween-80-treated 0.2 and 0.08 um pore size polycarbonate filters.

EXPERIMENTS

The general procedures for the disinfection

experiments were as described by Sobsey et al. (1991) with the following additions or modifications.

1) Monochloramine at a final concentration of 2.0 mg/L or chlorine dioxide at a final concentration of 0.5 mg/L was used.
2) Both MS2 and HAV were added to test samples to give titers of approximately 10^5 infectious units/ml.
3) Experiments were performed in phosphate-buffered HDF water adjusted to pH values of 6, 8 and 10.
4) In addition to 60 minute long experiments, three day long experiments using monochloramineat pH 8 were conducted, with additional samples taken at 1440, 2880 and 4320 minutes using monochloramine.
5) Two types of three day long "re-dosing" experiments were performed with monochloramine at a pH value of 8. At time=0, samples were placed in reaction tubes as usual. After the 1440 minute samples had been taken, one reaction tube was dosed with additional HAV and MS2 bacteriophage to achieve a final concentration of approximately 10^4 infectious units/ml. A second reaction tube was supplemented with additional monochloramine at a target concentration of 2.0 mg/L. A third tube received no supplemental viruses or monochloramine, as in previous 3 day-long experiments. Samples were

taken at specified time intervals as described above for 3 day-long experiments and virus control and halogen control tubes were also sampled periodically.

Data Analysis

Disinfection data as plaque forming units (PFU) per ml for MS2 and HAV were calculated as average values from triplicate cultures. For each time point, the average virus/phage concentration (Nt) was divided by the mean value of the virus/phage concentration of the controls (No). These values were then $\log_{10}$-transformed ($\log_{10}$ [Nt/No]) and the values averaged for each set of replicate experiments.

To compute the estimated time for 99.99% virus inactivation, linear regression was performed on each experiment and the time estimated from the best fit of the regression equation. This value was multiplied by the average disinfectant concentration throughout the experiment to obtain the concentration x time (CT) value.

MODELING

Analytic approach

Each set of data was interpreted through five distinct and separate theories of the relationship between

disinfectant concentration, time of exposure and fraction of viruses surviving treatment. The intent of the analysis was to determine (1) the ability of the various theories to adequately predict the data, (2) the parameter values obtained through the fitting of each theory to the data and (3) the sensitivity of predictions of 99.99 % reduction to variations in experimental run, virus type, and theoretical framework of analysis. The various theories employed in the study, their mathematical formulation, and their associated bases of axioms, are described below.

Theory 1: One Population

It is assumed here that inactivation proceeds through first-order kinetics with rate constant k (per unit concentration). All viruses possess identical values of this rate constant. The concentration of disinfectant decreases throughout the treatment period with first order kinetics and rate constant ϕ. Let Nt be the number of viruses present in the sample at time t after the onset of disinfection. The differential equation describing the rate of change of the number of viruses then is:

$$dNt/dt = -kC_o e^{-\phi t}$$

where C_o is the initial concentration of the disinfectant. The solution to the above equation is:

$$Nt = N_o e^{-kCo(1-e-\Phi t)/\Phi}$$

and where N_o is the initial number of viruses. The surviving fraction may then be obtained through division of both sides of the above equation by N_o, or

$$S(t) = e^{-kC_o(1-e-\lambda t)/\lambda}$$

Theory 2: One Hit-Two Populations I

It is assumed here that there are two separate subpopulations of viruses. Each subpopulation is inactivated by first order kinetics, the first with rate constant k1 (per unit concentration) and the second with rate constant k2 (per unit concentration). The concentration decreases throughout the treatment period, with removal rate constant Φ. If f1 is the fraction of viruses in the first subpopulation and f2 is the fraction of viruses in the second population, the solution is analogous to that obtained in theory 1 and yields:

$$S(t) = f_1 e^{-k_1 C_o(1-e-\lambda t)/\lambda} + f_2 e^{-k_2 C_o(1-e-\lambda t)/\lambda}$$

Theory 3 : One Hit-Two Populations II

The assumptions here are essentially those of theory 2, with the exception that the concentration of disinfectant is assumed constant at C_o throughout the period of treatment.

The survival fraction then is:

$$S(t) = f_1 e^{-k_1 C_o t} + f_2 e^{-k_2 C_o t}$$

Theory 4 : Multistate

It is assumed here that viruses must pass through two substates in being inactivated. The first substate represents sublethal damage to a virus. The second substate represents additional damage which is lethal to the virus. The fraction of initial viruses in the first substate is f_1. The fraction of viruses totally undamaged is f_0. The transition rate constant from state zero (undamaged) to state one (sublethal damage) is k_0 (per unit concentration). The transition rate constant from state one (sublethal damage) to state two (inactivated) is k_1. The concentration of disinfectant is assumed constant at C_o throughout the treatment period. The differential equation for the rate of change of the number of viruses in the three states then is:

State 0: $dN_0(t)/dt = -k_0 C_0 N_0(t)$

State 1: $dN_1(t)/dt = k_0 C_0 N_0(t) - k_1 C_0 N_1(t)$

State 2: $dN_2(t)/dt = k_1 C_0 N_1(t)$

The fractions of viruses surviving treatment at time t then is equal to the fraction in state zero plus the fraction in

state one (or 1 minus the fraction in state two). This surviving fraction is given by:

$$S(t) = f_0 e^{-k_0 C_0 t} + f_1 e^{-k_1 C_0 t} + \frac{f_0 k_0}{(k_1 - k_0)}(e^{-k_0 C_0 t} - e^{-k_1 C_0 t})$$

Theory 5 : Distributive Rate Constant

It is assumed here that the individual viruses are inactivated by the disinfectant through first-order kinetics. The inactivation rate constant, k, however, is assumed to be a distributed quantity with probability density function P(k). The concentration is assumed to change with removal constant λ and initial concentration C_o. The fraction of surviving viruses then is:

$$S(t) = \int_0^{\infty} P(k) e^{-kC_0(1-e^{-\lambda t})/\lambda} dk$$

In this study, it was assumed that P(k) is a lognormal distribution with a geometric standard deviation of 3.0. The above equation was integrated numerically to obtain the surviving fraction.

Measure of fit:

Each of the above theories was fit to the various sets of data to obtain estimates of the necessary parameters. "Best" fitting parameter estimates were determined through use of a least squares routine applied to

the log transformation of the predicted and measured values. The data points were assigned differential weight based on the temporal density of the data points throughout the domain. The equation of the least-square employed was:

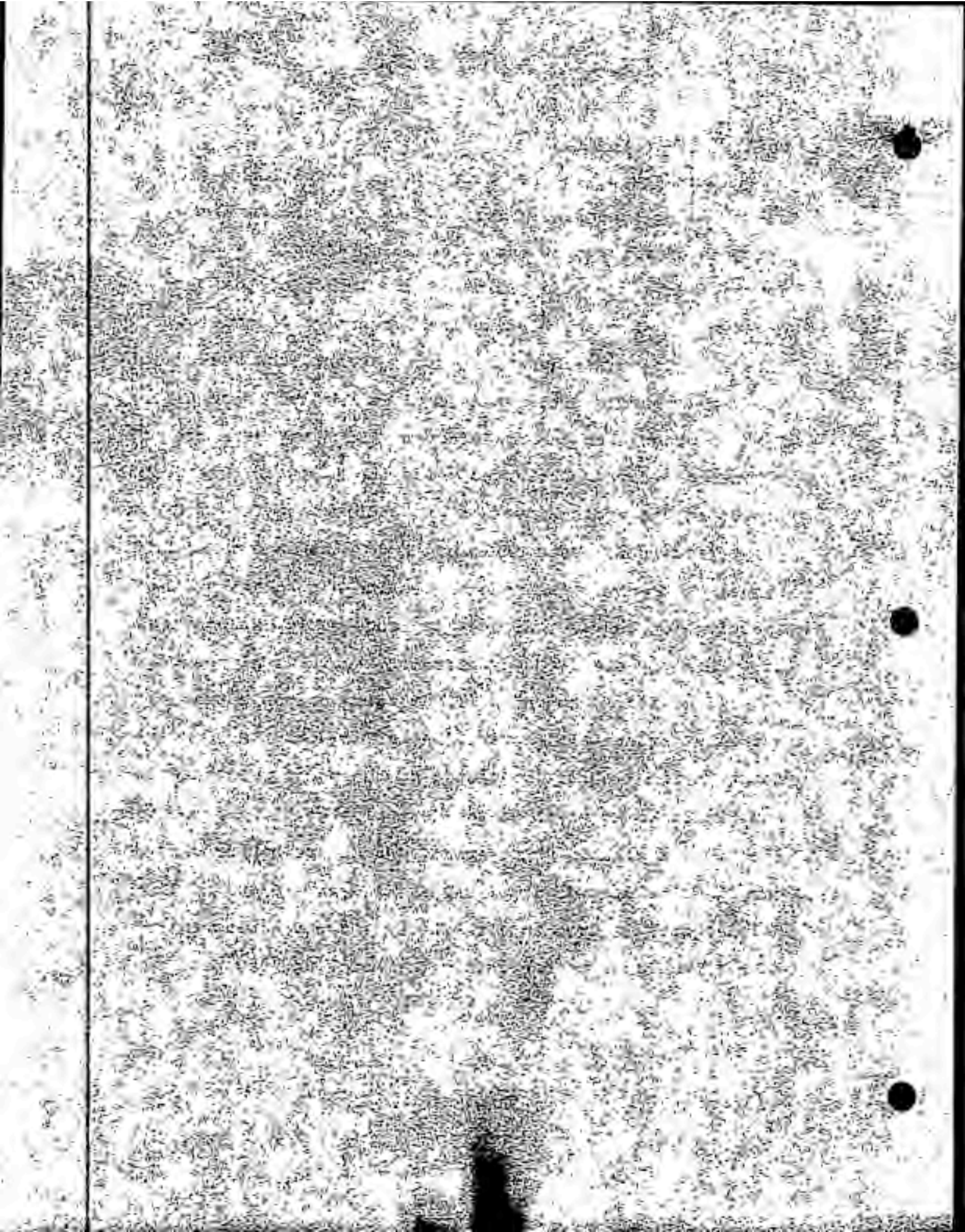
$$\text{Measure of fit} = \sum W_i \left(\ln(\text{pred})_i - \ln(\text{meas})_i \right)^2$$

where W_i is the weighting for the ith data point and the summation is over all data points for a given set of data.

DATA IN THE APPENDIX

A summary of the raw data for each experiment is presented in Appendix 1. This consists of the surviving fraction of viruses at each time point as well as the control samples at the start and conclusion of the experiment. When calculating the percent of original virus titer remaining at each time point (Nt/No), the average of the virus control titers were used as "No" to account for factors other than the disinfectant which may have been responsible for any observed reduction in titer. The graphs depicting the averaged values of inactivation for each time point (for experiments performed under the same conditions) are also presented in Appendix II. The error bars on the inactivation curves indicate the range of log Nt/No values as within one standard deviation of the value plotted.

In addition, Appendix III contains graphs comparing actual experimental inactivation curves with the inactivation kinetics predicted by the models. Both actual and predicted survival curves were plotted as the surviving fraction of viruses versus time. Graphs are not presented for chlorine dioxide at pH values of 9 and 10 due to the initial drastic drop in virus titer and the consequential lack of sufficient data points for modeling purposes.



RESULTS

MONOCHLORAMINE

Effect of pH levels on monochloramine The results of experiments on inactivation of dispersed HAV and MS2 by a dose of 2.0 mg/L monochloramine at 5° C are presented graphically in Figures 1-2, in which mean $\log_{10}$ Nt/No is plotted against contact time in minutes. Figure 1 plots HAV and MS2 individually with respect to inactivation at all three pH levels (6, 8 and 10). Figure 2 plots both viruses at a given pH (pH 6, 8, and 10), with data for each pH plotted separately.

Both MS2 and HAV reacted similarly to doses of monochloramine at the different pH values, indicating that MS2 is an adequate model indicator of HAV and perhaps other enteric viruses. After 60 minutes of contact, inactivation of MS-2 and HAV was about 1 $\log_{10}$ or 90 %. For HAV, inactivation was somewhat greater at pH 10, than at pH 6 and 8 (Figure 1). However, under no condition was there greater than one $\log_{10}$ virus reduction at 60 minutes.

Three day-long experiments. Figures 3-5 illustrate the results of standard three day-long experiments as well as experiments in which additional virus or disinfectant was added after 24 hours (Figures 4 and 5). It is evident from

the results shown in Figure 3 that inactivation kinetics of both MS2 and HAV are of the retardant die-off type. Inactivation proceeded at a slightly higher rate for MS2 than for HAV. At the end of three days, there was slightly more than a 2 $\log_{10}$ reduction of MS2. HAV was more persistent, with somewhat less than a two $\log_{10}$ reduction after 3 days.

The three day-long experiments in which supplemental monochloramine or virus stock was added are displayed in Figures 4 and 5. After 72 hours, the total $\log_{10}$ reduction of HAV and MS2 was approximately 1.5 and 2, respectively, when neither virus nor monochloramine was added. Both replicate experiments under each test condition exhibited similar patterns of inactivation (data not shown). When the reaction mixture was supplemented with additional monochloramine at a concentration of 2.0 mg/L at 24 hours, < 0.5 $\log_{10}$ unit of additional inactivation was observed at the end of the 72 hour period. In contrast, the virus inactivation kinetics in the reaction mixtures in which additional viruses were added at 24 hours suggest that "supplemental viruses" react with the same inactivation kinetics as the virus population present at the onset of the experiment. However, total inactivation of "supplemental viruses" did not exceed 1.0 and 1.5 $\log_{10}$ units for HAV and MS2, respectively.

Modeling The predicted times for inactivation of 2, 3 and 4 $\log_{10}$ (99, 99.9 and 99.99 %) of initial viruses by monochloramine are summarized in Tables 1-7 and they are shown graphically in Figure 10.

Generally, the one population model consistently predicted the shortest times followed by the simple linear regression method currently employed by the EPA. This is true for all pH values using monochloramine. It is of interest to compare the predicted times derived from the 60 minute-long and 3 day-long experiments in which monochloramine was used. The calculated times for a particular extent of inactivation vary considerably between the two types of experiments by more than two orders of magnitude. For example, the multistate model predicts 99.99 % inactivation times for MS2 of 544 and 8,356 minutes using the data of the 60-and 3-day-experiments respectively. In some cases, the time predicted for a 4 $\log_{10}$ inactivation cannot be determined. As reported in Table 7, both the two population with changing concentration model and the distributive rate constant model indicate that the monochloramine would be depleted prior to 99.99 % removal of viruses. This phenomenon is only discerned when the data from the 3-day long experiments are used in conjunction with the models.

Overall, all models (with the exception of the one population model) predicted longer times necessary to

achieve a 99.99% reduction than did the first order model. The measure of the goodness-of-fit of the models to the actual data revealed that the two population with changing concentration model predicted the data most accurately (data not shown).

In addition, to eliminate any doubt that the inactivation of viruses was due to monochloramine, and not dichloramine, the speciation of chloramines were examined during a pH 6 disinfection experiment (see Table 8). This pH value was chosen since it is more likely that dichloramine would be present under more acidic conditions. From the results, it can be seen that monochloramine is the dominant species and dichloramine would have a negligible effect on the inactivation kinetics observed.

FIGURE 1

INACTIVATION OF HAV AND MS2 BY MONOCHLORAMINE
(2 mg/l; 5°C; 0.01M PO_4 BUFFER, pH 6, 8 & 10)

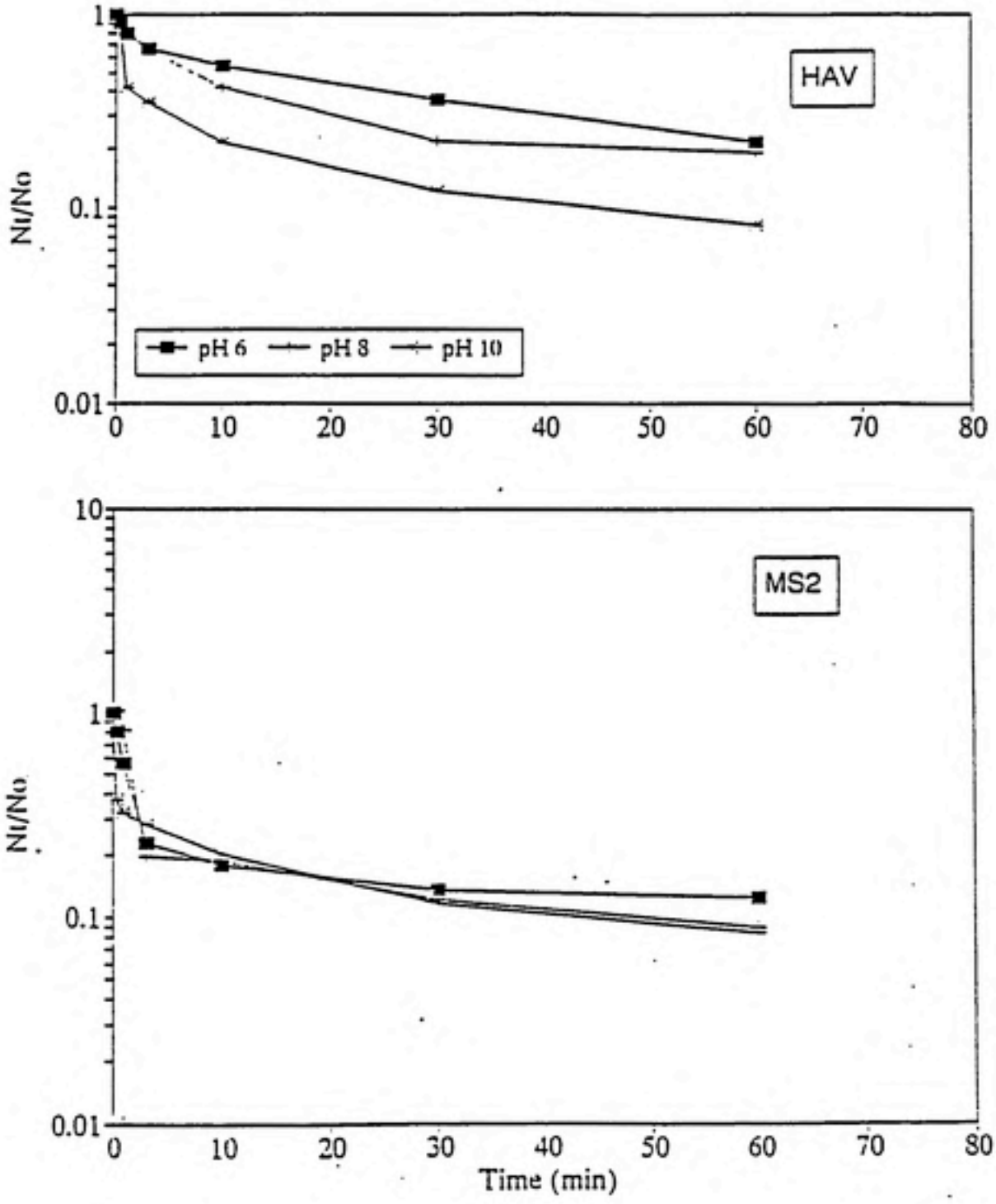


FIGURE 2

INACTIVATION OF HAV AND MS2 BY 2 mg/l MONOCHLORAMINE AT pH 6; 8 & 10 AND 5°C IN 0.01M PO_4 BUFFER

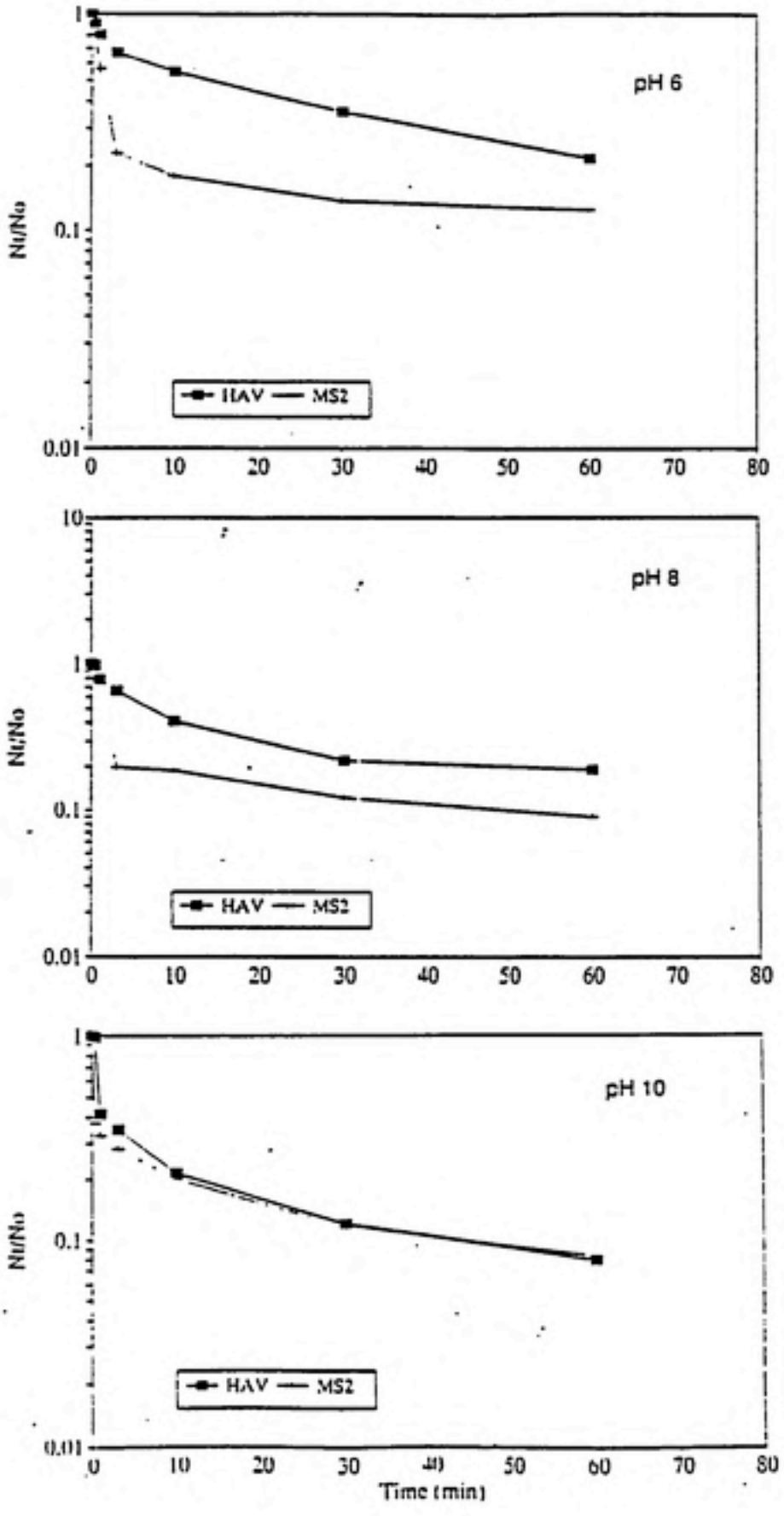


Figure 3: 3 day Monochloromine (2.0 mg/L); pH 8
5 degrees C; .01 M Phosphate buffer

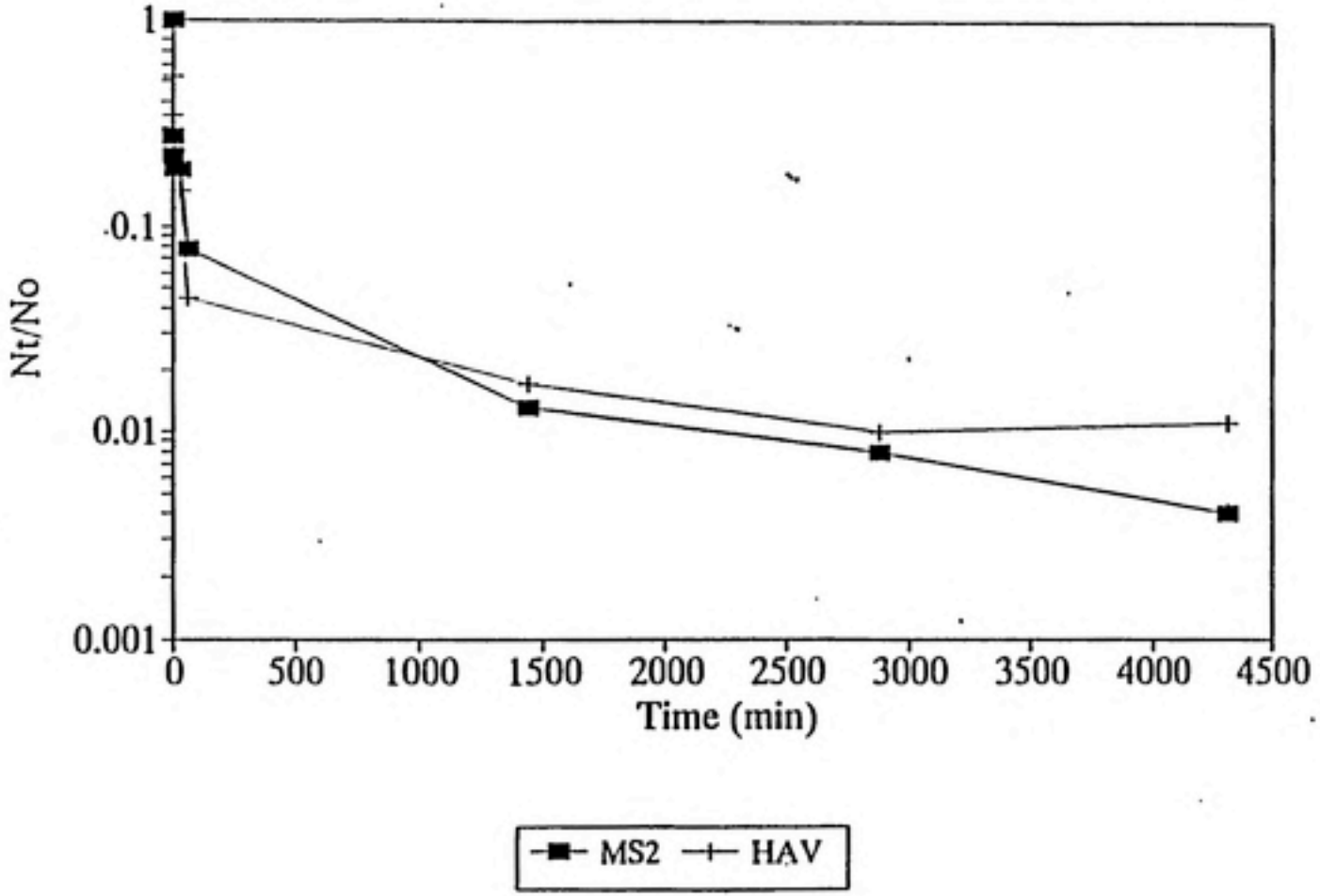


Figure 4: NH2Cl DISINFECTION; 3 DAY; pH 8

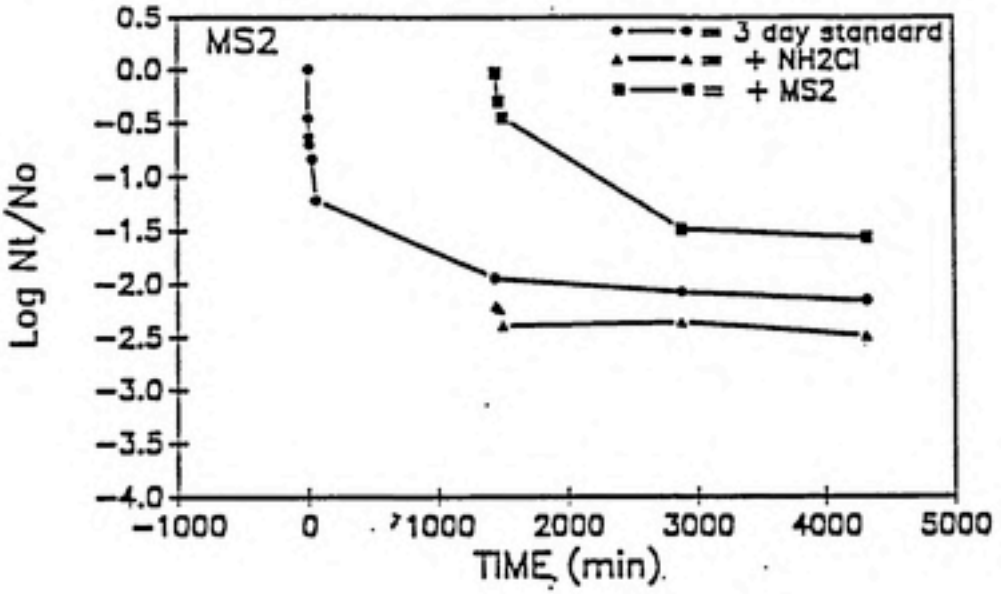


Figure 5:

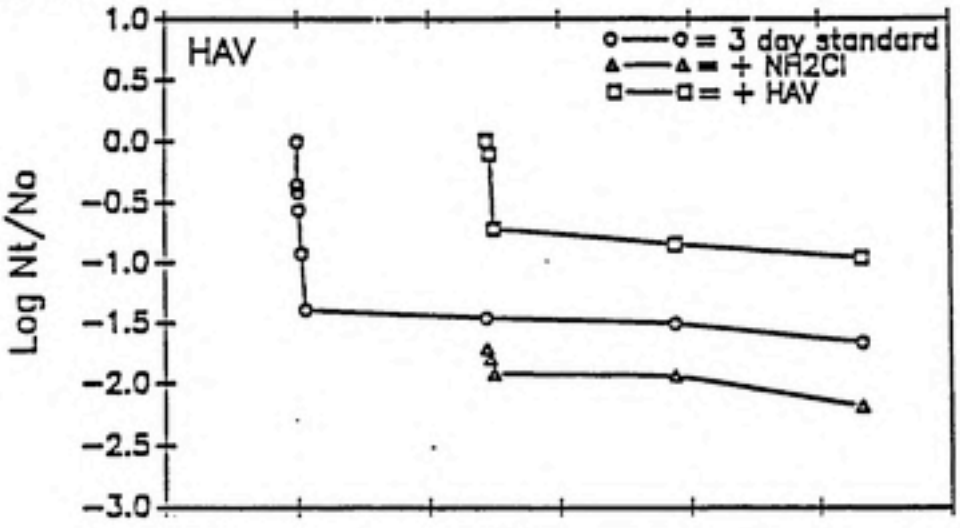


Figure 6:

Predicted times for 99.99% inactivation of MS2 and HAV

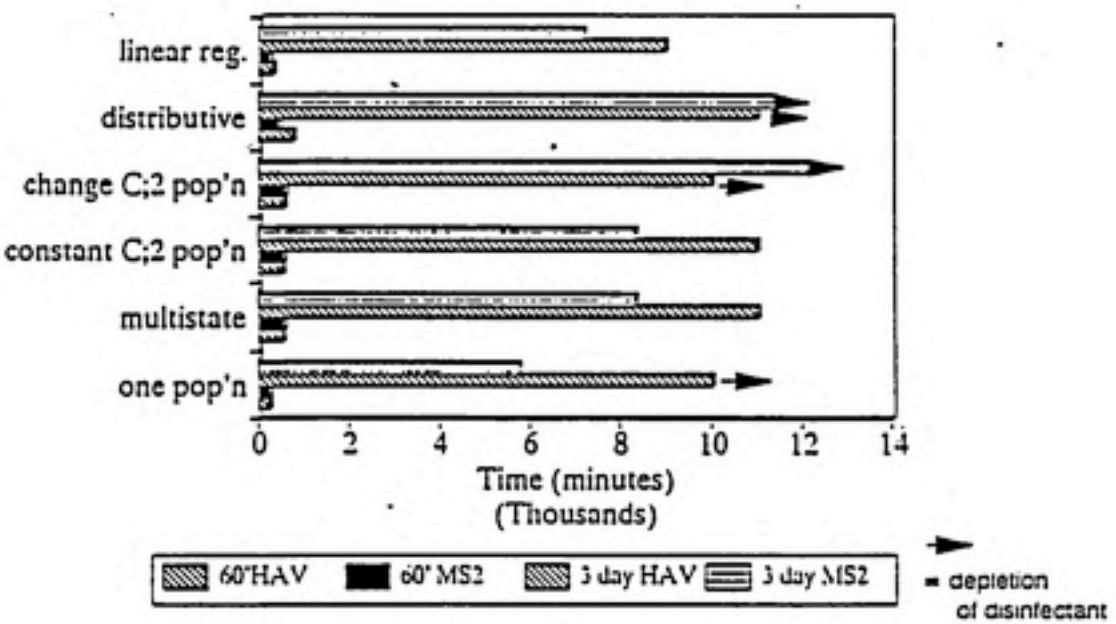


Table 1: Times for 99.99% inactivation of HAV and MS2 by doses of 2.0 mg/L preformed monochloramine at 5° C; 60 minute long experiments; pH 6

Model	virus	Time (min)	C x T value*
one pop'n	HAV	340	n.a.
multistate	HAV	440	n.a.
two pop'n constant C	HAV	440	n.a.
two pop'n change C	HAV	465	n.a.
distributive	HAV	1210	n.a.
linear reg.	HAV	380	733**
one pop'n	MS2	215	n.a.
multistate	MS2	760	n.a.
two pop'n constant C	MS2	950	n.a.
two pop'n change C	MS2	1085	n.a.
distributive	MS2	560	n.a.
linear reg.	MS2	300	579**

* Only applicable to linear regression where a direct relationship between time and concentration is assumed.

** avg. concentration of NH_2Cl (C)= avg. conc. at T0 and T60

Table 2: Times for 99.99% inactivation of HAV and MS2 by doses of 2.0 mg/L preformed monochloramine at 5° C; 60 minute long experiments; pH 8

Model	virus	Time (min)	C x T value*
one pop'n	HAV	235	n.a.
multistate	HAV	525	n.a.
two pop'n constant C	HAV	525	n.a.
two pop'n change C	HAV	560	n.a.
distributive	HAV	760	n.a.
linear reg.	HAV	321	626
one pop'n	MS2	235	n.a.
multistate	MS2	545	n.a.
two pop'n constant C	MS2	545	n.a.
two pop'n change C	MS2	580	n.a.
distributive	MS2	440	n.a.
linear reg.	MS2	235	449

* Only applicable to linear regression where a direct relationship between time and concentration is assumed.

** Avg. conc. of NH_2Cl (C) = avg conc. at T0 and T60

Table 3: Times for 99.99% inactivation of HAV and MS2 by doses of 2.0 mg/L preformed monochloramine at 5° C; 60 minute long experiments; pH 10

Model	Virus	Time (min)	C x T value*
one pop'n	HAV	155	n.a
multistate	HAV	390	n.a.
two pop'n constant C	HAV	390	n.a.
two pop'n change C	HAV	410	n.a.
distributive	HAV	440	n.a.
linear reg.	HAV	233	443
one pop'n	MS2	155	n.a.
multistate constant C	MS2	390	n.a.
two pop'n constant C	MS2	390	n.a.
two pop'n change C	MS2	410	n.a.
distributive	MS2	465	n.a.
linear reg.	MS2	274	523

* Only applicable to linear regression where a direct relationship between time and concentration is assumed.
** average conc. NH2Cl (C)= avg conc. at T0 and T60

Table 4: Predicted times for 2, 3, and 4 log_{10} cycles of inactivation of HAV and MS2 by doses of 2.0 mg/L preformed monochloramine at 5° C; 60 minute long experiments; pH 6

Model	virus	99% (inactivation)	99.9%	99.99%
one pop'n	HAV	167	254	343
multistate	HAV	209	324	439
two pop'n constant C	HAV	208	323	438
two pop'n change C	HAV	214	337	464
distributive	HAV	353	736	1218
linear reg.	HAV	190	280	380
one pop'n	MS2	106	160	215
multistate	MS2	300	531	761
two pop'n constant C	MS2	374	662	950
two pop'n change C	MS2	393	724	1084
distributive	MS2	173	351	563
linear reg.	MS2	142	225	300

Table 5: Predicted times for 2, 3, and 4 $\log_{10}$ cycles of inactivation of HAV and MS2 by doses of 2.0 mg/L preformed monochloramine at 5° C; 60 minute long experiments; pH 8

Model	virus	99% (inactivation)	99.9%	99.99%
one pop'n	HAV	116	176	237
multistate	HAV	240	384	528
two pop'n constant C	HAV	237	381	525
two pop'n change C	HAV	245	401	562
distributive	HAV	116	176	237
linear reg.	HAV	160	238	320
one pop'n	MS2	116	176	237
multistate	MS2	215	379	544
two pop'n constant C	MS2	213	378	542
two pop'n change C	MS2	219	397	583
distributive	MS2	137	278	443
linear reg.	MS2	110	170	240

Table 6: Predicted times for 2, 3, and 4 $\log_{10}$ cycles of inactivation of HAV and MS2 by doses of 2.0 mg/L preformed monochloramine at 5° C; 60 minute long experiments; pH 10

Model	virus	99% (inactivation)	99.9%	99.99%
one pop'n	HAV	77	116	156
multistate	HAV	161	277	392
two pop'n constant C	HAV	160	276	391
two pop'n change C	HAV	164	286	411
distributive	HAV	137	278	443
linear reg.	HAV	110	170	230
one pop'n	MS2	77	116	156
multistate	MS2	161	276	391
two pop'n constant C	MS2	160	276	391
two pop'n change C	MS2	164	286	411
distributive	MS2	125	252	401
linear reg.	MS2	122	200	270

Table 7: Predicted times for 2, 3, and 4 $\log_{10}$ cycles of inactivation of HAV and MS2 by doses of 2.0 mg/L preformed monochloramine at 5° C; 3 day-long experiments; pH 8

Model	virus	99% (inactivation)	99.9%	99.99%
one pop'n	HAV	3428	7964	(0.00034)*
multistate	HAV	4593	16,106	27,619
two pop'n constant C	HAV	4581	16,094	27,607
two pop'n change C	HAV	3400	(0.005)*	(0.005)*
distributive	HAV	2878	(0.0011)*	(0.0011)*
linear reg.	HAV	3620	5900	9000
one pop'n	MS2	2613	5085	12,817
multistate	MS2	2599	5477	8356
two pop'n constant C	MS2	2599	5477	8355
two pop'n change C	MS2	2271	9763	(0.0007)*
distributive	MS2	2201	7340	(0.0005)*
linear reg.	MS2	2500	5000	7200

* number is surviving fraction of viruses at point in which disinfectant is depleted.

Table 8 : Determination of speciation of chloramines; pH 6
2.0 mg/L preformed monochloramine at 5° C;

Time (min)	NH_2Cl (mg/L)	$NHCl_2$ (mg/L)	% as $NHCl_2$
0	2.00	0.03	1.48
3	2.00	0.06	2.91
10	1.80	0.10	5.26
20	1.81	0.12	6.22
30	1.76	0.11	5.88
40	1.70	0.11	6.08
50	1.72	0.12	6.32
60	1.72	0.12	6.32

CHLORINE DIOXIDE

Effect of pH levels on chlorine dioxide The inactivation kinetics of a 0.5 mg/L dose of chlorine dioxide on monodispersed MS2 and HAV are illustrated in figures 7-9. Figure 8 compares both viruses as to their $\log_{10}$ survival at each of the pH values of 6, 8, 9 and 10. Inactivation of each virus by chlorine dioxide at all four pH values is represented in graph 7.

Unlike monochloramine, disinfection kinetics due to chlorine dioxide were influenced substantially by pH. As the pH was raised, the resulting inactivation of both MS2 and HAV was increased. At pH 10, the viruses were inactivated within the first 20 seconds (>3 $\log_{10}$), as marked by the "limit of detection" points. This detection limit is based upon ability of the assay to detect at least one viable organism in the least dilute sample (ten-fold dilution), inoculated in triplicate for a total volume of 0.6 ml. At pH 6 and 8, both HAV and MS2 were considerably more persistent then they were at pH 9 and 10, with <3 $\log_{10}$ inactivation by 60 minutes. At pH 6 and 8, inactivation kinetics were similar. Therefore, the largest change in virus inactivation rates occurs between pH 8 and 9.

Modeling The predicted times for inactivation of 2, 3 and 4 $\log_{10}$ (99, 99.9 and 99.99%) of initial viruses by chlorine dioxide are summarized in Tables 9-12. Modeling was

performed to predict inactivation at pH levels of 6 and 8 only, due to limitations on detectibility of viruses after the initial time point at pH 9 and 10. The predicted times for 99.99% inactivation of both viruses at pH 6 and 8 according to each model are summarized in Tables 9 and 10, respectively. By way of example, the actual experimental inactivation data and the predicted data for each model are shown graphically in Figure 3, where $\log_{10}$ Nt/No is plotted versus contact time. At pH 6 and 8, where virus inactivation can be followed, the first-order model now used by the U.S. EPA and the one population model predicted the shortest times for virus inactivation. A 4 $\log_{10}$ (99.99%) inactivation time for MS2 at pH 6 and 8 could not be determined for one model incorporating a decreasing concentration of disinfectant because it was predicted that the chlorine dioxide would be exhausted before this reduction could be achieved. The models that appeared to best fit the experimental data, the multistate and the two population-constant concentration, often predicted longer times to achieve 99.9 and 99.99% virus inactivation than the first order and the one population models. In some cases, the times to achieve the desired degree of inactivation are higher by a factor of two-fold or more than the times estimated by the first-order model.

FIGURE 7

INACTIVATION OF HAV AND MS2 BY CHLORINE DIOXIDE
(0.5 mg/l DOSE; 5°C; 0.01M PO_4 BUFFER, pH 6, 8, 9 & 10)

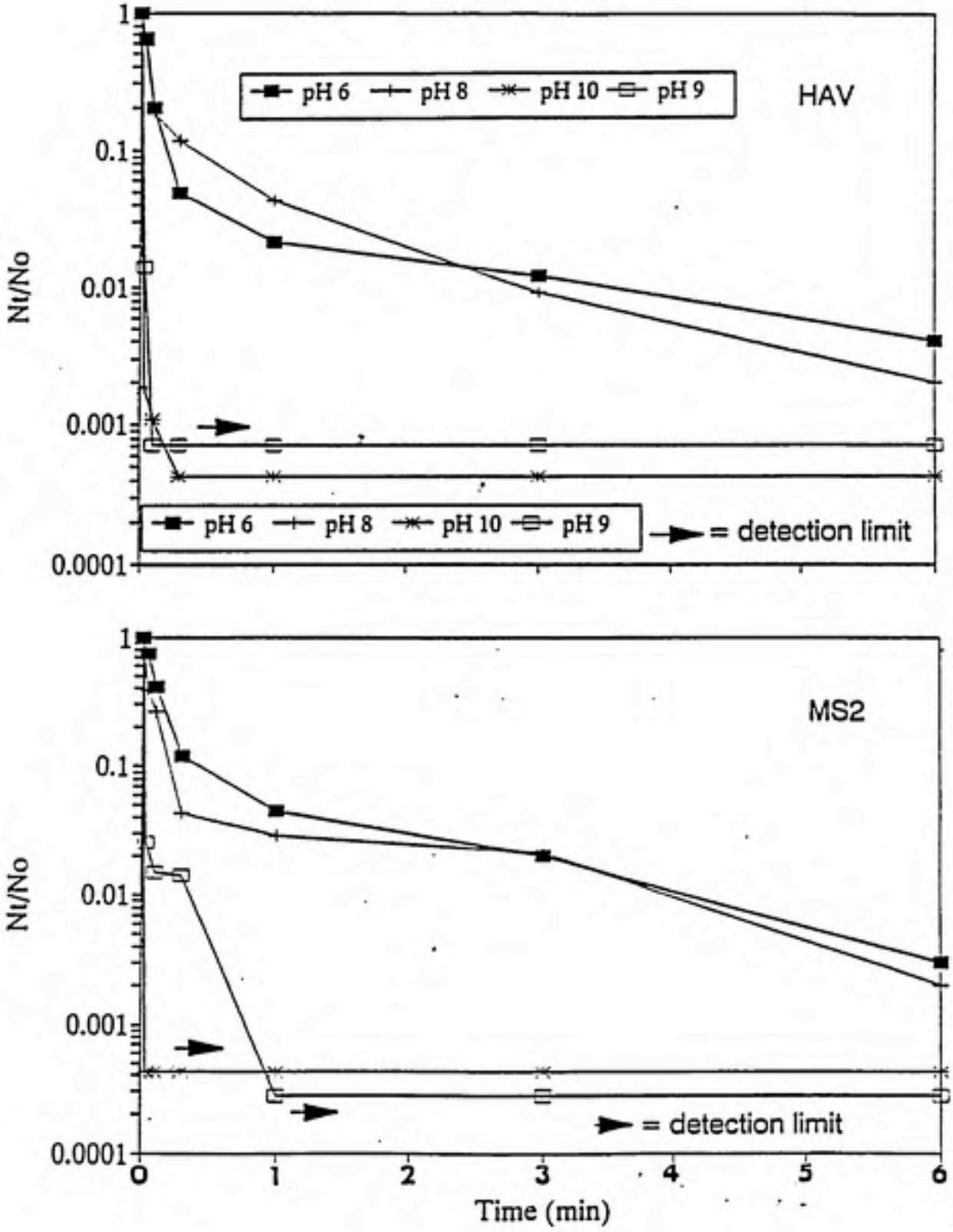


FIGURE 8

INACTIVATION OF HAV AND MS2 BY CHLORINE DIOXIDE (0.5 mg/l DOSE; 5°C; 0.01M PO_4 BUFFER, pH 6, 8, 9 & 10)

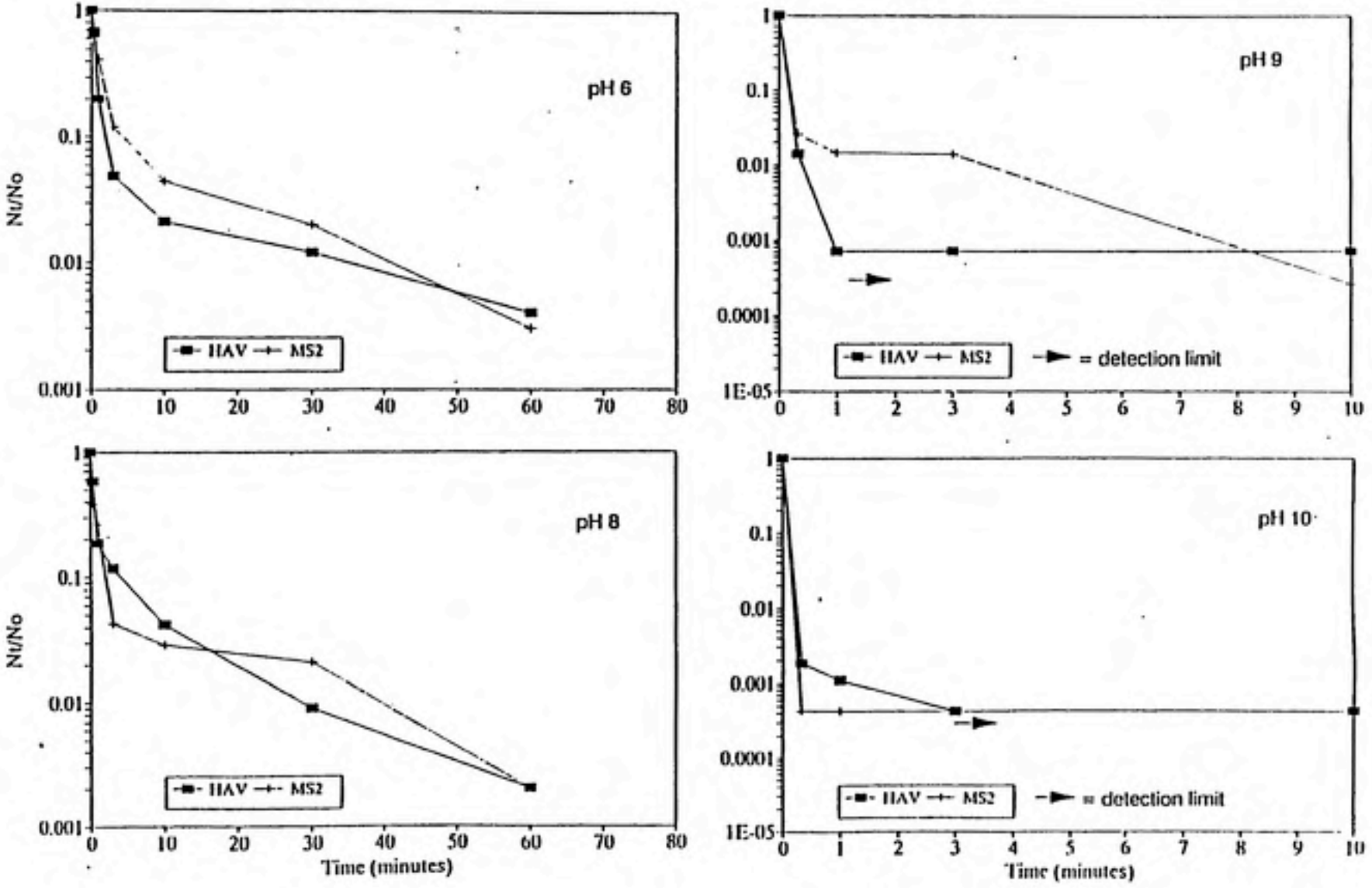


Table 9: Predicted times for 99.99% inactivation of HAV and MS2 by doses of 0.5 mg/L chlorine dioxide at 5° C; 60 minute long experiments; pH 6

Model	virus	Time (min)	C x T value*
one pop'n	HAV	62	n.a.
multistate	HAV	86	n.a.
two pop'n constant C	HAV	86	n.a.
two pop'n change C	HAV	136	n.a.
distributive	HAV	163	n.a.
linear reg.	HAV	104	40.6**
one pop'n	MS2	114	n.a.
multistate	MS2	132	n.a.
two pop'n constant C	MS2	131	n.a.
two pop'n change C	MS2	$(2.1e^{-4})$*	n.a.
distributive	MS2	163	n.a.
linear reg.	MS2	98	38.2**

* Only applicable to linear regression where a direct relationship between time and concentration is assumed.

** avg. conc. of ClO_2 (C)= avg conc. at T0 & T60= 0.39 mg/L

Table ID: Predicted times for 99.99% inactivation of HAV and MS2 by doses of 0.5 mg/L chlorine dioxide at 5° C; 60 minute long experiments; pH 8

Model	virus	Time (min)	C x T value*
one pop'n	HAV	89	n.a.
multistate	HAV	107	n.a.
two pop'n constant C	HAV	107	n.a.
two pop'n change C	HAV	204	n.a.
distributive	HAV	163	n.a.
linear reg.	HAV	83	31.5**
one pop'n	MS2	94	n.a.
multistate	MS2	111	n.a.
two pop'n constant C	MS2	110	n.a.
two pop'n change C	MS2	$(1.3e^{-4})$*	n.a.
distributive	MS2	163	n.a.
linear reg.	MS2	89	33.8**

* Only applicable to linear regression where a direct relationship between time and concentration is assumed.

** avg. conc. of ClO_2 (C)= avg conc. at T0 & T60= 0.38 mg/L

Table 11: Predicted times for 2, 3, and 4 $\log_{10}$ cycles of inactivation of HAV and MS2 by doses of 0.5 mg/L chlorine dioxide at 5° C; 60 minute long experiments; pH 6

Model	virus	99% (inactivation)	99.9%	99.99%
one pop'n	HAV	26	42	62
multistate	HAV	17	51	86
two pop'n constant C	HAV	16	51	86
two pop'n change C	HAV	14	56	136
distributive	HAV	28	68	163
linear reg.	HAV	42	75	100
one pop'n	MS2	40	69	114
multistate	MS2	41	86	132
two pop'n constant C	MS2	40	85	131
two pop'n change C	MS2	28	96	$(2.1e^{-4})$*
distributive	MS2	28	68	163
linear reg.	MS2	45	73	97

* number is surviving fraction of viruses at point in which disinfectant is depleted.

Table 12: Predicted times for 2, 3, and 4 $\log_{10}$ cycles of inactivation of HAV and MS2 by doses of 0.5 mg/L chlorine dioxide at 5° C; 60 minute long experiments; pH 8

Model	virus	99% (inactivation)	99.9%	99.99%
one pop'n	HAV	33	57	89
multistate	HAV	32	69	107
two pop'n constant C	HAV	31	69	107
two pop'n change C	HAV	27	78	204
distributive	HAV	28	68	163
linear reg.	HAV	38	65	85
one pop'n	MS2	35	60	94
multistate	MS2	29	70	111
two pop'n constant C	MS2	28	69	110
two pop'n change C	MS2	28	96	(0.00013)*
distributive	MS2	28	68	163
linear reg.	MS2	38	65	90

* number is surviving fraction of viruses at point in which disinfectant is depleted.

DISCUSSION

Monochloramine

When the inactivation kinetics of MS2 and HAV by monochloramine are compared, both viruses exhibit similar retardant die-off patterns. The kinetics results indicate that at pH 10, the two viruses have nearly identical inactivation rates. At the pH levels of 8 and 6, HAV was slightly more resistant to monochloramine than was MS2. This suggests that at alkaline conditions HAV may be more sensitive to monochloramine since its inactivation was somewhat increased from pH 8 to 10 while MS2 inactivation remained generally the same at all pH levels. Some investigators have reported that the inactivation efficiency of monochloramine increases with decreasing pH, and according to Hoff (1986), this may be due to the activity of free chlorine, which is in equilibrium with monochloramine. However, the results of this study, in which efforts were made to minimize the presence of free chlorine during monochloramine production, indicate that pH levels in the range of 6-10 have only a minor influence on virus inactivation kinetics.

The 3 day-long experiments were performed in order to determine if the tailing-off of virus inactivation observed in the 60' long experiments was consistent over

longer time periods. The results indicated that clearly this was the case. After 60 minutes, less than one additional $\log_{10}$ inactivation was observed after as long as 3 days. This tailing-off has been described by Prokop and Humphrey (1970) as a typical type of curve generated from disinfection data. Many researchers have attempted to describe this departure from linear, first-order kinetics (Gard 1957; Chang, 1971; Hom 1972). Several of the explanations offered are: 1) virus aggregation 2) variations within the population with respect to response to a particular disinfectant and 3) different states of infectivity or "virility" of the viruses, with some being healthy (more infectious) and others damaged (less or non-infectious).

Another step in further characterizing the disinfection kinetics of HAV and MS2 by NH_2Cl was to test the effect of adding supplemental virus or NH_2Cl on inactivation kinetics. When additional viruses were added, they followed the same inactivation kinetics as the original viruses did at the beginning of the experiment: an initial sharp decline, followed by a leveling off of inactivation. The overall $\log_{10}$ inactivation of viruses was less for the supplemental viruses than the viruses present at the start of the experiment, which could be due to the fact that the original concentration of monochloramine had decreased at day 1 by nearly 30%. Supplemental monochloramine but no

added viruses only marginally increased the amount of virus inactivation. These experiments indicate that the retardant die-off kinetics observed are probably a result of a resistant fraction of viruses in the population. This could be due to: (a) aggregation, in which viruses located within aggregates are protected from being destroyed, (b) various states of "health" of the viruses, or (c) subpopulations of viruses with different responses to inactivation by monochloramine. Similar results have been reported by Snead (1972), in which the model indicator virus f2 (a male-specific coliphage similar to MS-2) was used in re-dosing experiments with monochloramine.

Electron microscopy was used to visualize the physical state of the viruses in some samples taken at different time points during the disinfection experiments in order to determine if aggregates of viruses existed. This was done using the kinetic attachment method of Sharp (1974). When MS2 was examined, both aggregates and single virions were observed (data not shown). Whether the relative proportions of these two physical states of viruses correspond quantitatively to the inactivation kinetics observed in these disinfection experiments has yet to be determined.

Chlorine dioxide

As expected from the results of previous studies, the

rate of inactivation of the viruses by chlorine dioxide was greater at alkaline pH values. Figures 7 and 8 show that at all pH values tested, this assumption was validated. In fact, at pH 10 both HAV and MS2 were inactivated by more than 3 $\log_{10}$ units in the first three minutes. It has been suggested by others that the enhanced activity of chlorine dioxide at high pH is the result of the formation of free hydroxyl radicals and it is these which are responsible for the biocidal action (Brett and Ridgeway, 1981).

The inactivation kinetics of MS2 and HAV were nearly identical at corresponding pH values of 6, 8 and 10. It is interesting to note that, unlike their responses to monochloramine, HAV was slightly more susceptible to chlorine dioxide than was MS-2 at pH values of 6, 8 and 9 (see figure 8).

The retardant die-off kinetics observed with monochloramine were also observed when chlorine dioxide was used. Although the tailing-off effect is not as pronounced, the results from the experiments conducted at pH 6 and 8 indicate the presence of a heterogeneous population with respect to susceptibility to chlorine dioxide. Experiments of longer duration will serve to elucidate the pattern of inactivation of these viruses as was done previously with monochloramine. This is especially important at pH conditions where chlorine dioxide is not as potent a virucide. In addition, it is important to determine whether

other disinfectants which are also strong oxidants, exhibit the same virus inactivation patterns.

MODELS

The models developed to characterize the disinfection kinetics of viruses by monochloramine and chlorine dioxide vary in their basic assumptions. Therefore, their predicted times for a given amount of inactivation exhibit considerable variability. The one hit one population model consistently predicted the shortest times for 4 $\log_{10}$ inactivation. This model is similar to Chick's law, which forms the basis for the C x T concept, where $S(t) = e^{-kt}$. The major assumption of Chick's law is that inactivation of microorganisms is a first-order reaction. Due perhaps to their structural complexity and variation in susceptibility, it is the rule rather the exception that many viruses do not adhere to this tenet. The C x T concept is not applicable to the other models because according to the theoretical framework of the models, concentration and time cannot be interchanged to result in a certain C x T product. For example, a concentration of 1 mg/L chlorine dioxide applied for 60 minutes would not have the same effect as a 5 mg/L solution applied for 12 minutes, despite the fact that both conditions give a C x T value of 60 mg-min/L.

The times predicted for inactivation by chlorine dioxide and monochloramine are consistently longer using the

multistate, distributive rate constant and two-population models. This reinforces the idea that the simplicity of the Chick-Watson model renders it unreliable in predicting the inactivation kinetics of disinfectants over extended time periods.

For monochloramine, comparisons were also made between the predictions for surviving fraction of viruses when experimental data from either 60 minute long or 3 day-long experiments were used. These results indicated that the predicted times for 4 $\log_{10}$ (99.99 %) inactivation differ considerably, with more than an order of magnitude difference predicted for HAV by the two population and multistate models. In some instances, 4 $\log_{10}$ (99.99 %) inactivation times could not be determined because the models incorporating a decreasing concentration of disinfectant predicted that the monochloramine would be exhausted before this reduction could be achieved. A caveat can be inferred from the large differences in the predicted times for 4 $\log_{10}$ (99.99 %) inactivation when experiments of different duration are used. Using data from experiments of short contact time (e.g. 60 minutes) may severely underestimate inactivation time with respect to weak oxidants such as monochloramine. Such experiments are biased towards that fraction of viruses which have the higher rate constant of inactivation. In longer disinfection experiments, where data exhibit retardant die-off kinetics,

resulting predictions are based on a higher proportion of data points derived from that part of the virus population exhibiting a slower rate constant of inactivation.

Several assumptions in devising the models when applied to monochloramine may not be valid for chlorine dioxide. Therefore, these models should be considered as an endeavor to improve and expand the current methodology of modeling inactivation kinetics. They should not serve as a replacement for the current first-order model until more expansive applications of these models are tested on data of many inactivation kinetic experiments.

CONCLUSIONS

The results of this study indicate that monochloramine is a weak virus disinfectant, regardless of pH. Based on $\log_{10}$ inactivation times, as observed in the experiments and predicted from the various models, monochloramine should not be considered for use as a primary disinfectant. In addition, its use as a secondary disinfectant should also be cautioned due to its low efficiency of inactivating viruses that may be introduced subsequently into a distribution system (Snead, et al., 1980).

Chlorine dioxide was efficient in destroying both viruses in a relatively short period of time. If used at pH values of 9 and above, it is a potent virucide. Although it is used extensively in Europe as a primary disinfectant,

most water authorities in the U.S. only use chlorine dioxide as preoxidant to control for taste and odor problems or limit the formation of THMs. This difference in treatment practices may be due to the current EPA recommendation that the combined residual of ClO2, ClO2-, and ClO3- not exceed 1.0 mg/l in finished drinking water. Recent research efforts have indicated that the most effective way to minimize the formation of chlorate ion is to avoid those conditions that result in a low reaction rate, such as high pH values or low initial reactant concentrations and the presence of free HOCl-. Perhaps if these potentially harmful by-products could be kept at a minimal concentration, the use of chlorine dioxide in the U.S. will increase.

As with the results of modeling virus inactivation by monochloramine, the times predicted for inactivation by chlorine dioxide are higher using the multistate, distributive rate constant and two-population models. This reinforces the idea that the simplicity of the Chick-Watson model renders it unreliable in predicting the inactivation kinetics of disinfectants over extended time periods.

RECOMMENDATIONS

Further disinfection studies should be conducted using field samples to determine whether the relative resistance of HAV and other enteric viruses existing in natural waters and those cultured in the laboratory have

most water authorities in the U.S. only use chlorine dioxide as preoxidant to control for taste and odor problems or limit the formation of THMs. This difference in treatment practices may be due to the current EPA recommendation that the combined residual of ClO2, ClO2-, and ClO3- not exceed 1.0 mg/l in finished drinking water. Recent research efforts have indicated that the most effective way to minimize the formation of chlorate ion is to avoid those conditions that result in a low reaction rate, such as high pH values or low initial reactant concentrations and the presence of free HOCl-. Perhaps if these potentially harmful by-products could be kept at a minimal concentration, the use of chlorine dioxide in the U.S. will increase.

As with the results of modeling virus inactivation by monochloramine, the times predicted for inactivation by chlorine dioxide are higher using the multistate, distributive rate constant and two-population models. This reinforces the idea that the simplicity of the Chick-Watson model renders it unreliable in predicting the inactivation kinetics of disinfectants over extended time periods.

RECOMMENDATIONS

Further disinfection studies should be conducted using field samples to determine whether the relative resistance of HAV and other enteric viruses existing in natural waters and those cultured in the laboratory have

similar inactivation kinetics. Different natural isolates of the same enterovirus type can exhibit dramatic differences in inactivation kinetics (Payment and Trudel, 1985). Furthermore, viruses repeatedly exposed to disinfectants may become selectively more resistant (Bates, 1977).

Additional experiments should also use parameters of virus quality and physical state, disinfectant dose and residual, contact time, mixing and other hydraulic conditions which reflect conditions in actual water treatment plants and distribution systems. Studies should be done ultimately with natural virus populations that tend to be aggregated and solids-associated because such viruses may be more resistant to disinfection (Sobsey et al., 1991). It would also be valuable to use water that is not halogen demand-free in order to examine and compare the stability of monochloramine and chlorine dioxide in water more typical of natural systems.

The validity of using the C x T concept to ensure safe drinking water needs to be reassessed when weak oxidants such as monochloramine are being used. This may also be true of stronger disinfectants such as chlorine and chlorine dioxide where similar retardant die-off kinetics are observed under certain conditions.

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APPENDIX I
RAW DATA FROM DISINFECTION EXPERIMENTS

TEST CONDITIONS: NH2Cl; pH 6; 5 degrees C; MS2

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	45900	1.03	0.01		
1	34500	0.77	-0.011		
3	11900	0.27	-0.57		PFU/ml
10	8170	0.18	-0.74		
30	5330	0.12	-0.92	VC0	47600
60	4600	0.1	-0.98	VC60	41200
EXP. 2					
0.33	25300	0.466	-0.33		
1	15300	0.282	-0.55		
3	12000	0.221	-0.66		PFU/ml
10	10100	0.186	-0.73		
30	9480	0.175	-0.76	VC0	71600
60	8970	0.165	-0.78	VC60	37000
EXP. 3					
0.33	58600	1.1	0.04		
1	44300	0.83	-0.08		
3	10600	0.2	-0.7		PFU/ml
10	8820	0.17	-0.77		
30	6520	0.12	-0.91	VC0	56300
60	5940	0.11	-0.95	VC60	50300
AVERAGE					
0.33	513667	1.09	-0.095		
1	385667	0.82	-0.092		
3	93300	0.21	-0.701		PFU/ml
10	88267	0.19	-0.727		
30	56867	0.12	-0.913	VC0	58500
60	42433	0.09	-1.046	VC60	42833.33

TEST CONDITIONS: NH2Cl; pH 8; 5 degrees C; MS2

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	53000	1.06	0.027		
1	39000	0.783	-0.106		
3	9180	0.184	-0.734		PFU/ml
10	8150	0.164	-0.786		
30	5820	0.117	-0.932	VC0	58000
60	4150	0.083	-1.079	VC60	41500
EXP. 2					
0.33	58300	1.14	0.057		
1	43300	0.88	-0.057		
3	9330	0.19	-0.724		PFU/ml
10	8940	0.18	-0.742		
30	5300	0.11	-0.969	VC0	58700
60	4730	0.096	-1.02	VC60	40000
EXP. 3					
0.33	44800	1.069	0.029		
1	33400	0.797	-0.1		
3	9480	0.226	-0.65		PFU/ml
10	9390	0.224	-0.65		
30	5940	0.142	-0.84	VC0	49000
60	3850	0.092	-1.04	VC60	34800
AVERAGE					
0.33	52033	1.0697	0.0377		
1	38567	0.8200	-0.0877		
3	9330	0.2000	-0.7027		PFU/ml
10	8827	0.1893	-0.7260		
30	5687	0.1230	-0.9137	VC0	55233
60	4243	0.0903	-1.0463	VC60	38767

TEST CONDITIONS: NH2Cl; pH 10; 5 degrees C; MS2

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	8940	0.83	-0.08		
1	8410	0.78	-0.11		
3	6760	0.63	-0.2		PFU/ml
10	3730	0.35	-0.46		
30	1790	0.17	-0.78	VC0	11800
60	1880	0.17	-0.78	VC60	9800
EXP. 2					
0.33	9300	0.22	-0.6		
1	9180	0.22	-0.67		
3	8000	0.19	-0.73		PFU/ml
10	6450	0.15	-0.82		
30	4090	0.1	-1.02	VC0	54000
60	2390	0.06	-1.25	VC60	30900
EXP. 3					
0.33	11500	0.258	-0.59		
1	9330	0.21	-0.68		
3	8600	0.193	-0.714		PFU/ml
10	7030	0.158	-0.801		
30	4420	0.099	-1	VC0	49100
60	2800	0.063	-1.2	VC60	40000
AVERAGE					
0.33	9913	0.4360	-0.1233		
1	8973	0.4033	-0.4867		
3	7787	0.3377	-0.5480		PFU/ml
10	5737	0.2193	-0.6937		
30	3433	0.1230	-0.9333	VC0	38300
60	2357	0.0977	-1.0767	VC60	26900

TEST CONDITIONS: NH2Cl; pH 6; 5 degrees C; HAV

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	32300	0.887	-0.052		
1	31100	0.854	-0.07		
3	26400	0.725	-0.14		PFU/ml
10	24100	0.662	-0.18		
30	15000	0.412	-0.377	VC0	40000
60	8400	0.231	-0.63	VC60	32700
EXP. 2					
0.33	24800	0.8	-0.1		
1	21200	0.69	-0.16		
3	17600	0.57	-0.25		PFU/ml
10	13600	0.44	-0.36		
30	8790	0.28	-0.55	VC0	33000
60	6970	0.23	-0.65	VC60	28800
EXP. 3					
0.33	34400	1.04	0.018		
1	28900	0.88	-0.058		
3	24200	0.73	-0.135		PFU/ml
10	18600	0.564	-0.25		
30	13000	0.394	-0.41	VC0	34700
60	6520	0.197	-0.704	VC60	31200
AVERAGE					
0.33	30500	0.9090	-0.0447		
1	27067	0.8080	-0.0960		
3	22733	0.6750	-0.1750		PFU/ml
10	18767	0.5553	-0.2633		
30	12263	0.3620	-0.4457	VC0	35900
60	7297	0.2193	-0.6613	VC60	30900

TEST CONDITIONS: NH2Cl; pH 8; 5 degrees C; HA\

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	31700	0.984	-0.007		
1	25900	0.804	-0.095		
3	21200	0.658	-0.186		PFU/ml
10	17030	0.529	-0.277		
30	6450	0.2	-0.698	VC0	33900
60	6010	0.187	-0.729	VC60	30500
EXP. 2					
0.33	33800	1.037	0.016		
1	23800	0.73	-0.137		
3	22000	0.675	-0.171		PFU/ml
10	11100	0.34	-0.468		
30	5870	0.18	-0.745	VC0	35600
60	5300	0.163	-0.79	VC60	30300
EXP. 3					
0.33	20900	0.92	-0.04		
1	18800	0.83	-0.08		
3	14500	0.64	-0.19		PFU/ml
10	9090	0.4	-0.4		
30	6670	0.29	-0.53	VC0	25000
60	5330	0.23	-0.64	VC60	20300
AVERAGE					
0.33	28800	0.9803	-0.0103		
1	22833	0.7880	-0.1040		
3	19233	0.6577	-0.1823		PFU/ml
10	12407	0.4230	-0.3817		
30	6330	0.2233	-0.6577	VC0	31500
60	5547	0.1933	-0.7197	VC60	27033

TEST CONDITIONS: NH2Cl; pH 10; 5 degrees C; HAV

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	36700	0.89	-0.05		
1	10800	0.26	-0.58		
3	9640	0.23	-0.63		PFU/ml
10	5970	0.14	-0.84		
30	2030	0.05	-1.31	VC0	48800
60	1390	0.03	-1.47	VC60	33600
EXP. 2					
0.33	16600	1.07	0.03		
1	10200	0.66	-0.182		
3	8120	0.52	-0.281		PFU/ml
10	5000	0.323	-0.491		
30	4610	0.297	-0.53	VC0	13800
60	2940	0.19	-0.722	VC60	17300
EXP. 3					
0.33	11500	0.258	-0.59		
1	9330	0.21	-0.68		
3	8600	0.193	-0.714		PFU/ml
10	7030	0.158	-0.801		
30	4420	0.099	-1	VC0	24320
60	2800	0.063	-1.201	VC60	25600
AVERAGE					
0.33	21600	0.7393	-0.2033		
1	10110	0.3767	-0.4807		
3	8787	0.3143	-0.5417		PFU/ml
10	6000	0.2070	-0.7107		
30	3687	0.1187	-0.9467	VC0	28973.33
60	2377	0.0943	-1.1310	VC60	25500

TEST CONDITIONS: NH2Cl; pH 8; 5 deg. C; MS2; 3-day

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	4460000	0.2114	-0.6749		
3	1920000	0.091	-1.041		
10	4170000	0.1976	-0.704		
30	2780000	0.1318	-0.88		PFU/ml
60	2030000	0.0962	-1.017		
1440	113000	0.0054	-2.271	VC0	21500000
2880	71500	0.0034	-2.47	VC60	29400000
4320	29600	0.0014	-2.853	VC3day	12400000
EXP. 2					
0.33	2770000	1.4	0.146		
3	1040000	0.525	-0.28		
10	739000	0.373	-0.428		
30	536000	0.271	-0.568		PFU/ml
60	126000	0.064	-1.196		
1440	67600	0.034	-1.467	VC0	2210000
2880	39700	0.02	-1.698	VC60	2050000
4320	24200	0.012	-1.913	VC3day	1690000
AVERAGE					
0.33	3615000	0.8057	-0.26445		
3	1480000	0.308	-0.6605		PFU/ml
10	2454500	0.2853	-0.566		
30	1658000	0.2014	-0.724	VC0	11855000
60	1078000	0.0801	-1.1065	VC60	15725000
1440	90300	0.0197	-1.869	VC3day	7045000
2880	55600	0.0117	-2.084		
4320	26900	0.0067	-2.383		

TEST CONDITIONS: NH2Cl; pH 8; 5 deg. C; HAV; 3-day

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	80600	1.364	0.135		
3	29700	0.503	-0.299		
10	26400	0.447	-0.35		
30	11300	0.191	-0.719		PFU/ml
60	10400	0.176	-0.755		
1440	6880	0.116	-0.934	VC0	71400
2880	6520	0.11	-0.957	VC60	62800
4320	6220	0.105	-0.978	VC3day	43000
EXP. 2					
0.33	342000	0.776	-0.11		
3	196000	0.444	-0.352		
10	157000	0.356	-0.449		
30	92700	0.2102	-0.677		PFU/ml
60	1100	0.0249	-1.603		
1440	3240	0.007	-2.134	VC0	939000
2880	1030	0.002	-2.632	VC60	205000
4320	1480	0.003	-2.474	VC3day	180000
EXP. 3					
0.33	32700	0.142	-0.849		
3	41500	0.18	-0.746		
10	17900	0.078	-1.111		
30	19000	0.0823	-1.085		PFU/ml
60	4720	0.02	-1.69		
1440	1300	0.006	-2.25	VC0	426000
2880	1030	0.0045	-2.351	VC60	43900
4320	830	0.0036	-2.4450	VC3day	35500
AVERAGE					
0.33	151767	0.761	-0.275		
3	89067	0.376	-0.466		
10	67100	0.294	-0.637		
30	41000	0.161	-0.827		PFU/ml
60	5407	0.074	-1.349		
1440	3807	0.043	-1.773	VC0	478800
2880	2860	0.039	-1.980	VC60	103900
4320	2843	0.037	-1.966	VC3day	86166.67

TEST CONDITIONS: NH2Cl; 5 deg. C; MODELING; MS2

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No
Standard no suppiement			
0.33	205000	0.435	-0.361
3	100000	0.212	-0.673
10	83800	0.178	-0.75
30	70000	0.149	-0.828
60	15600	0.033	-1.48
1440	5780	0.012	-1.911
2880	4650	0.01	-2.006
4320	3470	0.007	-2.133
supplemental virus			
3	329000	0.537	-0.27
30	136000	0.222	-0.654
60	88700	0.145	-0.84
1440	43500	0.071	-1.149
2880	34700	0.057	-1.247
supplemental NH2Cl			
3	3330	0.007	-2.151
30	2830	0.007	-2.151
60	1620	0.003	-2.464
1440	1670	0.004	-2.45
2880	1300	0.003	-2.559

	PFU/ml
VC0	659000
VC60	514000
VC3day	242000

TEST CONDITIONS: NH2Cl; 5 deg. C; MODELING II; MS2

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No
Standard no supplement			
0.33	121000	0.284	-0.5466
3	109000	0.256	-0.592
10	93700	0.22	-0.6576
30	61000	0.143	-0.8441
60	46400	0.109	-0.9629
1440	4270	0.01	-1.999
2880	2890	0.007	-2.1685
4320	2700	0.006	-2.198
supplemental virus			
3	965000	1.5794	0.1985
30	685000	1.1211	0.0496
60	532000	0.8707	-0.06
1440	8860	0.0145	-1.838
2880	7700	0.0126	-1.8996
supplemental NH2Cl			
3	2300	0.007	-2.2677
30	2020	0.006	-2.3241
60	1980	0.005	-2.3241
1440	2230	0.005	-2.2811
2880	1620	0.004	-2.42

	PFU/ml
VC0	470000
VC60	383000
VC3day	84500

TEST CONDITIONS: NH2Cl: 5 deg. C; MODELING; HAV

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No
Standard no supplement			
0.33	102000	0.5231	-0.2814
3	84200	0.4316	-0.3647
10	55800	0.2862	-0.5434
30	37700	0.1933	-0.7137
60	5880	0.0302	-1.5207
1440	5180	0.0266	-1.5757
2880	4760	0.024	-1.6124
4320	2590	0.0133	-1.8767
supplemental virus			
3	5330000	0.8514	-0.0698
30	4920000	0.7859	-0.1046
60	689000	0.1101	-0.9583
1440	545000	0.0871	-1.0602
2880	456000	0.0728	-1.1376
supplemental NH2Cl			
3	2590	0.0133	-1.8767
30	2020	0.0104	-1.9847
60	1530	0.0078	-2.1053
1440	1600	0.0082	-2.0859
2880	6.7	0.0032	-2.4957

	PFU/ml
VC0	230000
VC60	159000
VC3day	105000

TEST CONDITIONS: NH2Cl; 5 deg. C; MODELING II; HAV

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No
Standard no supplement			
0.33	120000	0.428	-0.369
3	95000	0.338	-0.47
10	74500	0.265	-0.576
30	20500	0.073	-1.136
60	15600	0.056	-1.255
1440	12800	0.046	-1.341
2880	11500	0.041	-1.388
4320	8620	0.0133	-1.513
supplemental virus			
3	7640000	1.218	0.086
30	5020000	0.8	-0.097
60	2080000	0.332	-0.479
1440	1470000	0.234	-0.63
2880	106000	0.169	-0.772
supplemental NH2Cl			
3	7860	0.028	-1.553
30	6880	0.024	-1.611
60	5230	0.019	-1.73
1440	4680	0.017	-1.778
2880	3940	0.014	-1.853

	PFU/ml
VC0	377000
VC60	300000
VC3day	165000

TEST CONDITIONS: ClO2; pH 6; 5 degrees C; MS2

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	127000	1.15	0.06		
1	76100	0.68	-0.162		
3	22100	0.2	-0.699		PFU/ml
10	8480	0.077	-1.115		
30	3590	0.032	-1.488	VC0	118000
60	1360	0.012	-1.91	VC60	103000
EXP. 2					
0.33	43500	0.902	-0.045		
1	25800	0.535	-0.271		
3	3020	0.063	-1.203		PFU/ml
10	1680	0.035	-1.458		
30	545	0.011	-1.947	VC0	52100
60	15	0.0003	-3.503	VC60	44400
EXP. 3					
0.33	33000	0.385	-0.415		
1	16000	0.187	-0.729		
3	11800	0.138	-0.861		PFU/ml
10	3030	0.035	-1.452		
30	1760	0.021	-1.688	VC0	96100
60	940	0.011	-1.96	VC60	75200
AVERAGE					
0.33	67833	0.8123	-0.1333		
1	39300	0.4673	-0.3873		
3	12307	0.1337	-0.9210		PFU/ml
10	4397	0.0490	-1.3417		
30	1965	0.0213	-1.7077	VC0	88733.33
60	772	0.0078	-2.4577	VC60	74200

TEST CONDITIONS: ClO2; pH 8; 5 degrees C; MS2

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	194000	0.469	-0.329		
1	187000	0.452	-0.345		
3	8550	0.021	-1.685		PFU/ml
10	5150	0.012	-1.905		
30	5850	0.014	-1.85	VC0	524000
60	33	8E-05	-4.099	VC60	303000
EXP. 2					
0.33	213000	0.88	-0.055		
1	185000	0.765	-0.117		
3	655	0.027	-1.567		PFU/ml
10	491	0.02	-1.693		
30	421	0.017	-1.76	VC0	275000
60	100	0.0004	-3.384	VC60	209000
EXP. 3					
0.33	2820	0.215	-0.667		
1	1830	0.14	-0.854		
3	1780	0.136	-0.867		PFU/ml
10	1240	0.095	-1.024		
30	906	0.069	-1.16	VC0	18800
60	500	0.038	-1.418	VC60	7410
AVERAGE					
0.33	136607	0.5213	-0.3503		
1	124610	0.4523	-0.4387		
3	3662	0.0613	-1.3730		PFU/ml
10	2294	0.0423	-1.5407		
30	2392	0.0333	-1.5900	VC0	272600
60	211	0.0128	-2.9670	VC60	173136.7

TEST CONDITIONS: ClO2; pH 10; 5 degrees C; MS2

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	33	0.0001	-3		
1	33	0.0001	-3		
3	33	0.0001	-3		PFU/ml
10	33	0.0001	-3		
30	33	0.0001	-3	VC0	38800
60	33	0.0001	-3	VC60	28800
EXP. 2					
0.33	33	0.0006	-3.238		
1	33	0.0006	-3.238		
3	33	0.0006	-3.238		PFU/ml
10	33	0.0006	-3.238		
30	33	0.0006	-3.238	VC0	106000
60	33	0.0006	-3.238	VC60	6100
EXP. 3					
0.33	33	0.0006	-3.213		
1	33	0.0006	-3.213		
3	33	0.0006	-3.213		PFU/ml
10	33	0.0006	-3.213		
30	33	0.0006	-3.213	VC0	100000
60	33	0.0006	-3.213	VC60	7780
AVERAGE					
0.33	33	0.0004	-3.1503		
1	33	0.0004	-3.1503		
3	33	0.0004	-3.1503		PFU/ml
10	33	0.0004	-3.1503		
30	33	0.0004	-3.1503	VC0	82266.67
60	33	0.0004	-3.1503	VC60	14226.67

TEST CONDITIONS: ClO2; pH 6; 5 degrees C; HAV

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	92400	0.811	-0.091		
1	42100	0.369	-0.433		
3	8400	0.074	-1.133		PFU/ml
10	1590	0.014	-1.856		
30	933	0.008	-2.087	VC0	120000
60	238	0.002	-2.68	VC60	108000
EXP. 2					
0.33	29500	0.683	-0.166		
1	5650	0.131	-0.883		
3	1910	0.044	-1.354		PFU/ml
10	1470	0.34	-1.468		
30	683	0.016	-1.801	VC0	45900
60	183	0.004	-2.373	VC60	40500
EXP. 3					
0.33	26700	0.516	-0.287		
1	8630	0.167	-0.778		
3	1830	0.035	-1.451		PFU/ml
10	966	0.019	-1.729		
30	766	0.015	-1.829	VC0	69600
60	433	0.008	-2.077	VC60	33800
AVERAGE					
0.33	49533	0.6700	-0.1813		
1	18793	0.2223	-0.6980		
3	4047	0.0510	-1.3127		PFU/ml
10	1342	0.1243	-1.6843		
30	794	0.0130	-1.9057	VC0	78500
60	285	0.0047	-2.3767	VC60	60766.67

TEST CONDITIONS: ClO2; pH 8; 5 degrees C; HAV

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	10700	0.744	-0.128		
1	5060	0.352	-0.454		
3	4750	0.33	-0.481		PFU/ml
10	600	0.042	-1.38		
30	100	0.007	-2.158	VC0	24200
60	10	0.0007	-3.158	VC60	4550
EXP. 2					
0.33	11300	0.312	-0.505		
1	3240	0.09	-1.048		
3	2060	0.057	-1.245		PFU/ml
10	1390	0.038	-1.415		
30	867	0.024	-1.62	VC0	66400
60	400	0.011	-1.956	VC60	5950
EXP. 3					
0.33	36700	0.856	-0.068		
1	8940	0.208	-0.681		
3	3670	0.086	-1.068		PFU/ml
10	1970	0.046	-1.338		
30	200	0.005	-2.331	VC0	60900
60	33	0.0008	-3.114	VC60	24800
AVERAGE					
0.33	19567	0.6373	-0.2337		
1	5747	0.2167	-0.7277		
3	3493	0.1577	-0.9313		PFU/ml
10	1320	0.0420	-1.3777		
30	389	0.0120	-2.0363		50500
60	148	0.0042	-2.7427		11766.67

TEST CONDITIONS: ClO2; pH 10; 5 degrees C; HAV

SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	33	0.0006	-3.216		
1	33	0.0006	-3.216		
3	33	0.0006	-3.216		PFU/ml
10	33	0.0006	-3.216		
30	33	0.0006	-3.216	VC0	105000
60	33	0.0006	-3.216	VC60	3670
EXP. 2					
0.33	742	0.0043	-2.369		
1	367	0.0021	-2.674		
3	17	0.0001	-4		PFU/ml
10	17	0.0001	-4		
30	17	0.0001	-4	VC0	295000
60	17	0.0001	-4	VC60	51700
EXP. 3					
0.33	17	0.0006	-3.255		
1	17	0.0006	-3.255		
3	17	0.0006	-3.255		PFU/ml
10	17	0.0006	-3.255		
30	17	0.0006	-3.255	VC0	54700
60	17	0.0006	-3.255	VC60	6520
AVERAGE					
0.33	264	0.0018	-2.9457		
1	139	0.0011	-3.0483		
3	22	0.0004	-3.4903		PFU/ml
10	22	0.0004	-3.4903		
30	22	0.0004	-3.4903	VC0	151566.7
60	22	0.0004	-3.4903	VC60	20630

TEST CONDITIONS: ClO2; pH 9; 5 degrees C; HAV

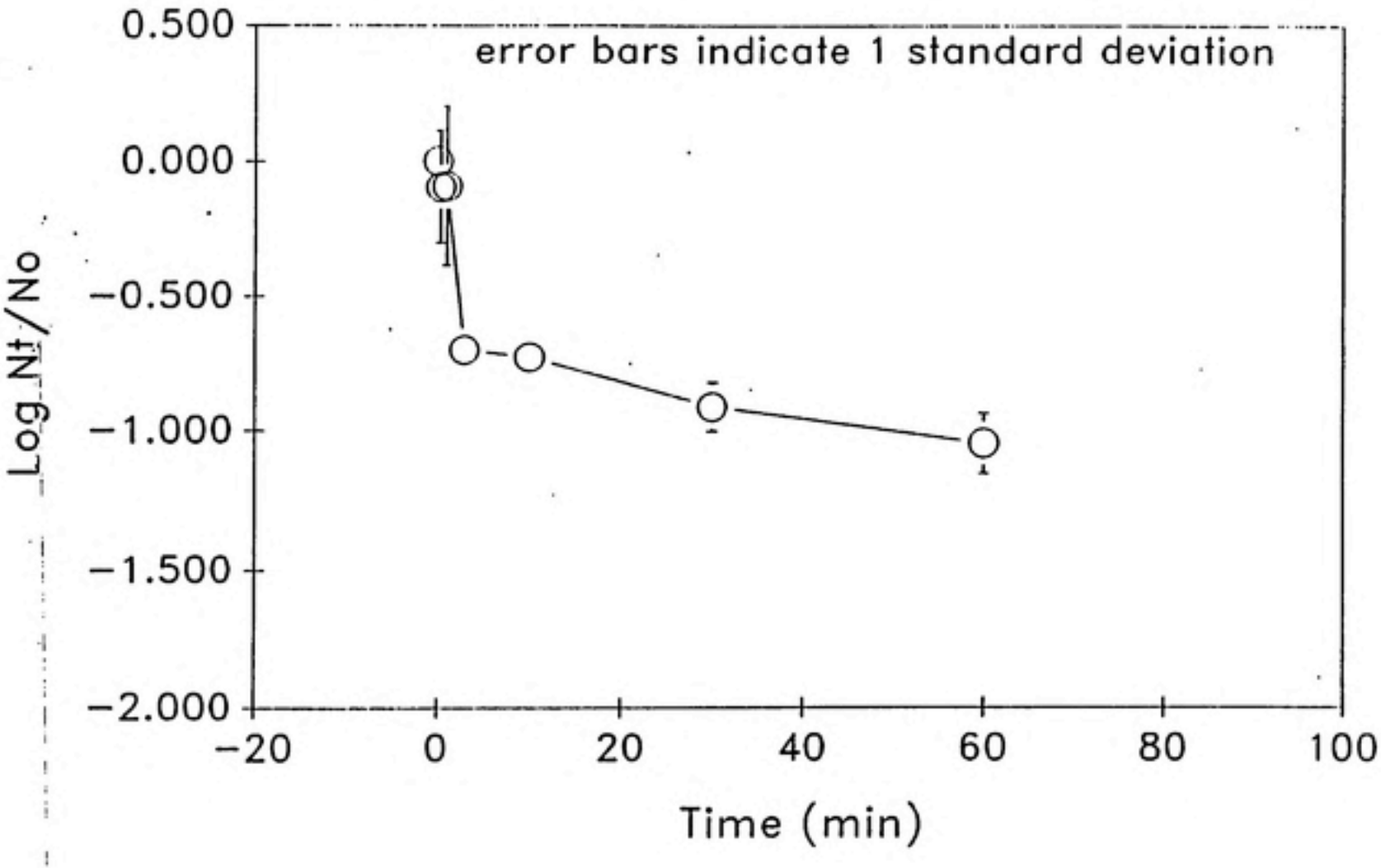
SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	17	0.0002	-3.6959		
1	17	0.0002	-3.6959		
3	17	0.0002	-3.6959		PFU/ml
10	17	0.0002	-3.6959		
30	17	0.0002	-3.6959	VC0	124000
60	17	0.0002	-3.6959	VC60	44800
EXP. 2					
0.33	383	0.0278	-1.5567		
1	17	0.00123	-2.9094		
3	17	0.00123	-2.9094		PFU/ml
10	17	0.00123	-2.9094		
30	17	0.00123	-2.9094	VC0	189000
60	17	0.00123	-2.9094	VC60	88300
AVERAGE					
0.33	200	0.014	-2.6263		
1	17	0.000715	-3.30265		
3	17	0.000715	-3.30265		PFU/ml
10	17	0.000715	-3.30265		
30	17	0.000715	-3.30265	VC0	156500
60	17	0.000715	-3.30265	VC60	66550

TEST CONDITIONS: ClO2; pH 9; 5 degrees C; MS2

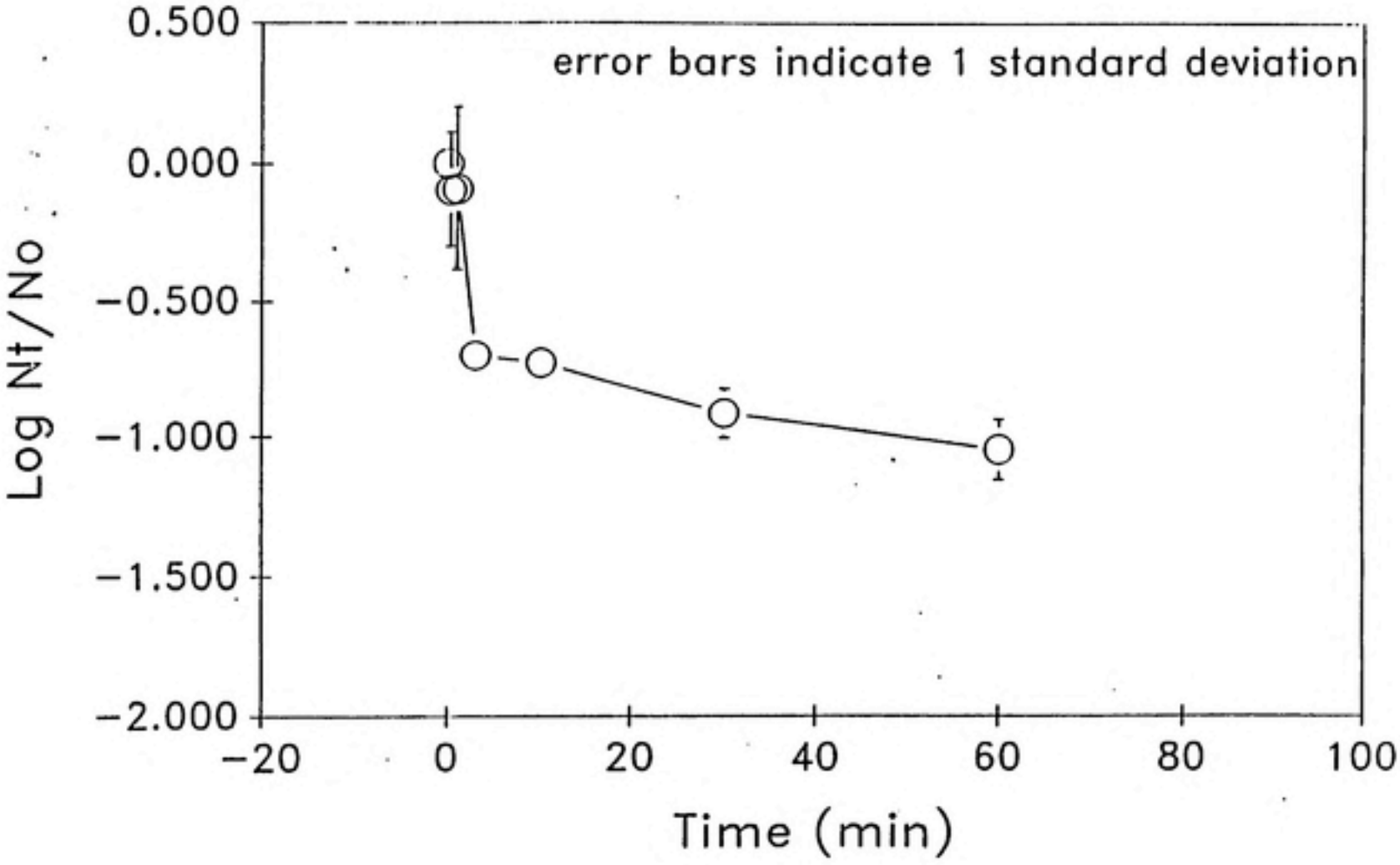
SAMPLE min.	PFU/ml	Nt/No	LOG Nt/No		
EXP. 1					
0.33	1680	0.0348	-1.4582		
1	786	0.0146	-1.8359		
3	200	0.0037	-2.4314		PFU/ml
10	17	0.0003	-3.5019		
30	17	0.0003	-3.5019	VC0	64400
60	17	0.0003	-3.5019	VC60	43600
EXP. 2					
0.33	1640	0.0239	-1.6221		
1	985	0.0143	-1.8435		
3	67	0.001	-3		PFU/ml
10	17	0.0002	-3.6065		
30	17	0.0002	-3.6065	VC0	74000
60	17	0.0002	-3.6065	VC60	63300
EXP. 3					
0.33	933	0.0189	-1.7237		
1	773	0.0157	-1.8054		
3	207	0.0042	-2.3778		PFU/ml
10	27	0.00055	-3.2624		
30	17	0.00034	-3.4633	VC0	78700
60	17	0.00034	-3.4633	VC60	20000
AVERAGE					
0.33	1144	0.02586	-1.6013		
1	1067	0.01486	-1.8283		
3	164	0.0141	-2.6031		PFU/ml
10	37	0.00026	-3.4569		
30	17	0.00028	-3.5239	VC0	72366.67
60	17	0.00028	-3.5239	VC60	42300

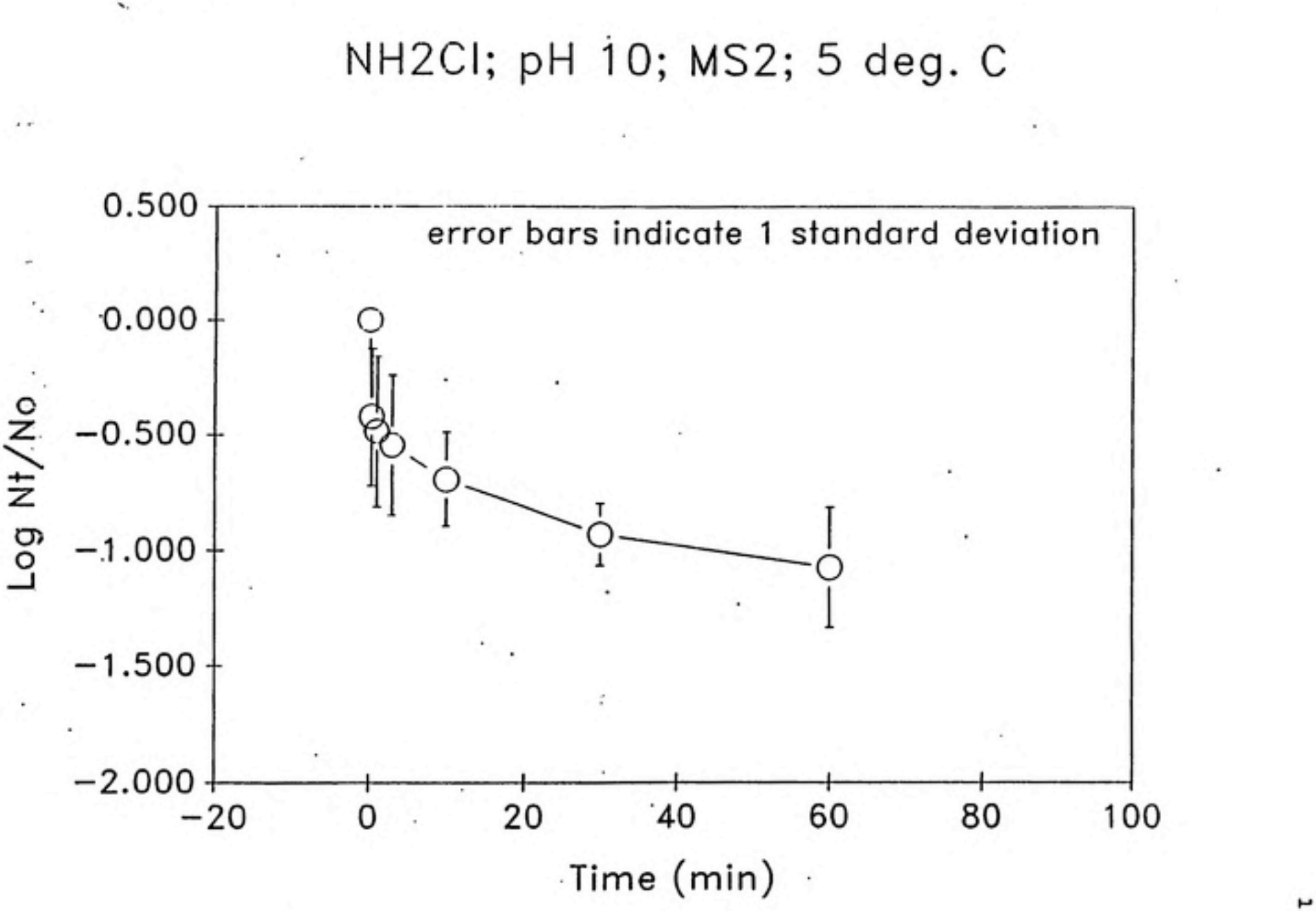
APPENDIX II

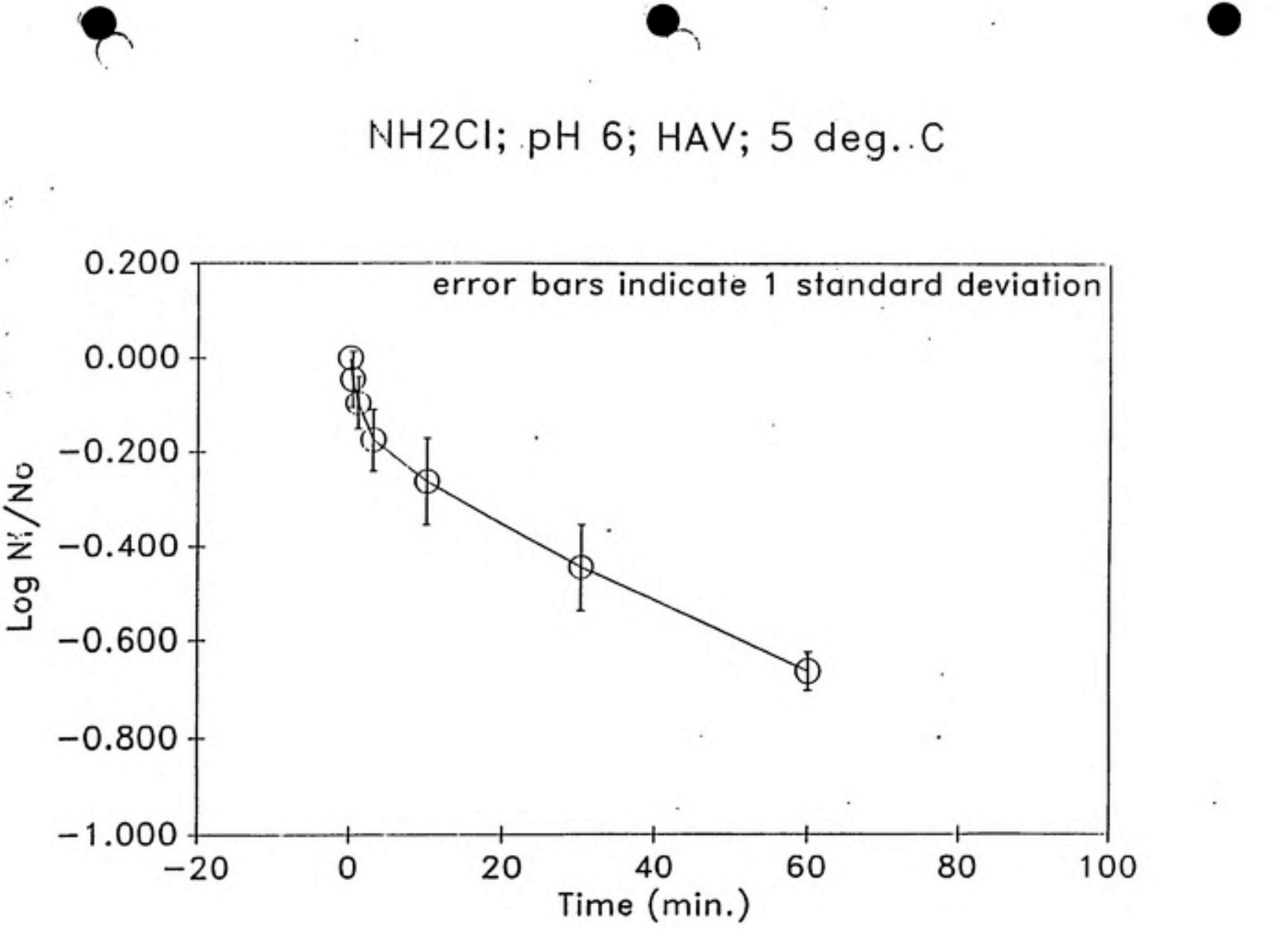
DISINFECTION EXPERIMENTS WITH ERROR BARS


NH2Cl; pH 6; MS2; 5 deg. C
error bars indicate 1 standard deviation
0.500
0.000
−0.500
−1.000
−1.500
−2.000
Log Nt/No
−20
0
20
40
60
80
100
Time (min)

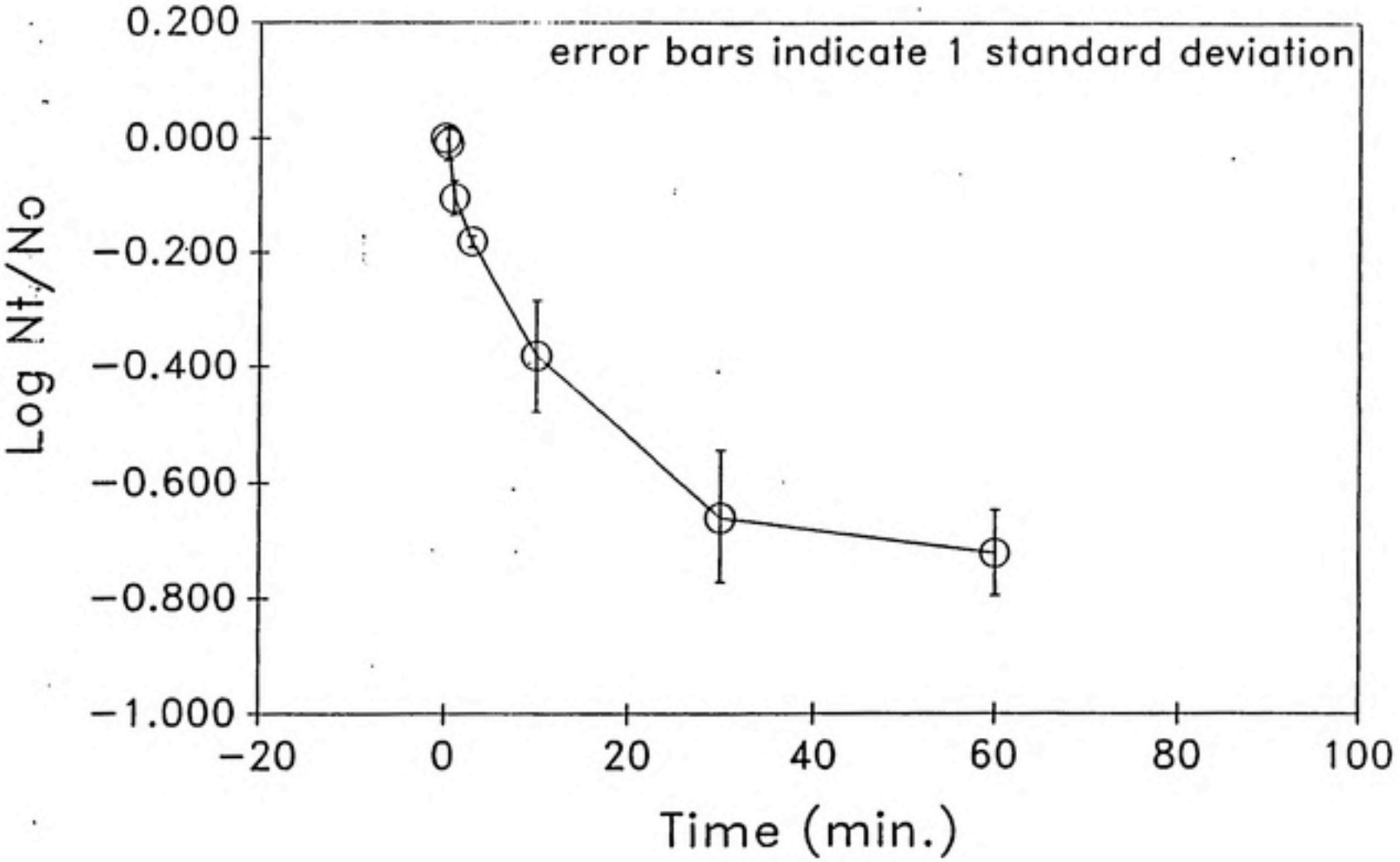
NH2Cl; pH 8; MS2; 5 deg. C



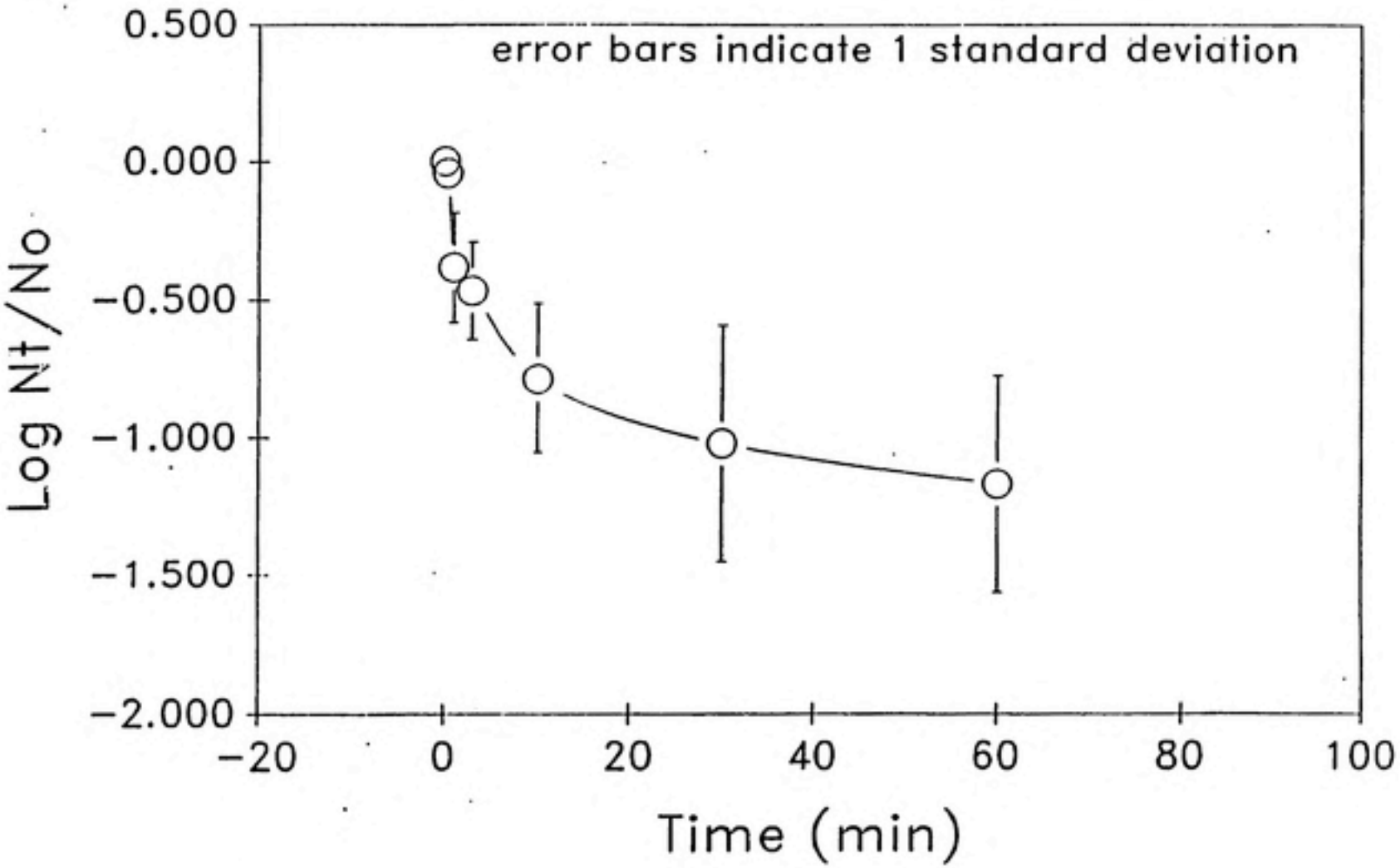

NH2Cl; pH 10; MS2; 5 deg. C
error bars indicate 1 standard deviation
0.500
0.000
−0.500
−1.000
−1.500
−2.000
Log Nt/No
−20
0
20
40
60
80
100
Time (min)


NH2Cl; pH 6; HAV; 5 deg..C
error bars indicate 1 standard deviation
0.200
0.000
−0.200
−0.400
−0.600
−0.800
−1.000
Log Ni/No
−20
0
20
40
60
80
100
Time (min.)

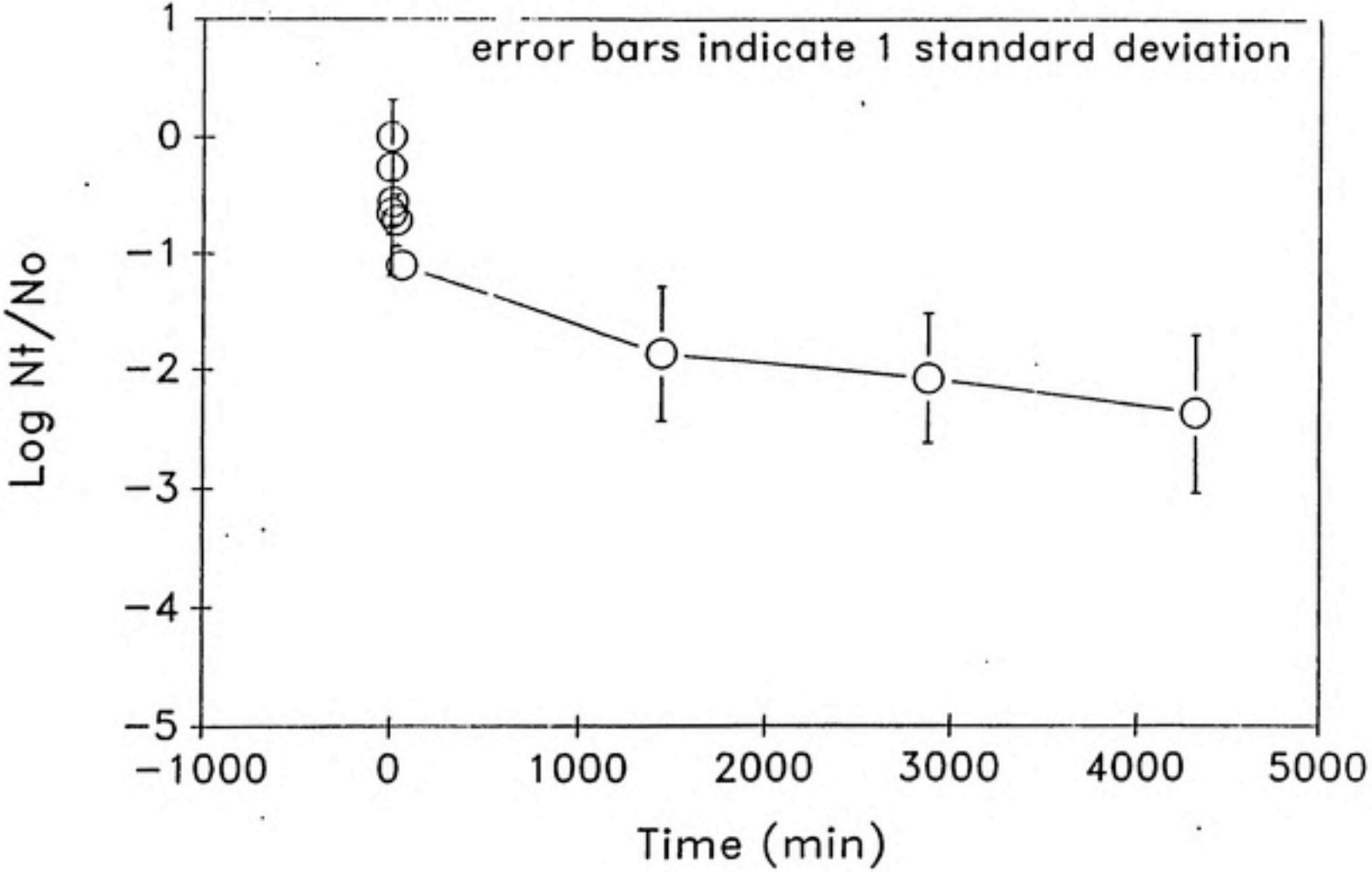
NH2Cl; pH 8; HAV; 5 deg. C



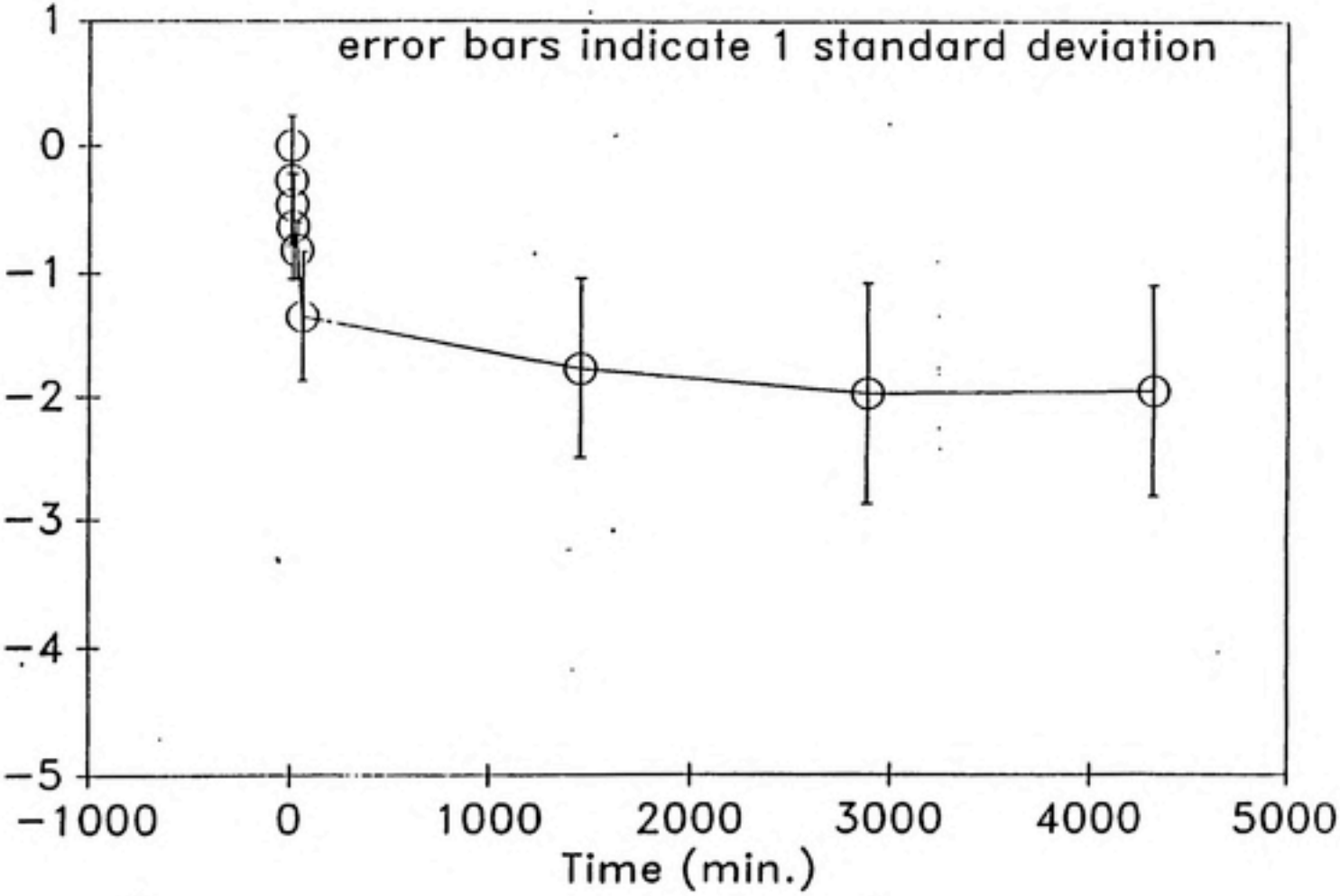
NH2Cl; pH 10; HAV; 5 deg. C



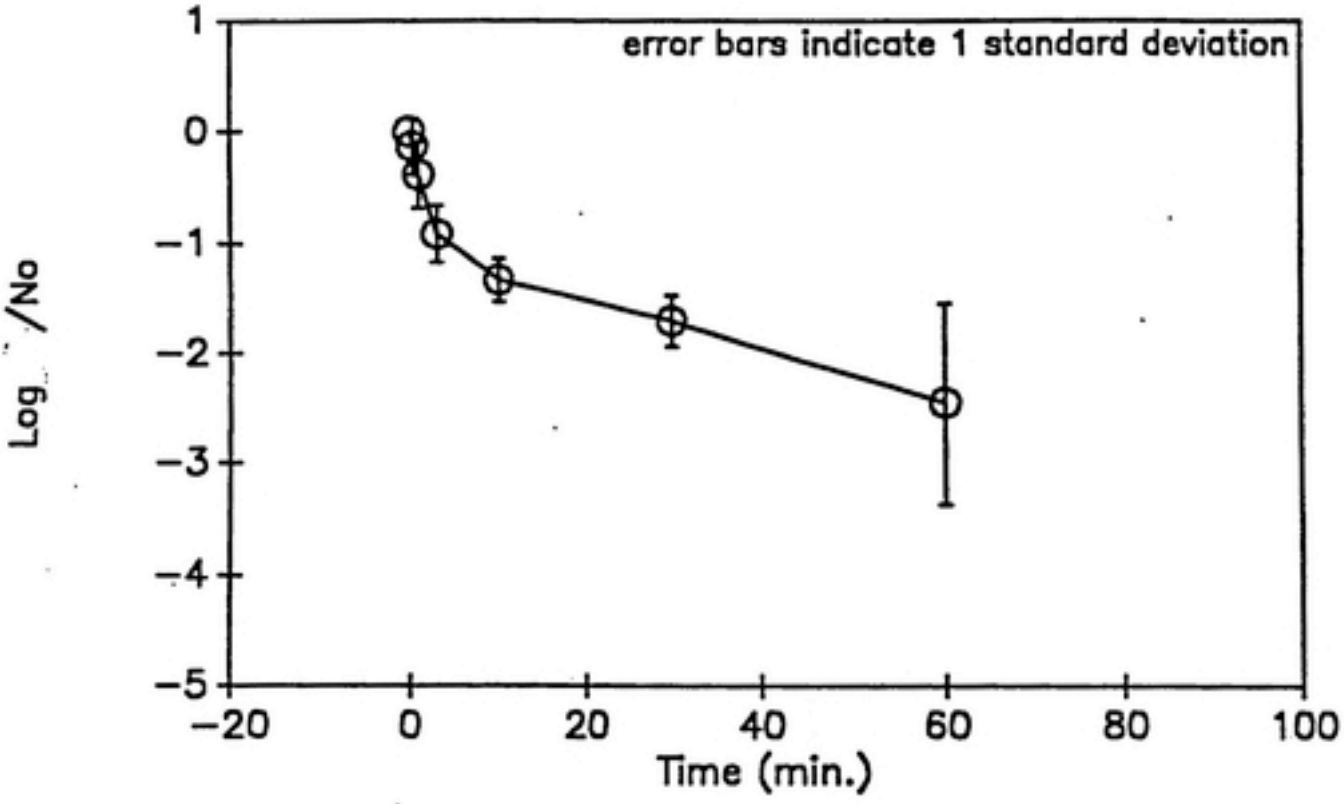
3-day exp.; NH2Cl; pH 8; MS2; 5 deg. C

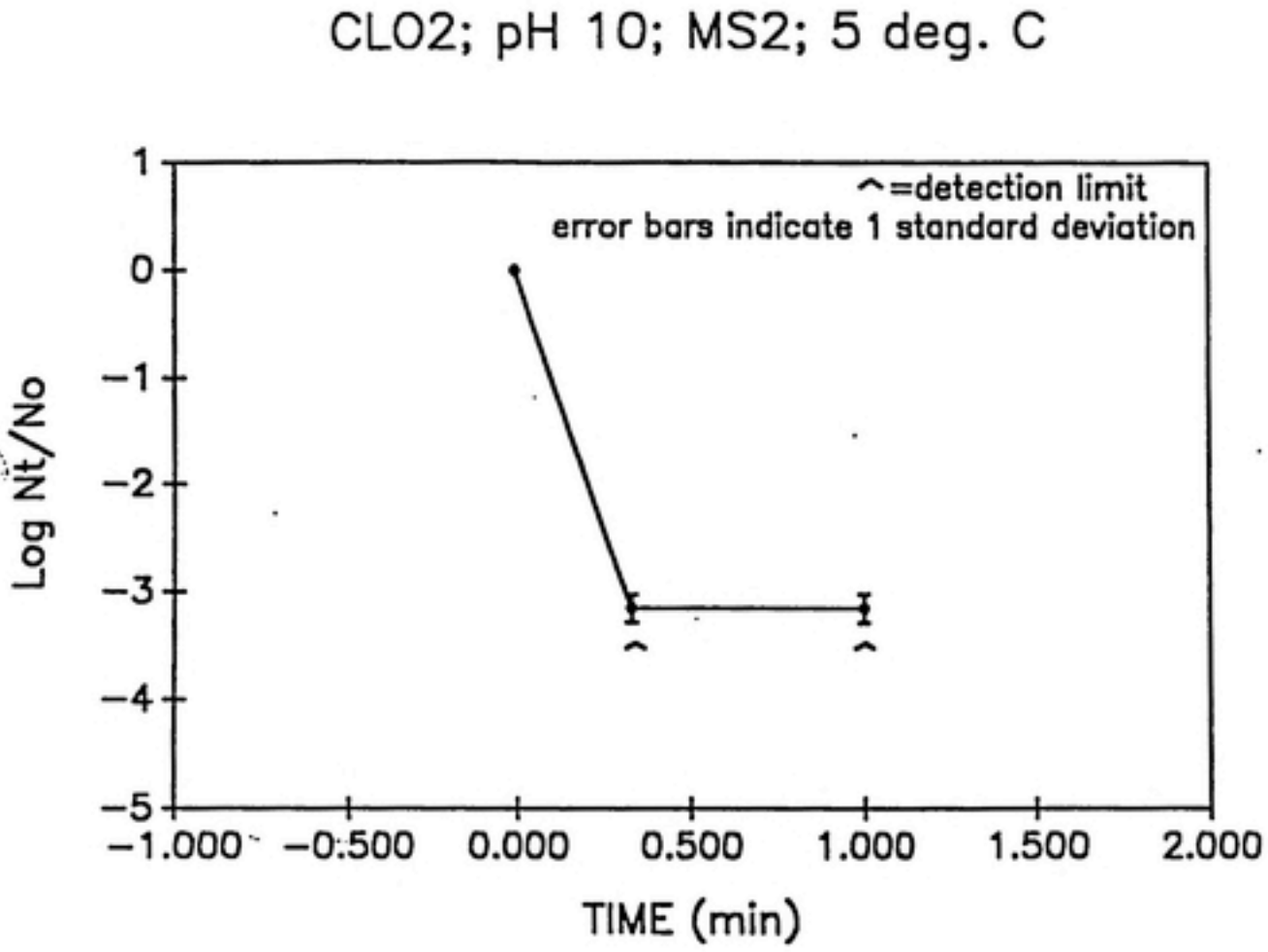


3-day exp.; NH2Cl; pH 8; HAV; 5 deg. C

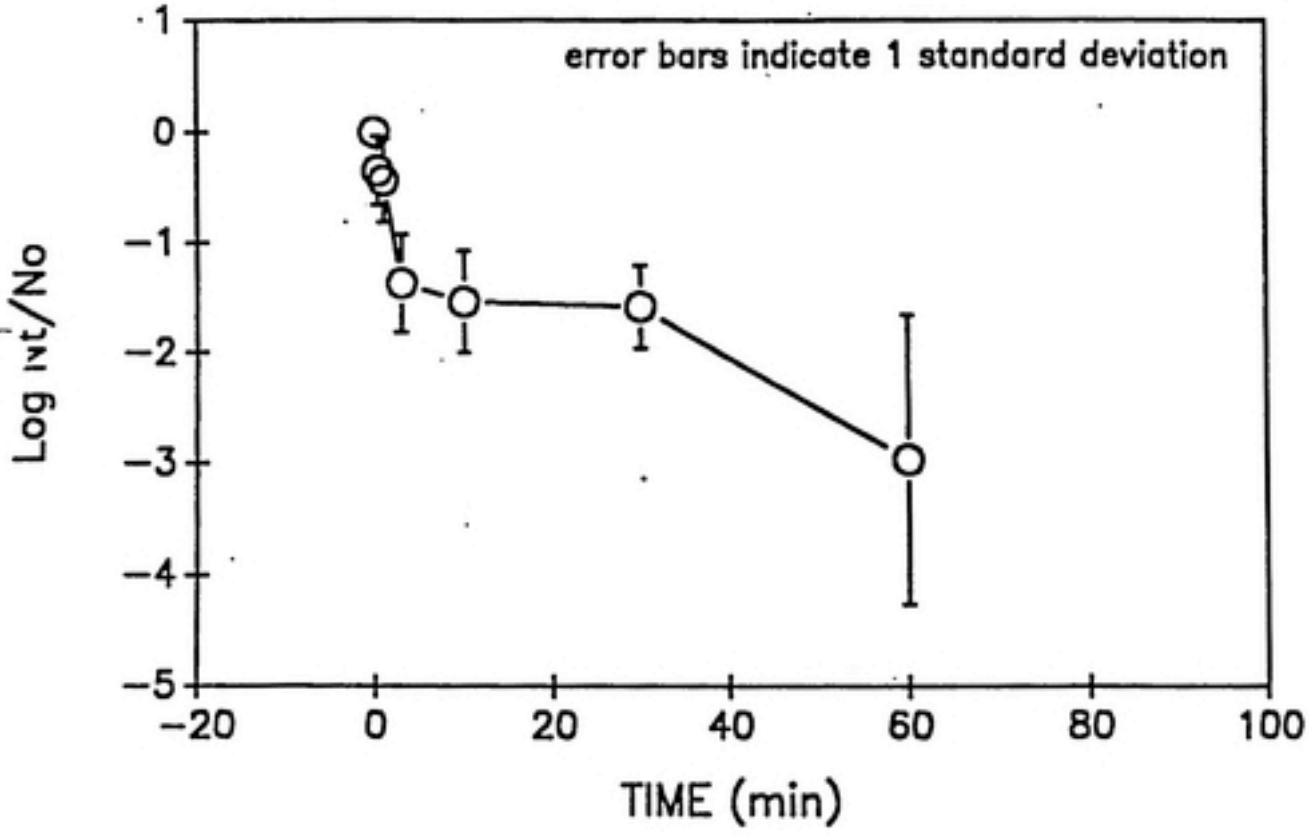


CLO2; pH 6; MS2; 5 deg. C

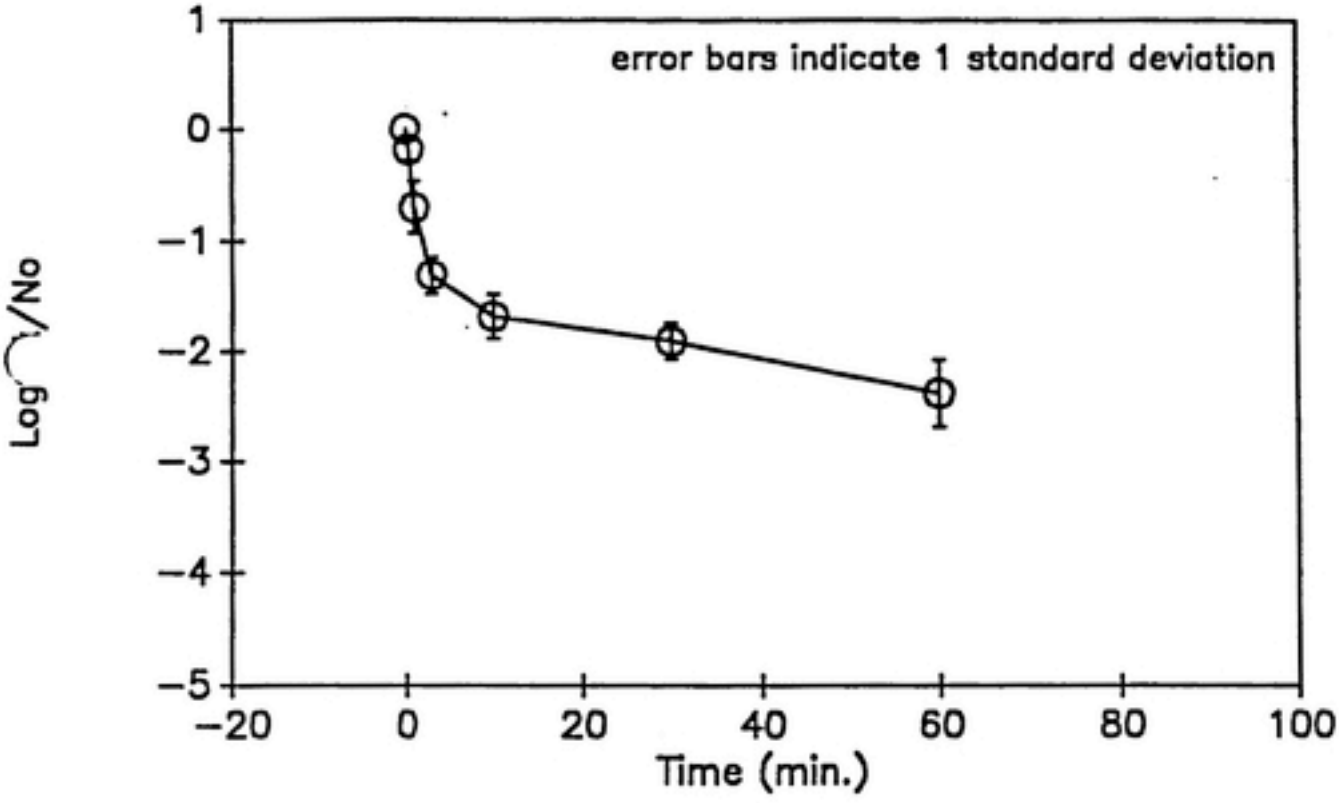



CLO2; pH 10; MS2; 5 deg. C
^=detection limit
error bars indicate 1 standard deviation
Log Nt/No
1
0
−1
−2
−3
−4
−5
−1.000
−0.500
0.000
0.500
1.000
1.500
2.000
TIME (min)

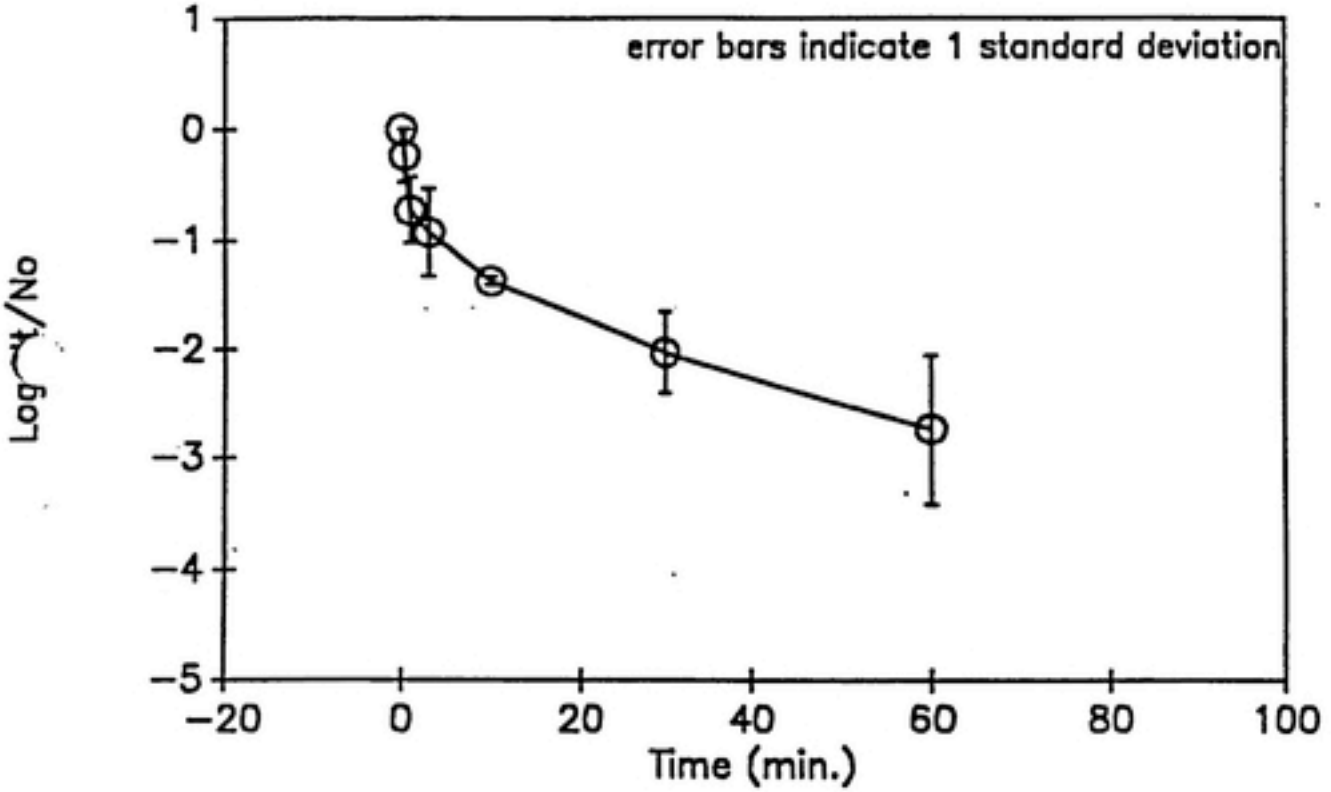
EPA DISINFECTION : CLO2 ; pH 8 : MS2



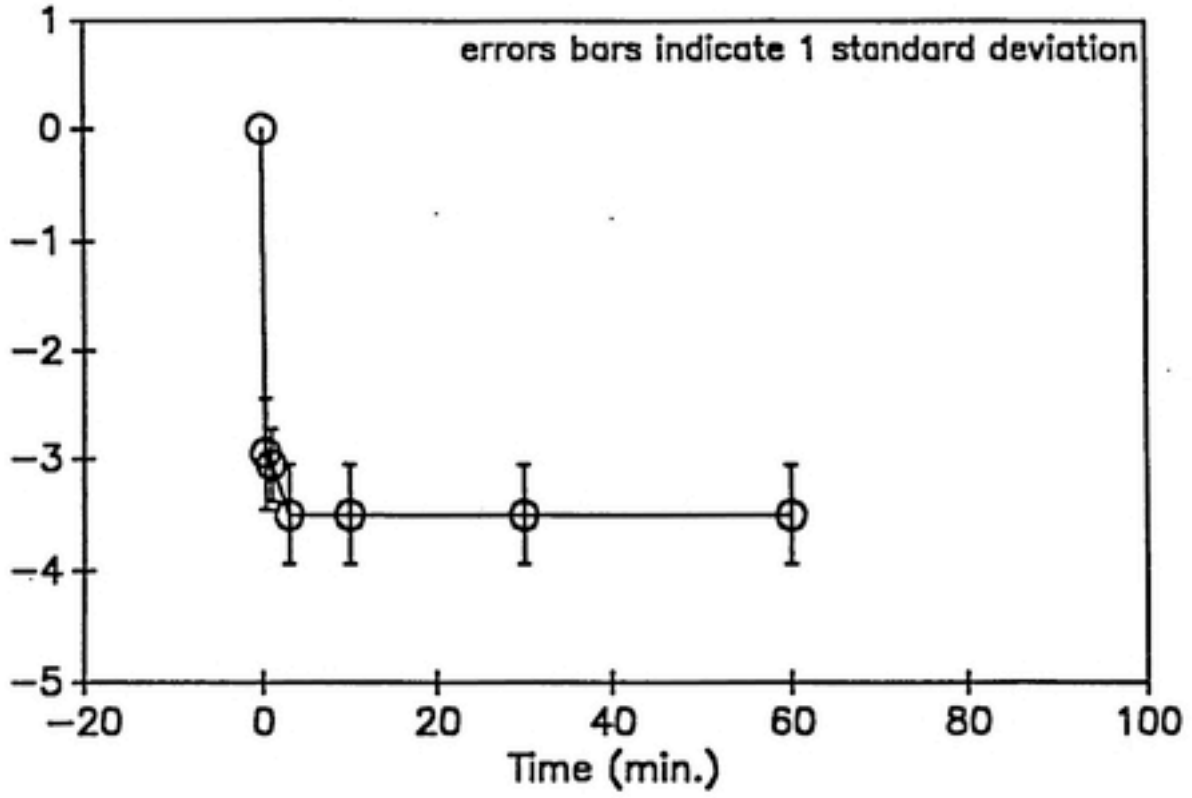
CLO2; pH 6; HAV; 5 deg. C



CLO2; pH 8; HAV; 5 deg. C



CLO2; pH 10; HAV; 5 deg. C



APPENDIX III
FIGURES OF SURVIVING FRACTION OF VIRUSES PREDICTED BY MODELS

FIGURE 9

SURVIVING FRACTIONS OF HAV AND MS2 EXPOSED TO 0.5 MG/L CHLORINE DIOXIDE AT pH 6 AND 8 AND 5°C AS PREDICTED BY ALTERNATIVE MODELS

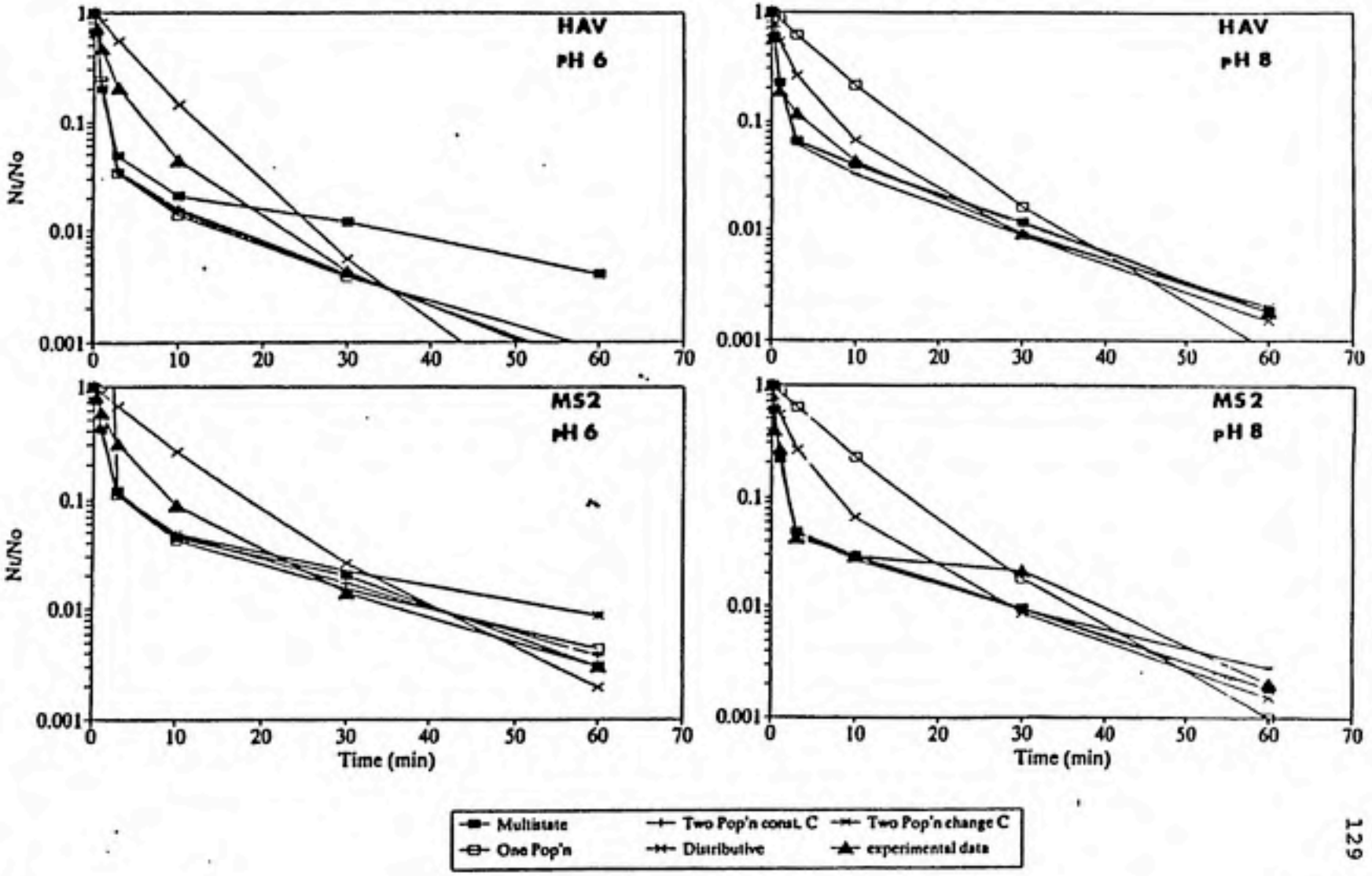
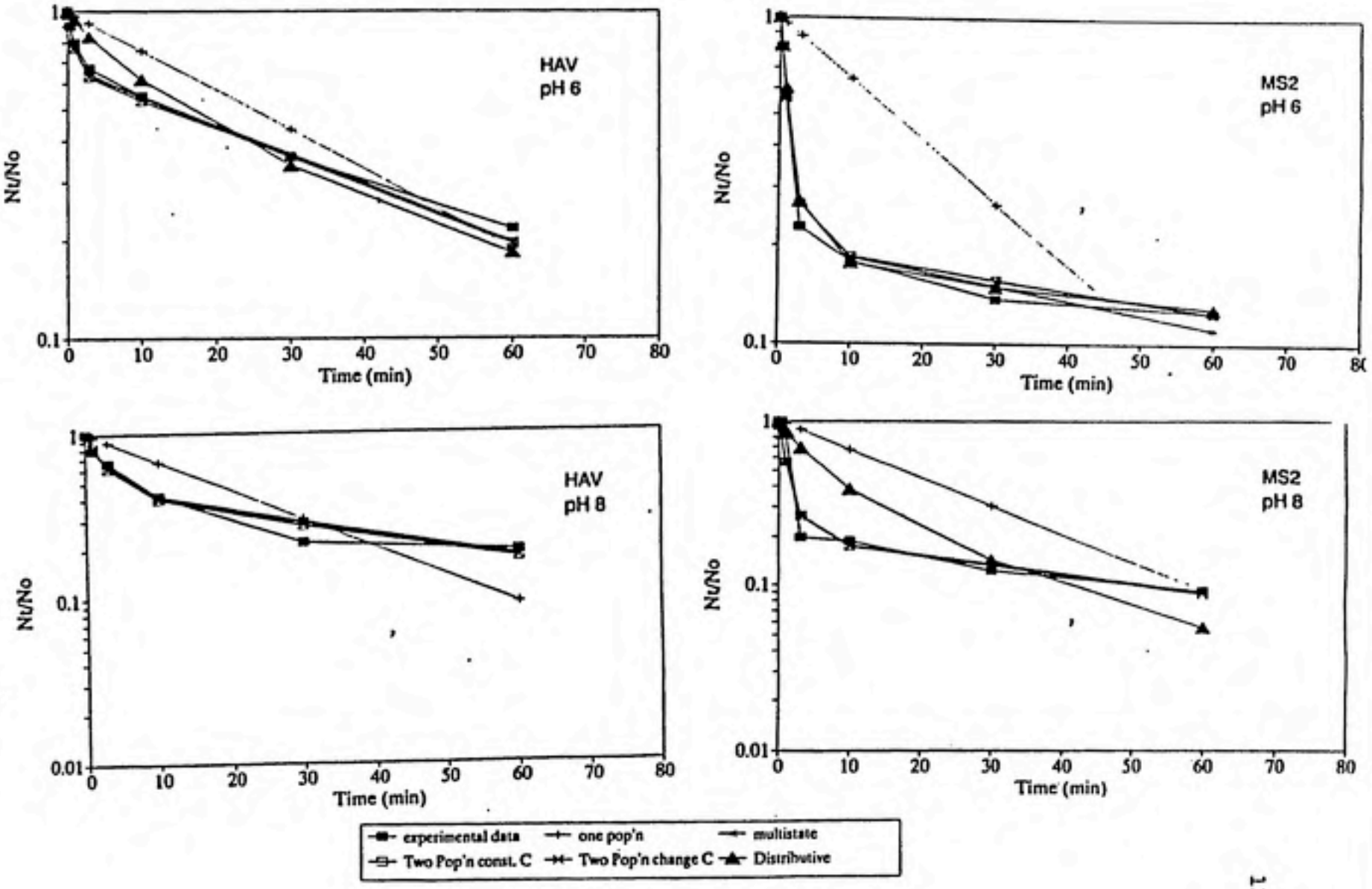


FIGURE 10
SURVIVING FRACTIONS OF HAV AND MS2 EXPOSED TO 2.0 MG/L MONO-CHLORAMINE AT pH 6 AND 8 AND 5 C AS PREDICTED BY ALTERNATIVE MODELS



APPENDIX IV
CHLORINE DIOXIDE GENERATION

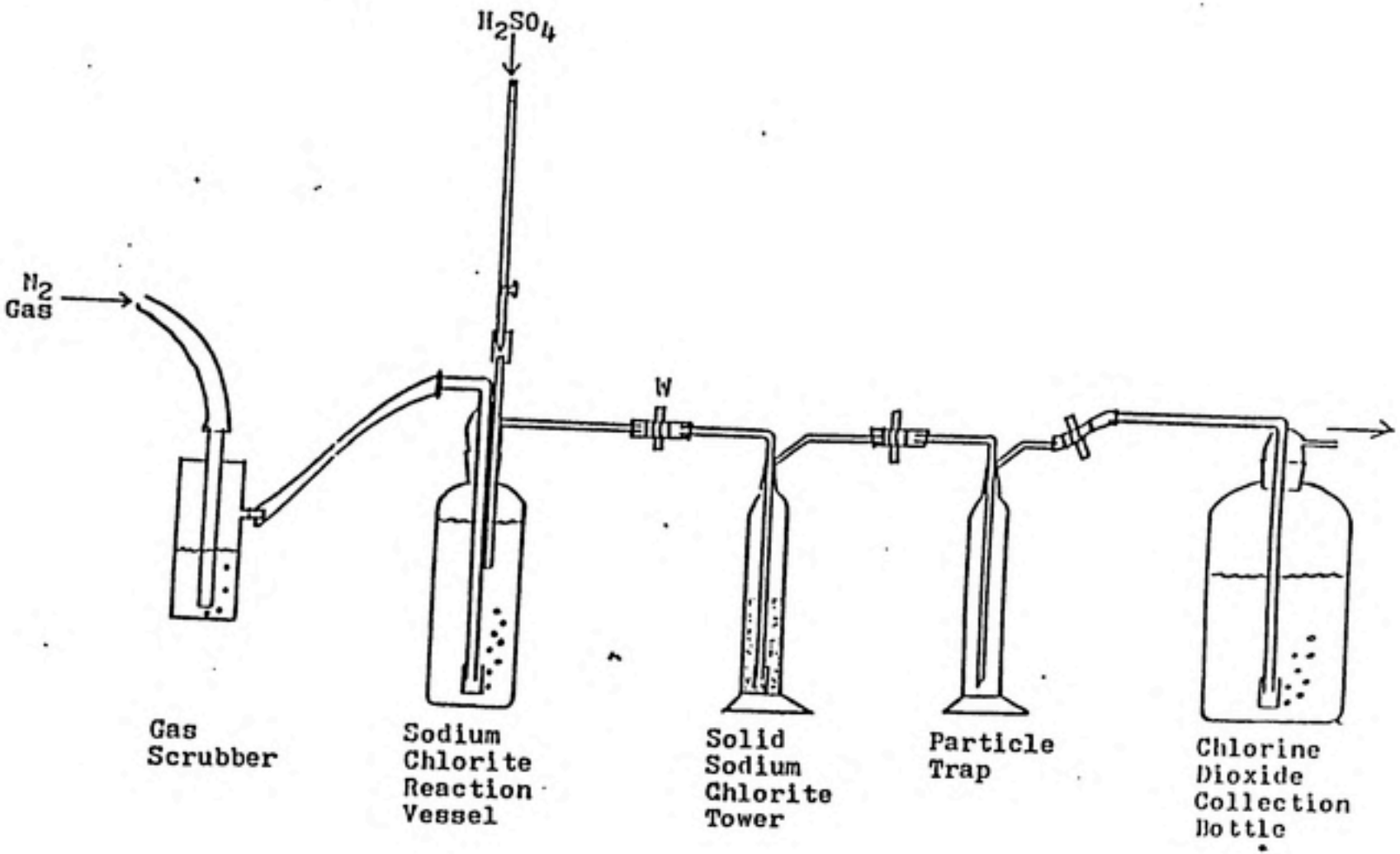


Figure 1. Chlorine Dioxide Generation Apparatus