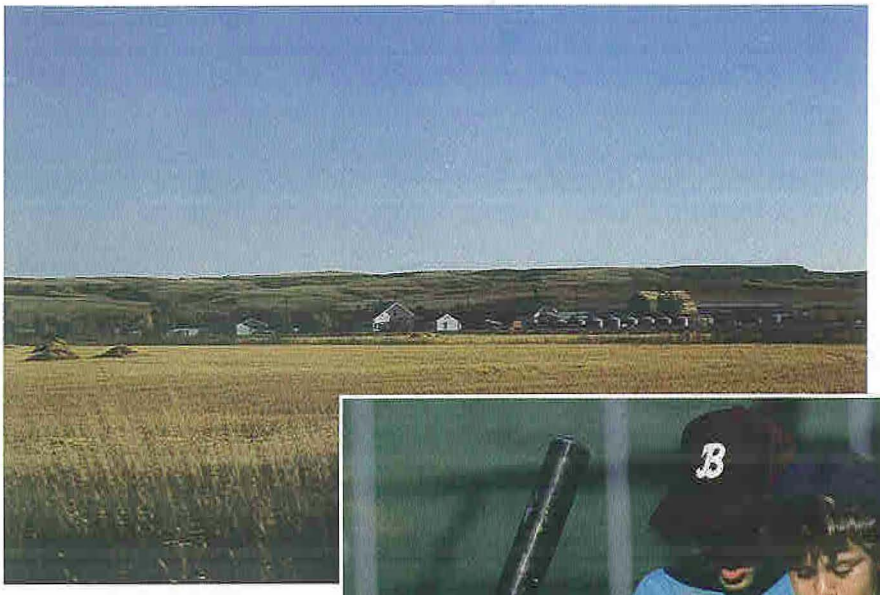

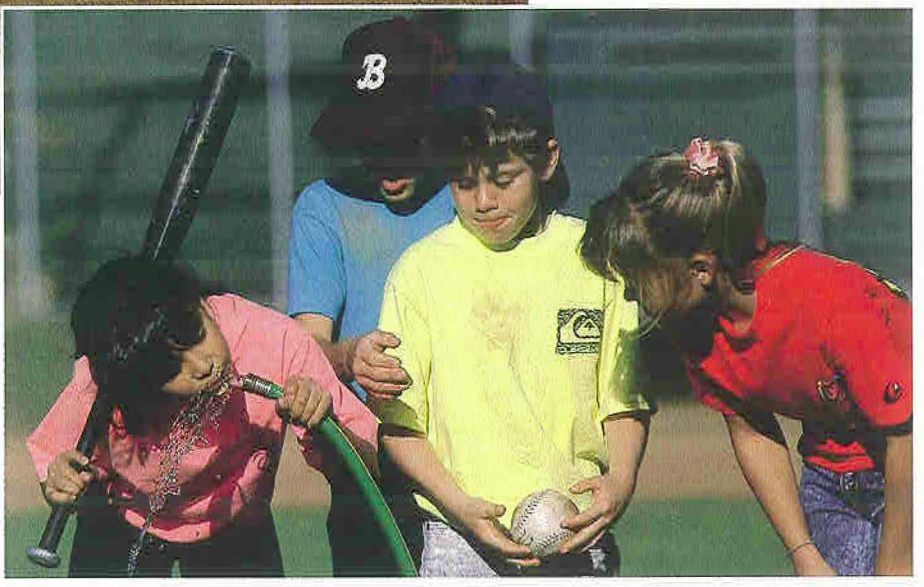

North Dakota State Water Commission and the Garrison Diversion Conservancy District

Northwest Area Water Supply Project Chloramine Challenge Study Final Report

December 1995

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Subject: Final Report
Chloramine Challenge Study Northwest Area Water Supply Project

Gentlemen:

Houston Engineering, Inc. in association with American Engineering PC, and Montgomery Watson Americas, Inc. are pleased to submit the Final Report for the Northwest Area Water Supply Project (NAWS) Chloramine Challenge Study.

The Final Report was prepared as a scientific evaluation to document the efficiency and applicability of both chloramination and ozonation in meeting the biota transfer concerns of the Engineering - Biology Task Group. The study also evaluated appropriate criteria for application of the disinfectants such as dose, contact time, disinfectant demand and disinfection by-product formation. Both chloramines and ozone will meet the disinfection requirements of the USEPA Safe Drinking Water Act (SDWA) for the Lake Audubon to Minot raw water supply system prior to reaching the divide between the Missouri River Basin and the Hudson Bay Basin.

The Draft Final Report is organized into six sections as follows:

Section 1.	Introduction
Section 2.	Experimental Methods
Section 3.	Experimental Results - Chemistry
Section 4.	Experimental Results - Microbiology
Section 5.	Summary
Section 6.	References

Technical Appendix A provides supporting documentation, and Technical Appendix B describes the experimental protocol for conducting the microbial inactivation studies.

We appreciate the opportunity to work with the State Water Commission staff on this project as an important element in the implement of the NAWS project.

Respectfully submitted,

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Section 1 Introduction

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BACKGROUND

The State of North Dakota proposes to construct the Northwest Area Water Supply (NAWS) system to deliver water for municipal, rural and industrial use from the Missouri River to cities and rural water systems in ten counties in northwestern North Dakota. The NAWS project is composed of 3 separate systems: the West system, Parshall System, and East System. The East System would supply about 10.2 million gallons per day (MGD) average daily flow and 28 MGD peak daily flow to customers in the Hudson Bay drainage. Because this project will deliver water from the Missouri river into the Hudson Bay drainage, there is concern that biota from the Missouri River including fish, fish viruses, and parasites could enter Canadian watersheds and have a negative impact on Canadian fisheries.

To address the concerns of this border issue, an Engineering-Biology Task Group was appointed in December, 1993 by the Joint Technical Committee to the Garrison Consultative Group. The report prepared by the Engineering-Biology Task Group was submitted to the Garrison Joint Technical Committee in May of 1994. The Garrison Consultative Group then met on September 23, 1994 in Ottawa, Ontario to discuss the findings of the report.

As identified under Option 1 in the report prepared by the Engineering-Biology Task Group, water from either Lake Audubon or Lake Sakakawea would be transported by pipeline from the Missouri drainage to Minot, ND in the Hudson Bay drainage where it would be treated to USEPA drinking water standards. This option was identified as technically feasible, provided that the water flowing out of the Missouri drainage could be pretreated to standards of drinking water disinfection under the criteria of the Surface Water Treatment Rule (SWTR) to ensure biota removal before crossing the drainage divide. Houston Engineering in association with American Engineering and Montgomery Watson was retained by the North Dakota State Water Commission to conduct a disinfection challenge study to investigate the effectiveness of chloramination and ozonation for disinfection and pretreatment of the transported water to drinking water disinfection standards to ensure biota removal.

The experimental protocol was developed to investigate a number of specific concerns related to pretreatment of the proposed raw water supply. These include influences of seasonal water quality characteristics of the raw water sources, seasonal temperature characteristics, and disinfection to SWTR requirements, i.e., 3-log (99.9%) inactivation of *Giardia* cysts and at least 4-log (99.99 %) inactivation of viruses. The disinfection challenge study was initially scoped to include examination of chlorine demand, disinfection by-product (DBP) formation, and MS2 virus inactivation in waters from Lake Audubon and Lake Sakakawea, and *Giardia* inactivation in water from Lake Audubon. However, because ozone is recognized as an extremely effective disinfectant often used for the inactivation of microbial contaminants, this study was expanded to include investigation of ozone decay and demand, ozone DBPs and ozone inactivation of *Giardia*.

The USEPA Guidance Manual to the SWTR stipulates that inactivation credit for disinfection is based upon the "C x T" method, where C is the concentration of disinfectant (mg/L) and T is the contact time in minutes. In general, viruses and protozoan cysts like *Giardia* have proven to be relatively resistant to chloramines; therefore CT values for chloramine inactivation are large as compared to CT values for free chlorine. However, a major drawback to the use of free chlorine as disinfectant is the formation of halogenated DBPs. Unlike chloramines, free chlorine readily reacts with natural organic matter to form DBPs such as trihalomethanes (THMs) and haloacetic acids (HAA5)[1]. THMs are currently regulated at 100 µg/L under the current drinking water regulations. However under Stage I of the D/DBP Rule, the maximum contaminant level (MCL) will decrease to 80 µg/L and the MCL for HAA(5) will be set at 60 µg/L. Although there are no proposed levels of DBPs for Stage II of the D/DBP rule, concentrations of 40 µg/L and 30 µg/L for THMs and HAA(5), respectively were discussed during the USEPA regulatory negotiations. In light of current and anticipated DBP regulations, this study was designed to assure sufficient inactivation of viruses and *Giardia* during the pipeline disinfection treatment (disinfectant dose x residence time) without formation of high concentrations of DBPs that exceed the D/DBP regulations.

[1] Haloacetic acids are designated as HAA(5), since under Stage I of the D/DBP rule the MCL will be based upon the sum of 5 of the 9 HAA compounds.

With these constraints in mind, the general disinfection strategy in this study was to provide a short period of free chlorine contact time before ammonia addition to form chloramines. The free chlorine would provide initial disinfection and the ammonia addition would prevent excessive DBP formation. A very conservative approach to the study was employed. Most DBP formation experiments were conducted at warm temperatures (20°C), which would maximize DBP formation. The disinfection experiments were performed at low temperatures (4°C) at which temperature microbial inactivation would be slow. Thus, worst-case DBP formation and microbial inactivation scenarios were employed throughout the study.

The required CT to meet USEPA disinfection regulations also considers the physical characteristics of the raw water transport system. Contact time is achieved in the proposed raw water pipeline from Lake Audubon to the top of the divide between the Missouri River drainage and the Hudson Bay drainage. The pipeline as defined in the NAWS Pre-Final Design Final Report, consists of 64,000 linear ft. of 42-inch diameter pipe and about 37,000 ft. of 38-inch diameter pipe to the top of the divide. The pipeline will be used as the contact structure for the disinfectants which will operate in a plug flow regime. At 10.2 MGD, the total contact time in the pipeline to the divide would be over 16 hours. At peak daily flow, the contact time would be about 5.9 hours. These contact times represent the design constraints for the disinfection processes.

EXPERIMENTAL APPROACH

This disinfection challenge study was conducted in three major phases, each of which was designed to build upon the results of the previous phase (see Table 1-1). The purpose of the Phase I experiments was to determine the maximum free chlorine contact time (prior to quenching with ammonia) that could be employed without formation of excessive disinfection by-products (DBPs) during the time of pipeline transmission to the divide. For the by-product formation studies, a residence time of 16.5 hours was chosen to represent the effective disinfectant contact time in the pipeline, as this represents residence time to the divide under average daily flow conditions of 10.2 MGD. Results from the by-product formation studies conducted in

Phase I at 20°C were used to select the free chlorine contact time (at 4°C) to be used in the MS2 virus and *Giardia* inactivation experiments of Phases II and III, respectively.

The objectives of Phase II experiments were to determine: 1) DBP formation for Lake Audubon and Lake Sakakawea waters at 20°C; and 2) determine viral inactivation of MS2 bacteriophage by free chlorine and pre-formed chloramines. In Phase II, ozone demand and decay were also determined for Lake Audubon and Lake Sakakawea waters at 4°C. For the MS2 virus inactivation studies conducted in Phase II, the pipeline transmission time of 5.9 hours corresponding to the hydraulic detention time in the transmission line at peak daily flow of 28 MGD was used as the primary time constraint for disinfection.

The objectives of the Phase III experiments were to conduct three seasonal samplings of the source waters and to evaluate the following: 1) chlorine demand and DBP formation for Lake Audubon and Lake Sakakawea waters at 20°C; 2) ozone demand and decay and ozone DBPs for Lake Audubon and Lake Sakakawea waters at 20°C and; 3) chloramine and ozone inactivation of *Giardia* for Lake Audubon water. Samples of lake waters were collected during the Winter (March), Spring (May), and Summer (August) of 1995.

The general protocols for determination of chlorine demand, ozone demand and decay, DBP formation, MS2 virus inactivation and *Giardia* inactivation are detailed in the Experimental Methods section.

Table 1.1
NAWS Disinfectant Challenge Study
Schedule of Phases

	7-Nov-94	9-Jan-95	12-Mar-95	30-May-95	21-Aug-95
Phase I					
	DBP formation				
Phase II		DBP formation			
		ozone demand and decay			
		MS2 virus inactivation			
Phase III			DBP formation	DBP formation	DBP formation
			ozone demand and decay	ozone demand and decay	ozone demand and decay
			Giardia inactivation	Giardia inactivation	Giardia inactivation

Section 2 Experimental Methods

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RAW WATER SAMPLING

During each phase of the disinfection challenge study, specified water volumes were obtained from Lake Audubon and Lake Sakakawea for the appropriate water quality analyses and disinfection assays. At the time of sampling, measurements were made of water temperature, sampling depth (feet) and other selected water quality parameters. Upon receipt of samples at the laboratory, the following water quality parameters were measured: temperature, pH, total organic carbon (TOC), turbidity, alkalinity, hardness, ammonia, UV-254 absorbance, total dissolved solids (TDS).

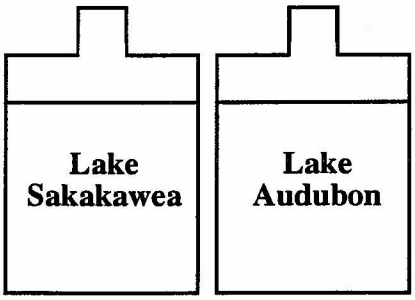
On each of the sampling dates, the water samples obtained from each source were shipped at 4°C overnight to Montgomery Watson's Applied Research Laboratory in Monrovia, California for analysis of DBP formation, chlorine demand, and ozone demand and decay. For the DBP formation and the ozone demand and decay experiments, 5 gallons were required for each type of analysis. During Phase II, 20 gallons from each water source were required for MS2 inactivation experiments. During Phase III, 10 gallons were sampled from both lakes for DBP formation and ozone demand and decay studies. An additional 30 gallons were required from Lake Audubon for the *Giardia* inactivation studies and were shipped overnight to Drexel University in Philadelphia, Pennsylvania.

CHLORINE DEMAND EXPERIMENTS

Chlorine demand studies were conducted at each seasonal sampling period of the disinfection challenge study. The chlorine demands of the waters were determined at 4°C in Phase I and at 20°C in Phase II and Phase III, at ambient pH so that proper chlorine dosing for the evaluation of DBP formation could be determined and a proper free chlorine contact time could be determined for the studies of *Giardia* inactivation with monochloramine. A schematic design for the chlorine demand experiments is provided in Figure 2.1. Raw waters were dosed at a range of free chlorine concentrations and chlorine demands were measured after free chlorine contact times of 1, 5, 10, 30 and 60 minutes. The goal of the prechlorination step was to provide contact time for effective inactivation of viruses and *Giardia*, and to enable maintenance of a

Figure 2.1 - Chlorine Demand and DBP Formation Experiments

Step 1 - Chlorine Demand Test



5-6 mg/L Chlorine

Measure Chlorine Residual at
- 1 minute
- 10 minutes
- 3 hours

Purpose: To establish approximate chlorine demand to aid in selecting chlorine doses for Step 2.

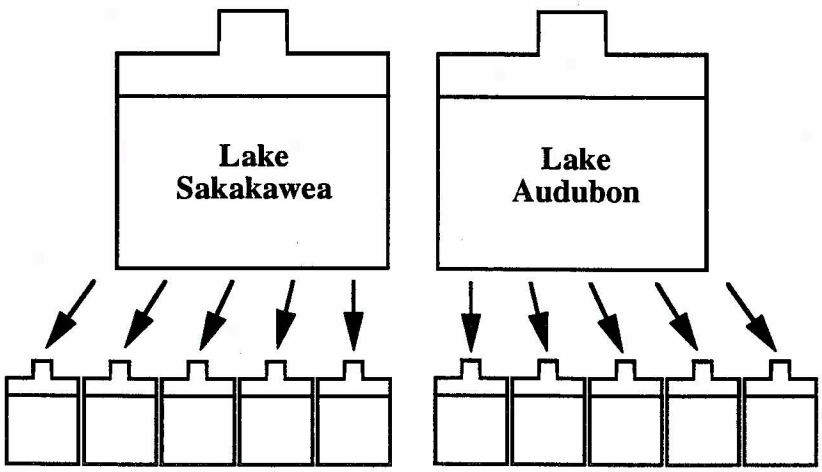
Step 2 - Chlorine Demand Experiments

Add a range of chlorine doses based on results of Step 1.

Measure Chlorine Residual at
- 1 minute
- 5 minutes
- 10 minutes
- 30 minutes
- 60 minutes

Purpose: To establish the exact chlorine dose to use for each water for Step 3 to acheive a residual of 3.5 - 4.0 mg/L.

Step 3 - 16.5 Hour DBP Formation Experiments



Add chlorine doses determined in Step 2 to batches of water.

Quench with ammonia at 1, 5, 10, 30 and 60 minutes.

Chlorine to ammonia ratio 4:1

Sample for THMs and HAAs at 16.5 hours.

chloramine residual of 3.5 to 4.0 mg/L after the 16.5 hours of the DBP formation experiments. To achieve these chloramine residuals at hour 16.5 of the DBP formation experiments, appropriate chlorine doses were chosen based on the chlorine demand results.

OZONE DEMAND AND DECAY EXPERIMENTS

Ozone demand and decay experiments were conducted on waters from both Lake Audubon and Lake Sakakawea during Phase II at a temperature of 4°C and also during Phase III at a temperature of 20°C. A total of five transferred ozone doses were employed to evaluate ozone demand and decay for each seasonal sampling period. From the results of these studies, the ozone demand was determined from the linear relationship between the transferred dose and ozone residual. The ozone decay coefficient was estimated by an exponential fit to the first-order decay of ozone residual over time. These demand and decay studies were conducted in order to provide supporting ozonation information for potential design purposes.

DBP FORMATION EXPERIMENTS

DBP formation experiments were conducted at each sampling period for the two lake waters. Phase I DBP experiments were conducted at 4°C, while Phase II and Phase III experiments were conducted at 20°C. All DBP experiments were conducted at the measured ambient pH. The chlorine doses were chosen based on the seasonal results obtained from the chlorine demand experiments (procedure described above), and were targeted to obtain a chlorine residual in the range of 3.5 to 4.0 mg/L at the time of quenching.

At the start of the DBP experiments, raw waters were dosed at appropriate free chlorine concentrations ranging from 3.8 to 5.6 mg/L. These chlorine doses were selected to achieve chlorine residuals in the range of 3.5 to 4.0 mg/L after the designated free chlorine contact times of: 1, 5, 10, 30 and 60 minutes (refer to Figure 2.1). At the end of the chosen free chlorine contact times, ammonia was added to the reaction vessel at a chlorine to ammonia ratio of 4:1 in order to form chloramines. The samples were then mixed for five minutes and transferred to storage for 16.5 hours in darkness at 20°C. The storage time of

16.5 hours was used to simulate plug flow pipeline transmission conditions. At the end of the holding time, samples were analyzed for trihalomethanes and haloacetic acids. Experimental conditions for DBP formation experiments are presented in Table 2-1.

Table 2-1
Experimental Conditions Of DBP Formation Experiments

Parameter	Number of Variables	Description
Waters	2	Lake Audubon and Lake Sakakawea
Chloramine Residual Target	1	3.5 - 4.0 mg/L
Total Disinfectant Contact Time	1	16.5 hours
Free Chlorine Contact Times	5	1, 5, 10, 30 & 60 minutes
Temperature	1	20°C
pH	1	Ambient
Cl_2:NH_3 Ratio	1	4 to 1

MS2 BACTERIOPHAGE INACTIVATION EXPERIMENTS

MS2 bacteriophage was chosen as the model virus for these inactivation studies since it is referenced in challenge studies in the Guidance Manual to the SWTR. The MS2 bacteriophage inactivation experiments were performed to determine the actual CT values for a 4-log inactivation of virus at the divide. Two types of experiments were conducted: free chlorine and chloramine inactivation experiments. The experimental apparatus and the experimental procedures for the MS2 inactivation studies are described in the following sections.

Experimental Apparatus

The experimental apparatus employed during the chloramine disinfection experiments for both MS2 bacteriophage and *Giardia muris* cysts consisted of two three-liter glass reaction vessels which served as disinfection and control reactors, a temperature control circulating water bath, and magnetic stirring devices to maintain completely mixed conditions within the reactors. The reactors were equipped with indentations that provide baffling and eliminate vortexing of the reactor contents during mixing. Mixing was provided by a Teflon™ coated magnetic stir bar driven by a large magnetic stirring device. The control reactor was used to confirm that the microbial populations were stable throughout the inactivation studies. Control studies were also performed during MS2 inactivation studies in order to determine the viability of MS2 bacteriophage over the time course of experiments in the absence of any disinfectant.

The two three-liter glass reaction vessels were equipped with two side arms used for dosing the reactor and withdrawing samples. The large screwcapped top for each vessel was used for filling and spiking the reactor with MS2 bacteriophage virus and *Giardia* cysts. The second, smaller screwcapped opening was used for disinfectant addition. A special adapter was fitted to the second sidearm in order control the flow of water from the reactor when withdrawing samples. The reactors were maintained under a positive pressure of 1 to 2 pounds-per-square-inch (psi) during disinfection experiments. This pressure provided the driving force for sample withdrawal, as well as reduction in the volatilization of the disinfectant. Positive pressure was provided by an air vacuum pump that introduced air through a special fitting attached to the side arm that contained the sample withdrawal tube. When the sample stopcock was opened, the reactor contents flowed from the reactor.

MS2 Virus Propagation and Enumeration

MS2 bacterial virus (ATCC, catalog number 15597-B1) was employed as the model virus for the microbial challenge studies. MS2 virus was propagated by inoculating a flask containing 100 mL of viral host bacteria, *E. coli* (ATCC

catalog number 15597), to which 1 mL of 0.1 M sterile calcium chloride ($CaCl_2$) was added. Bacteria were grown in a flask that was incubated in a water bath at 37°C. When the density of bacteria reached approximately $1x10^8$ colony forming units (cfu)/mL, an aliquot of the virus stock (approximately 10^{12} plaque forming units (pfu)/mL) was added to provide a multiplicity of infection (MOI) of 0.1. Results were expressed in pfu/mL, where plaques are indicated by clearings in the bacterial lawn. This MOI was employed to assure two rounds of virus replication. Incubation of the bacterial culture continued until the host cells lysed (ruptured), after which 0.01 gram of crystallized lysozyme and 3 mL of sterile 0.2 M EDTA (Ethylendiaminetetraacetate) were added followed by additional incubation for 1 hour in a shaking water bath at 37°C. The propagated virus and cellular debris were then centrifuged for 20 minutes at 3000xG, filter sterilized and refrigerated at 4°C until needed.

MS2 was assayed by the agar overlay technique described by Adams (1959) with the following modifications noted below. Host cultures of *E. coli* were grown on the day of the assay in TYE broth at 37°C under aerated conditions for 5 to 6 hours and dispensed in 20 mL aliquots in sterile dropper bottles. Just prior to use, 1.0 mL of 0.1 M sterile $CaCl_2$ solution was added to the dropper bottle. After the MS2 samples were serially diluted in 0.001 M phosphate-saline buffer (PBS), 0.1 mL was added to 2 mL of trypticase yeast extract (TYE) soft agar, which was maintained at 46 to 48°C. Three to four drops of the host *E. coli* were added, and then the soft agar was mixed gently and poured on a TYE hard agar petri dish. After the soft agar solidified, the petri dishes were incubated at 37°C for 24 hours, after which time the plaques were counted. All dilutions were plated in duplicate.

MS2 Bacteriophage Spiking

Spiking was performed by addition of MS2 bacteriophage to the disinfection and control reactors containing 2,000 mL of raw water equilibrated at 4°C. The bacteriophage spike was prepared immediately prior to each inactivation experiment by adding 0.5 mL of a concentrated phage stock solution to 500 mL of sterile deionized water to provide an approximate density of 10^9 (pfu)/mL. The disinfection and control reactors were spiked with 2 mL of this working stock, resulting in a final bacteriophage density of approximately 10^6 (pfu)/mL

within the reactors. All microbial inactivation studies on both MS2 bacteriophage were conducted at 4.0°C ± 0.5°C in order to simulate cold water inactivation conditions. Temperature was controlled by pumping cooling water through the water jackets of the disinfection reactors. Cooling water was provided from a temperature controlled circulating water bath. A total of 84 virus assays were conducted for the MS2 inactivation studies. The experimental conditions of MS2 inactivation studies are provided in Table 2-2.

Table 2-2
Experimental Conditions of MS2 Inactivation by Chloramination Experiments

Parameter	Number of Variables	Description
Waters	2	Lake Audubon and Lake Sakakawea
Temperature	1	4°C
Chlorine Dose	1	Sufficient to provide a 3.5-4.0 mg/L monochloramine residual after 16.5 hours
Residual	1	3.5-4.0 mg/L
Cl_2:NH_3 Ratio	1	4:1
Free Chlorine Contact Time	7	0, 15, 30, 60, 90, 120, 180, and 300 seconds
pH	1	Ambient
Replicate Experiments	6	
Total Assays	84	

Giardia Inactivation Experiments - Chloramination Studies

Giardia inactivation experiments were conduced by Dr. Charles Haas of Drexel University. *Giardia* cysts were obtained from Dr. Ernest Meyer at the Oregon Health Sciences University in Portland, Oregon where they had been cultured in specific-pathogen-free mice. The experiments were conducted in a manner similar to the MS2 bacteriophage inactivation

experiments. A concentrated preparation of *Giardia muris* cysts was added to the disinfection and control reactors containing 2,000 mL of raw water equilibrated at 4°C such that the final cyst concentration within each reactor was 10^3 to 10^4 cysts/mL. Prior to the addition of disinfectant, an initial sample was taken from each reactor and analyzed for pH, total cyst concentration and cyst viability.

A free chlorine contact time of 5 minutes (as selected during the chlorine demand studies and MS2 inactivation studies in Phase I and Phase II) was used in the monochloramine experiments of *Giardia* inactivation (with the exception of the preammoniation studies). A single monochloramine dose of 4.5 mg/L, with a chlorine to ammonia ratio of 4:1, was utilized throughout the *Giardia* chloramine disinfection experiments. After initial addition of disinfectant, samples were withdrawn from the disinfection reactor at specified time intervals and analyzed for chlorine residual to verify that the desired chlorine dose had been achieved. *Giardia* inactivation was measured at a number of disinfectant contact times between 5 minutes and 300 minutes for the prechlorination studies (Table 2-3), and contact times between 5 minutes and 300 minutes for the preammoniation studies.

Table 2-3
Experimental Conditions of *Giardia* Inactivation by Chloramination Experiments

Parameter	Number of Variables	Description
Waters	1	Lake Audubon
Temperature	1	4°C
Chlorine Dose	1	Sufficient to provide a 3.5-4.0 mg/L monochloramine residual after 16.5 hours
Residual	1	3.5-4.0 mg/L
Cl_2:NH_3 Ratio	1	4:1 after 5 minutes free chlorine demand met
Free Chlorine Contact Time	1	5 minutes
pH	1	Ambient
Prechlorination Sampling Contact Times	7	0, 5, 60, 90, 120, 150, 180, 210, and 300 minutes
Replicate Experiments	6	

Control experiments for the concentration and viability of *Giardia* in the disinfectant-free reactor were also conducted with the following objectives: 1) to determine whether *Giardia* cysts adhered to the surfaces of the reactor; and 2) to determine the viability of *Giardia* cysts over the time course of the experiment in the absence of any disinfectant. Samples for control experiments were collected from the control reactor at the same time points as the disinfection reactor and were subjected to the same analyses for residual chlorine concentration.

Giardia Excystation and Enumeration Methods

In vitro excystations were performed using a modified procedure developed by Sauch (1988). The procedure is performed in the following steps. A 10 mL aliquot of sample water was concentrated to 1 mL by centrifugation in a

conical-bottom centrifuge tube, to which was added 10 mL of reducing solution (Hank's balanced salt solution, supplemented with 32 mM glutathione and 57 mM L-cysteine HCl) and 10 mL of 0.1 sodium bicarbonate. This suspension (pH 4.7) was vortexed and incubated for 30 minutes at 37°C. The cysts were then centrifuged (2 minutes at 650xG) and washed once in 20 mL of excystation medium by centrifugation (2 minutes at 650xG). After washing, the cysts were suspended in 1 mL of a 0.5 percent solution of pre-warmed and buffered proteose peptone in PBS at pH 7.2 (0.8 g NaCl, 0.2 g KH_2PO_4, 2.9 g Na_2HPO_4 (12 H_2O), 0.2 g KCl, 1,000 mL final volume), and incubated at 37°C in a heated water bath for 30 minutes.

After final incubation, a volumetric method of cyst enumeration proposed by Haas et al., (1994) was performed in order to compute the survival ratio from the inactivation studies. According to this method, a volume of concentrated sample was selected, the number of trophozoites (TR) was counted, and the equivalent volume of sample from which those trophozoites were enumerated was determined. The survival ratio is then computed from this information.

Giardia Inactivation Experiments - Ozonation Studies

For the *Giardia* inactivation experiments with ozone, three reaction vessels were run in parallel in order to measure *Giardia* decay in the absence of disinfectant, loss of disinfectant residual, and inactivation of *Giardia* caused by disinfectant. Ozone inactivation studies were conducted at various ozone doses and at various disinfectant contact times. Experimental conditions are presented in Table 2-4.

Table 2-4
Experimental Conditions of *Giardia* Inactivation by Ozonation Experiments

Parameter	Number of Variables	Description
Waters	1	Lake Audubon
Temperature	1	4°C
Ozone Dose	1	Transferred doses from 0.08 to 1.9 mg/L
Ozone Residual	1	From: 0 mg/L at 30 seconds to 0.25 mg/L at 20 minutes
pH	1	Ambient
Winter Sampling Contact Times	7	0, 1, 5, 10, 15, 20 and 25 minutes
Spring and Summer Sampling Contact Times	7	0, 0.5, 1, 2, 3, 4, and 6 minutes
Residual Measurements	7	At each microbial sampling point

Experiments were begun with the addition of a suspension of *Giardia* at the desired concentration density to a 3-L beaker containing source water. This homogeneous solution was then added in 1,000 mL volumes to the three reactor vessels. At time zero, an ozone solution targeted to achieve a selected ozone dose was added to reactors 2 and 3 in a volume approximating 5% of the *Giardia* suspension volume. At the pre-determined times indicated in Table 2-4, samples were withdrawn from the control and survival reactors, and disinfectant residuals were immediately quenched with excess sterile sodium thiosulfate (0.1 mL of a 10 percent solution). Samples were then measured for viability of *Giardia* (hence inactivation of *Giardia* in disinfection reactors) and ozone residual concentration.

Control experiments for the ozone inactivation studies were also conducted to identify any change in the viability of *Giardia* cysts over the time course of experiments.

Section 3 Experimental Results Chemistry

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RAW WATER QUALITY

Water samples were obtained from Lake Audubon and Lake Sakakawea during five sampling events over a 10 month period. Table 3-1 presents a summary of the sampling dates and the experiments conducted.

Table 3-1
Sampling Events And Analyses

Sample Date	Lake(s)	Experiment	Temperature °C
Phase I			
November 7, 1994	Sakakawea and Audubon	DBP formation	4
Phase II			
January 9, 1995	Sakakawea and Audubon	MS2 virus inactivation	4
	Sakakawea and Audubon	Ozone Demand and Decay	4
	Sakakawea and Audubon	DBP formation	20
Phase III			
March 12, 1995 - Winter	Sakakawea and Audubon	DBP formation	20
	Sakakawea and Audubon	Ozone Demand and Decay	20
	Audubon	*Giardia*+Cl_2	4
	Audubon	*Giardia*+O_3	4

Table 3-1 (continued)
Sampling Events And Analyses

Sample Date	Lake(s)	Experiment	Temperature °C
May 30, 1995 - Spring	Sakakawea and Audubon	DBP formation	20
	Sakakawea and Audubon	Ozone Demand and Decay	20
	Audubon	*Giardia*+Cl_2	4
	Audubon	*Giardia*+O_3	4
August 21,1995 - Summer	Sakakawea and Audubon	DBP formation	20
	Sakakawea and Audubon	Ozone Demand and Decay	20
	Audubon	*Giardia*+Cl_2	4
	Audubon	*Giardia*+O_3	4

At the time of each sampling event, on-site raw water quality measurements were made of the following parameters as shown in Tables 3-2 and 3-3.

Table 3-1 (continued)
Sampling Events And Analyses

Sample Date	Lake(s)	Experiment	Temperature °C
May 30, 1995 - Spring	Sakakawea and Audubon	DBP formation	20
	Sakakawea and Audubon	Ozone Demand and Decay	20
	Audubon	*Giardia*+Cl_2	4
	Audubon	*Giardia*+O_3	4
August 21,1995 - Summer	Sakakawea and Audubon	DBP formation	20
	Sakakawea and Audubon	Ozone Demand and Decay	20
	Audubon	*Giardia*+Cl_2	4
	Audubon	*Giardia*+O_3	4

At the time of each sampling event, on-site raw water quality measurements were made of the following parameters as shown in Tables 3-2 and 3-3.

Table 3-2
Raw Water Quality - Lake Audubon

Parameter	Phase I	Phase II	Phase III-Sampling Season		
			Winter	Spring	Summer
Sampling Date	11/7/94	1/9/95	3/12/95	5/30/95	8/21/95
Sampling Depth, feet	13.5	15	15	15	12
Lake Level (feet above sea level)	1845.2	1844.9	1844.7	1847.0	1846.8
Temperature, °C	5.1	2	2	14	21
pH	7.5	n/a	n/a	n/a	n/a
Dissolved Oxygen, mg/L	11.1	n/a	n/a	n/a	n/a
Conductivity, µS/cm	880	n/a	n/a	n/a	n/a

n/a = not available

Table 3-3
Raw Water Quality - Lake Sakakawea

Parameter	Phase I	Phase II	Phase III-Sampling Season		
			Winter	Spring	Summer
Sampling Date	11/7/94	1/9/95	3/12/95	5/30/95	8/21/95
Sampling Depth, feet	50	48	54	55	65
Lake Level (feet above sea level)	1840.0	1837.1	1835.1	1839.6	1850.6
Temperature, °C	6.9	2	2	14	20
pH	7.4	n/a	n/a	n/a	n/a
Dissolved Oxygen, mg/L	10.5	n/a	n/a	n/a	n/a
Conductivity, µS/cm	710	n/a	n/a	n/a	n/a

n/a = not available

Upon receipt of water samples at Montgomery Watson's Applied Research Laboratory, the water was analyzed for additional water quality parameters. These results are shown in Tables 3-4 and 3-5.

Table 3-4
Water Quality Upon Receipt at MW Research Laboratory*
Lake Audubon

Parameter	Phase I	Phase II	Sampling Season		
			Winter	Spring	Summer
Temperature (upon receipt) °C	3.8	1	4	13	n/a
pH	8.5	8.4	8.0	7.9	8.2
Total Organic Carbon, mg/L	5.6	6.4	6.1	5.3	5.8
Turbidity, NTU	3.5	0.6	0.4	6.4	1.8
Alkalinity, mg/L as $CaCO_3$	190	240	230	205	200
Hardness, mg/L as $CaCO_3$	250	280	270	236	238
Ammonia, mg/L	<0.1	<0.1	< 0.1	0.15	< 0.1
UV-254 Absorbance (unfiltered)	0.095	n/a	0.085	0.94	0.100
TDS, mg/L			512	550	560

*Parameters were measured in the Applied Research Laboratory of Montgomery Watson, Monrovia, CA

Note: All parameters with the exception of the temperature were analyzed at 20°C.

n/a = not available

Table 3-5
Water Quality Upon Receipt at MW Research Laboratory*
Lake Sakakawea

Parameter	Phase I	Phase II	Sampling Season		
			Winter	Spring	Summer
Temperature (upon receipt), °C	3.7	1	5	8	n/a
pH	8.3	8.4	7.9	8.1	8.1
Total Organic Carbon, mg/L	4.1	4.2	4.0	3.6	4.0
Turbidity, NTU	3.5	1.9	0.8	2.0	3.0
Alkalinity, mg/L as $CaCO_3$	165	190	170	175	165
Hardness, mg/L as $CaCO_3$	218	234	218	208	224
Ammonia, mg/L	<0.1	<0.1	< 0.1	0.17	<0.1
UV-254 Absorbance (unfiltered)	0.097	n/a	0.089	0.086	0.086
TDS, mg/L			276	450	444

*Parameters were measured in the Applied Research Laboratory of Montgomery Watson, Monrovia, CA

Note: All parameters with the exception of the temperature were analyzed at 20°C.

n/a = not available

CHLORINE DEMAND EXPERIMENTS

The chlorine demands of the waters were determined for each seasonal sampling event and for two water temperatures (4°C and 20°C) so that proper dosing could be determined for the chloramine DBP formation experiments at a contact time of 16.5 hours used to represent average daily flow. Chlorine demands for a ten minute free chlorine contact time are shown below in Tables 3-6 and 3-7, along with the additional chlorine demands expressed over the 16.5 hour chloramination period for Lake Audubon and Lake Sakakawea.

Table 3-6
Chlorine Demand - Lake Audubon

Season	Temperature °C	10 minute Chlorine Demand (mg/L)	16.5 hour post-Chloramination Demand (mg/L)*	Total Demand (mg/L)
Phase I- 11/7/94	4	0.70	0.25	0.95
Phase II- 1/9/95	20	1.4	0.30	1.7
Phase III				
3/12/95	20	1.1	0.30	1.4
5/30/95	20	0.80	0.40	1.2
8/21/95	20	1.05	0.35	1.4

*represents demand due to NH_2Cl after post-ammoniation of chlorinated raw water

Table 3-7
Chlorine Demand - Lake Sakakawea

Season	Temperature °C	10 minute Chlorine Demand (mg/L)	16.5 hour post-Chloramination Demand (mg/L)*	Total Demand (mg/L)
Phase I- 11/7/94	4	0.50	0.20	0.70
Phase II- 1/9/95	20	0.75	0.20	0.95
Phase III				
3/12/95	20	0.80	0.20	1.0
5/30/95	20	0.75	0.30	1.05
8/21/95	20	0.65	0.20	0.85

*represents demand due to NH_2Cl after post-ammoniation of chlorinated raw water

For all sampling events chlorine demand was slightly higher for Lake Audubon than for Lake Sakakawea, which is consistent with the higher TOC levels. The larger chlorine demand suggests that water from Lake Audubon has a higher concentration of dissolved oxidizable material and additionally may be likely to produce higher concentrations of disinfection by-products in the raw water line than water from Lake Sakakawea.

OZONE DEMAND AND DECAY EXPERIMENTS

Ozone demand and decay experiments were conducted on waters sampled during Phase II and Phase III of this study. Analysis was made on a seasonal basis in order to evaluate the dose of ozone necessary for oxidation of raw water dissolved organic matter, in the case that ozone was employed for inactivation of viruses and *Giardia* cysts. Calculated values for ozone demand (based upon extrapolation of the linear relationship between residual vs. transferred dose) and ozone decay coefficients (based upon first-order exponential decay) are provided in Tables 3-8 and 3-9 for experiments conducted during Phases II and III. (See Appendix A for ozone demand graphs and decay curves.)

Table 3-8
Ozone Decay And Demand-Lake Audubon

Sampling Date	Temperature	Decay Coefficient (min^{-1})	Ozone Demand (mg/L)
1/9/95	4	0.19	1.3
3/12/95	20	0.39	3.1
5/30/95	20	0.36	2.5
8/21/95	20	0.50	2.5

Table 3-9
Ozone Decay And Demand- Lake Sakakawea

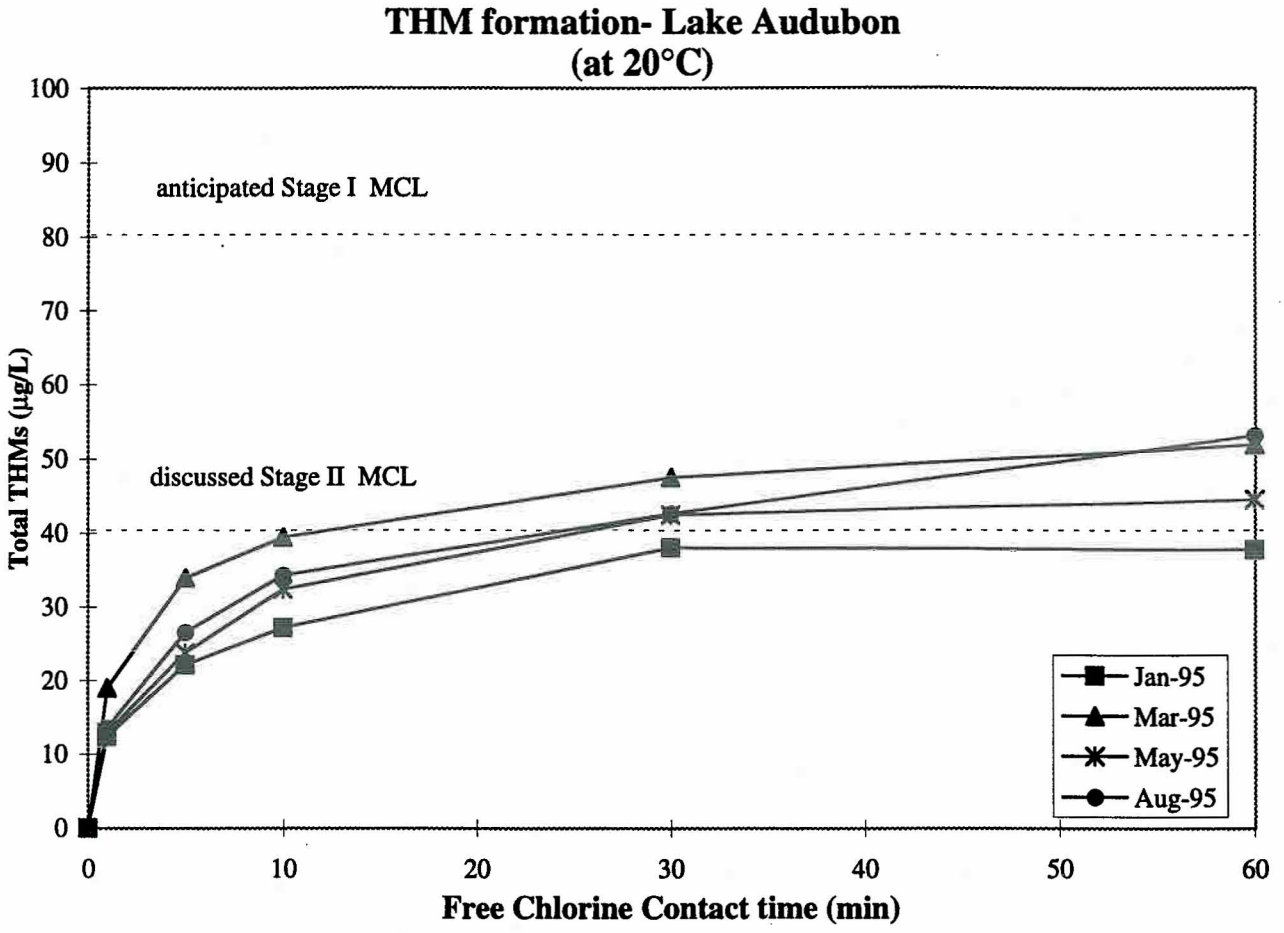
Sampling Date	Temperature	Decay Coefficient (min^{-1})	Ozone Demand (mg/L)
1/9/95	4	0.18	1.1
3/12/95	20	0.38	2.9
5/30/95	20	0.29	2.5
8/21/95	20	0.34	2.4

In general, the ozone demands and the ozone decay coefficients from these seasonal sampling events indicate that ozone decay occurs at a slower rate in Lake Sakakawea (i.e., a smaller ozone dose will produce a greater ozone residual) than in Lake Audubon, but the differences are small. The average of ozone demands for waters from Lake Sakakawea is slightly lower than for Lake Audubon, suggesting that water from Lake Sakakawea has a lower concentration of dissolved oxidizable material relative to the concentration in Lake Audubon. As expected, the ozone demands and ozone decay coefficients were lower at the lower temperature of 4°C.

DBP FORMATION

DBP formation experiments were conducted for all water samples taken from Lake Audubon and Lake Sakakawea over the 10 month sampling period in order to determine the seasonal range in DBP formation. In addition, the influence of free chlorine contact time on DBP formation was evaluated for the chloramination experiments. A total disinfectant contact time equal to 16.5 hours was evaluated for each experiment in order to simulate the hydraulic detention time in the pipeline for average daily flow of 10.2 MGD. For samples obtained during November 1994 and January 1995, a comparison was made between experiments conducted at 4°C and 20°C in order to determine the impact of temperature on DBP formation.

The formation of DBPs (at 20°C) for Lake Sakakawea and Lake Audubon waters at various free chlorine contact times is shown in Figure 3.1 and



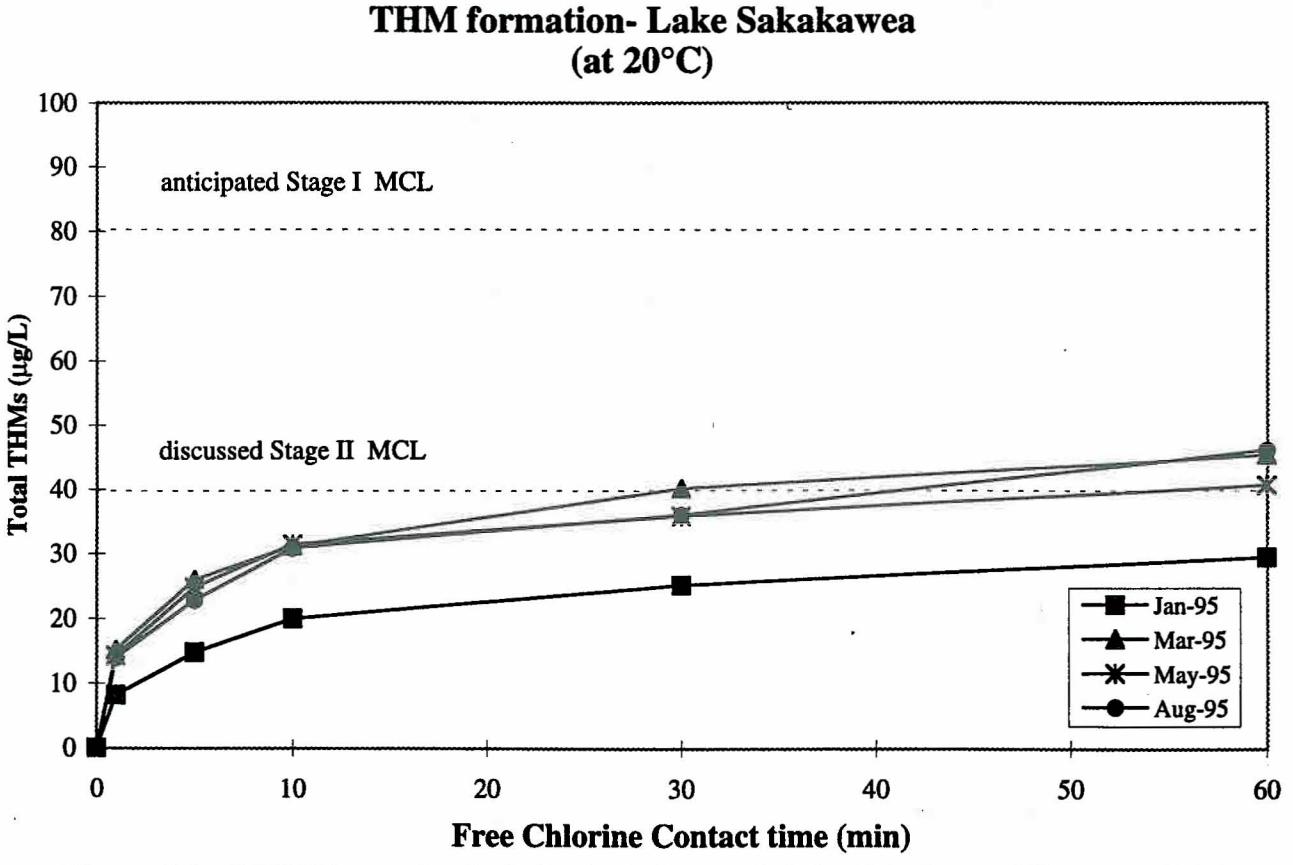
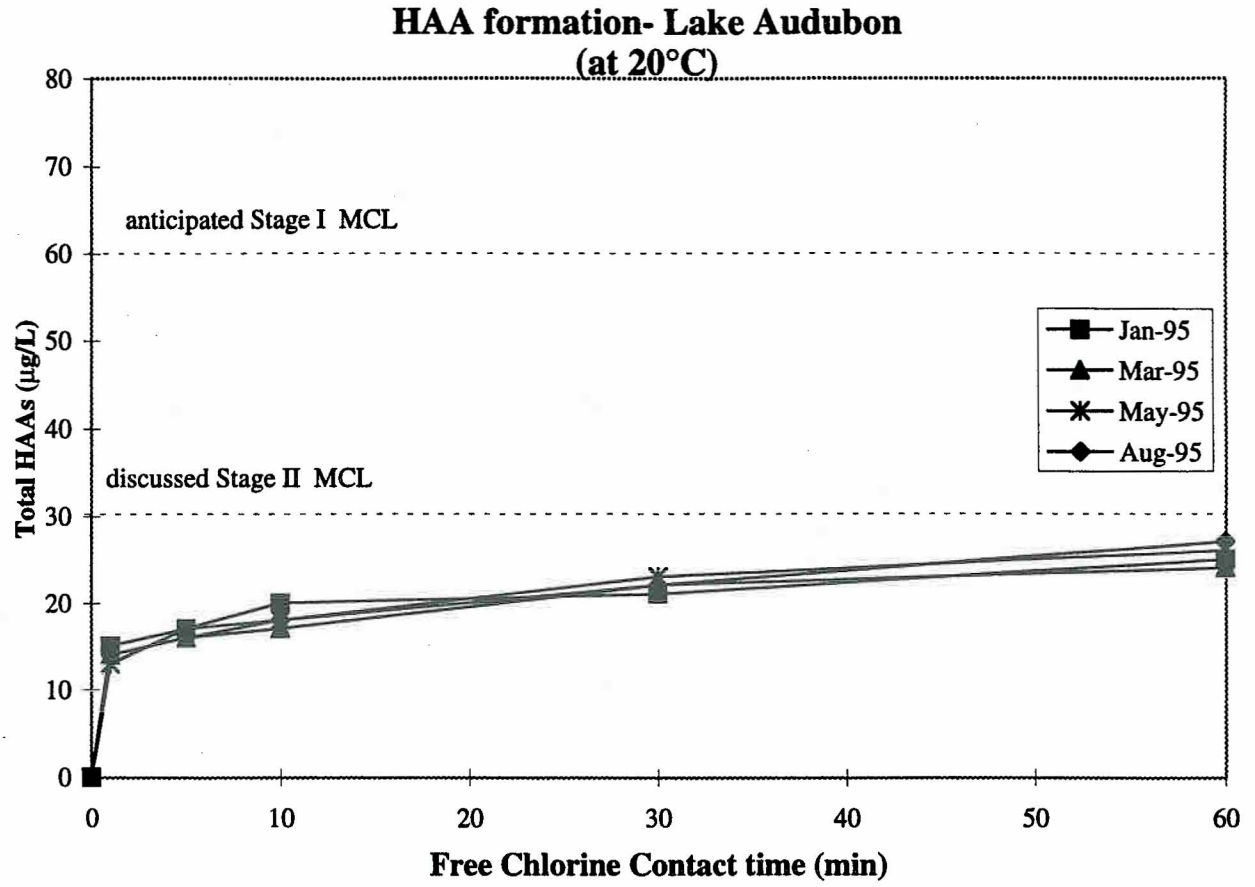


Figure 3.1 - THM Formation in Lake Audubon and Lake Sakakawea Waters at 20°C



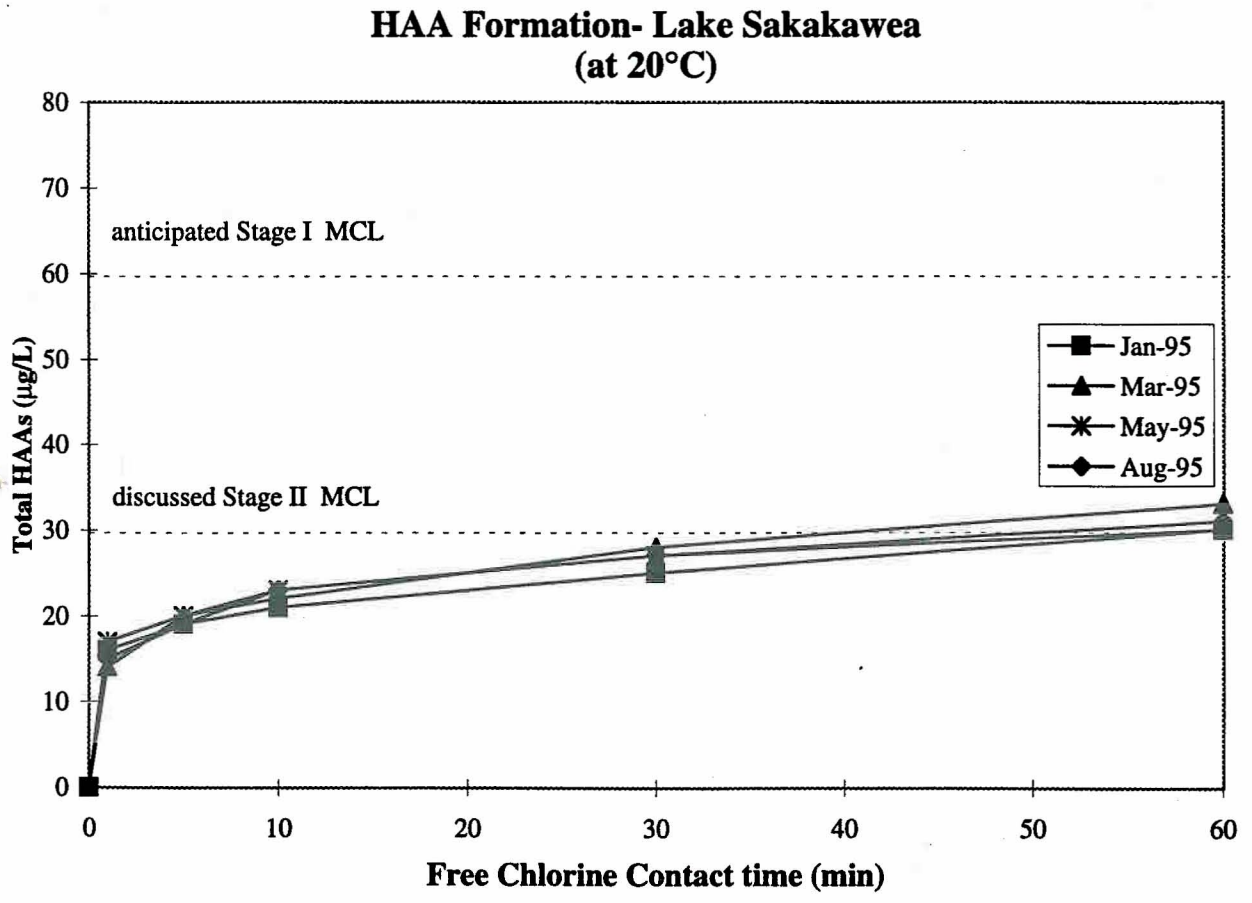


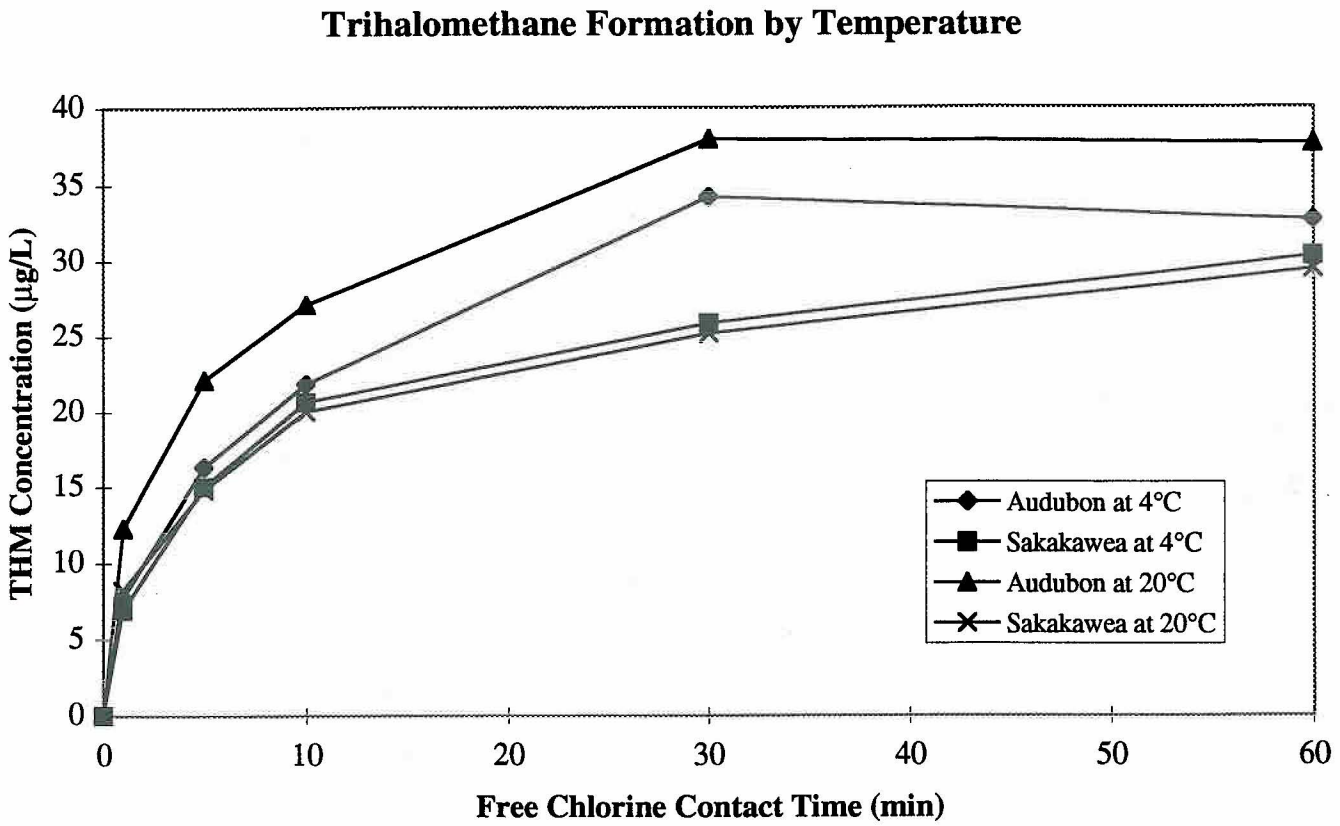
Figure 3.2 - HAA Formation in Lake Audubon and Lake Sakakawea Waters at 20°C

Figure 3.2. The influence of temperature (at 4 and 20°C) on DBP formation is shown in Figure 3.3. (See Appendix A for seasonal DBP results in tabular format.)

The results presented in Figure 3.1 indicate that, in general, slightly higher concentrations of halogenated THMs were formed in Lake Audubon water than in Lake Sakakawea water. This result is consistent with the higher chlorine demand expressed by the waters from Lake Audubon (and higher TOC concentrations for this water). The formation of THMs was also slightly higher for both lake waters during Phase III sampling (months of March, May, August) than during Phase II (month of January). For Lake Audubon, the highest THM concentrations were generated in March; this may have been a result of a change in the character of the natural organic matter dissolved in the lake water due to early spring run-off, but this has not been confirmed. As shown in Figure 3.2, HAA concentrations proved to be slightly higher for Lake Sakakawea than for Lake Audubon waters.

Figure 3.3 shows that levels of DBPs were generally greater at 20°C than at 4°C; an exception was for THM formation on Lake Sakakawea where temperature did not appear to impact THM concentrations. However, it is important to note that the 20°C experiments were conducted on an aliquot of water sampled two months after the water sampled for the 4°C experiments. Therefore, water quality differences may have played a role.

The measured concentrations of THMs and HAAs were in all cases below the anticipated MCLs for DBPs as proposed in Stage I of the D/DBP rule (80 and 60 µg/L for THMs and HAA(5), respectively), even after up to 60 minutes free chlorine contact time. This indicates that for a high temperature condition of (20°C) a free chlorine contact time of 60 minutes could be employed without excessive DBP formation. For free chlorine contact times shorter than 10 minutes, THMs and HAAs were below the MCLs discussed at the USEPA regulatory negotiations for the Stage II D/DBP rule (40 µg/L for THMs and 30 µg/L for HAA(5)). The possibility of additional DBP formation at the Minot WTP needs to be considered during selection of a design free chlorine contact time.



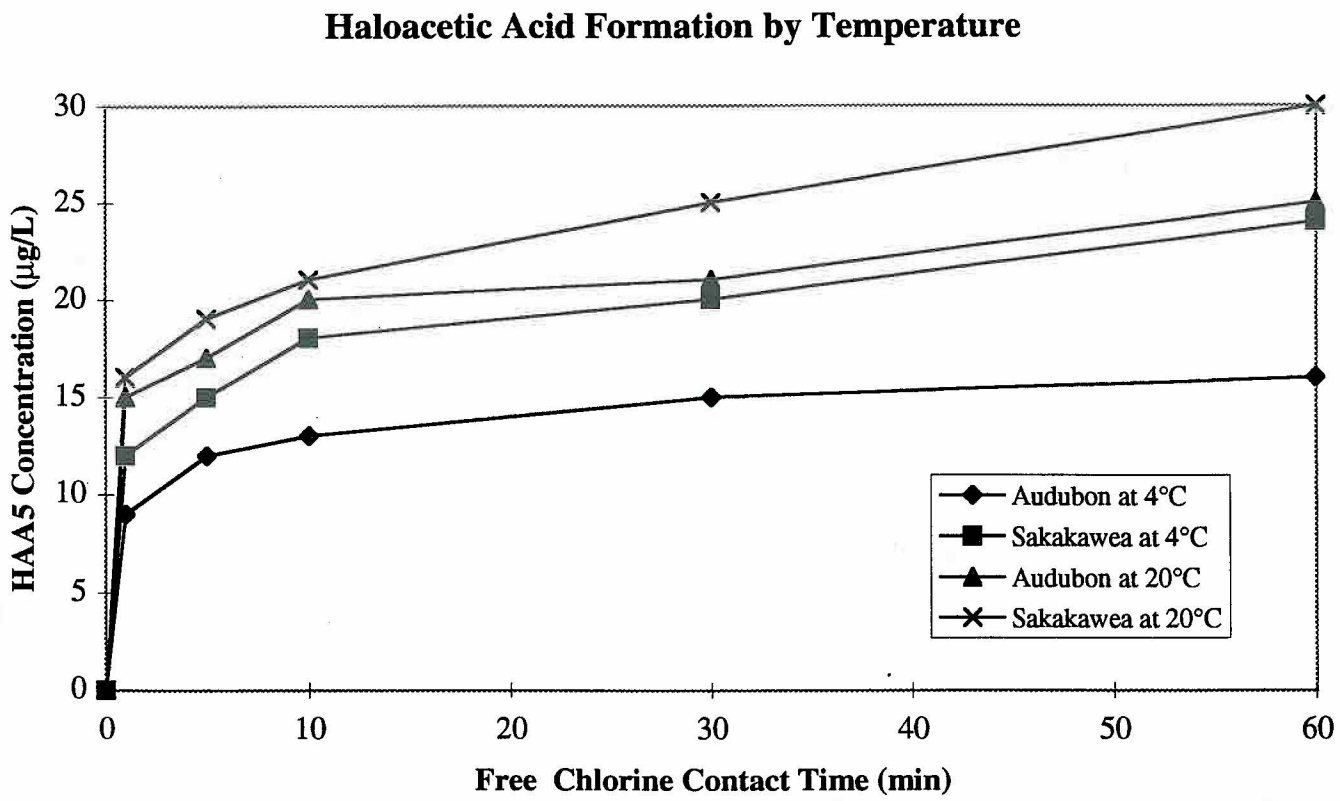


Figure 3.3 - THM and HAA Formation in Lake Audubon and Lake Sakakawea Waters at 4°C and 20°C

OZONE DBPS

Results of the ozonation DBP formation experiments for Phase III studies at the highest evaluated ozone dose are presented in the Tables 3-10 and 3-11 below.

Table 3-10
Ozonation By-Products - Lake Audubon

Aldehyde	Units	3/12/95	5/30/95	8/21/95
Bromide	mg/L		0.10	0.10
Bromate	µg/L		<10	<10
Acetaldehyde	µg/L	7	6	6
Butanal	µg/L	<1	<1	<1
Formaldehyde	µg/L	29	25	29
Glyoxal	µg/L	9	12	11
M-Glyoxal (Pyruvic Aldehyde)	µg/L	7	7	7
Pentanal	µg/L	<1	<1	<1
Propenal	µg/L	ND	1	1
TOTAL ALDEHYDES	µg/L	52	51	54

Conditions: 20°C, ambient pH, and highest ozone dose (6 mg/L).

Table 3-11
Ozonation By-Products - Lake Sakakawea

Aldehyde	Units	3/12/95	5/30/95	8/21/95
Bromide	mg/L		0.1	0.04
Bromate	µg/L		<10	<10
Acetaldehyde	µg/L	9	3	4
Butanal	µg/L	<1	<1	<1
Formaldehyde	µg/L	25	23	25
Glyoxal	µg/L	8	8	8
M-Glyoxal (Pyruvic Aldehyde)	µg/L	6	5	5
Pentanal	µg/L	<1	<1	<1
Propenal	µg/L	1	<1	<1
TOTAL ALDEHYDES	µg/L	49	39	42

Conditions: 20°C, ambient pH, and highest ozone dose (6 mg/L).

Aldehyde concentrations were slightly higher in the Lake Audubon waters (51 to 54 µg/L) than in the Lake Sakakawea samples (39 to 49 µg/L). The predominant aldehyde detected was formaldehyde, which comprised approximately 50 percent of the total aldehyde concentration. Bromate is an ozonation by-product that is often detected when elevated levels of bromide are present in the raw water. Levels of bromate in the ozonated waters of Lake Audubon and Lake Sakakawea were below the analytical detection limit of 10 µg/L. Under Stage I of the proposed D/DBP Rule, bromate will be regulated at 10 µg/L.

Section 4 Experimental Results Microbiology

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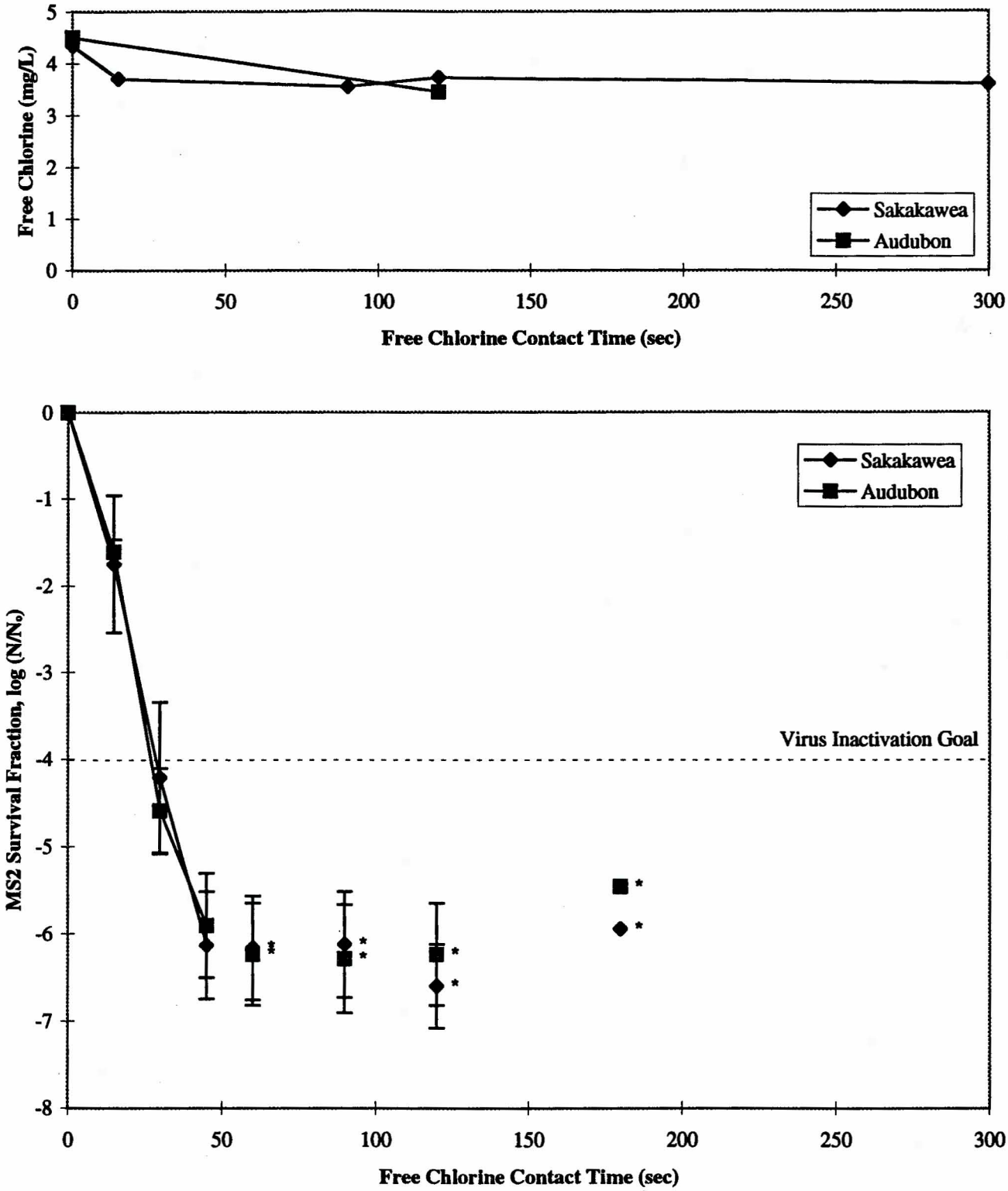
The objectives of the chloramine disinfection experiments were to determine the CT values that provide various levels of inactivation of MS2 bacteriophage and *Giardia* cysts in water from Lake Audubon. The experimentally determined CT values for inactivation by chloramination were then compared to the CT values designated for inactivation in the USEPA Guidance Manual to the SWTR. Similar experimental objectives were tested for the *Giardia* inactivation studies conducted with ozone in Lake Audubon water.

MS2 BACTERIOPHAGE INACTIVATION

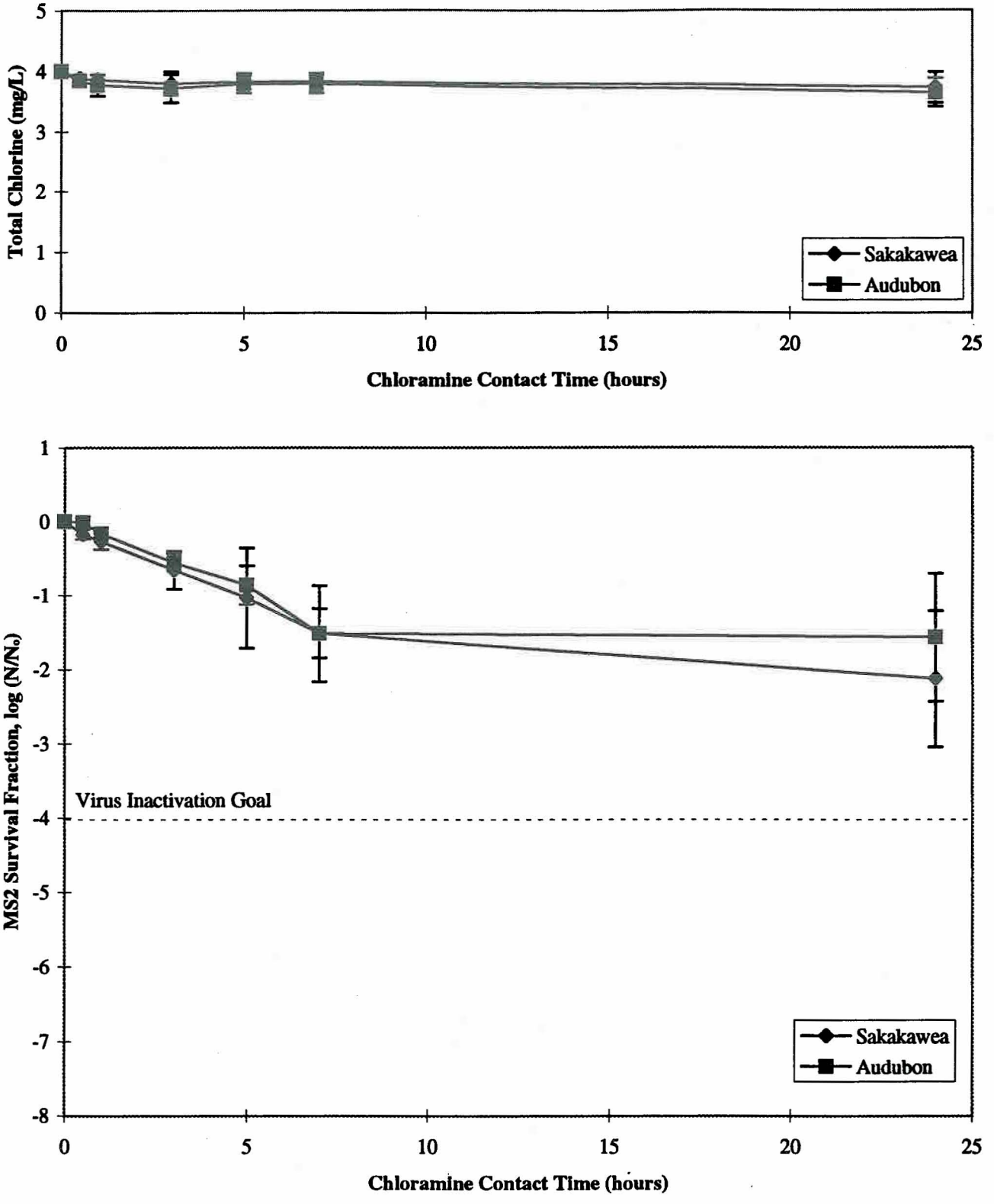
The results of the virus inactivation studies are shown in Figures 4.1 and 4.2. The data are presented as the log of the MS2 survival fraction. A survival fraction of -1 log signifies that 10 percent of virus are still active (or 90 percent inactivated). A survival fraction of -2 logs indicates that 1 percent of the virus are still active (or 99 percent inactivated). As designed in the experimental plan, the trials were conducted to yield a free chlorine residual of approximately 4 mg/L after accounting for the chlorine demand. The chloramine residuals measured during the MS2 disinfection studies were very similar for the waters from Lakes Audubon and Sakakawea, so the MS2 inactivation results for the two sources could be compared. The stars next to the averaged data points indicate that the virus were inactivated to levels below the sensitivity limit of the virus assay. In most trials, 6 logs or greater sensitivity were achieved.

Control experiments were conducted during MS2 inactivation studies to determine whether MS2 concentrations remained stable in the reactors over the duration of experiments. Control experiments were conducted in parallel with both sets of inactivation studies for free chlorine and chloramine. As shown in Figure 4.3, no significant changes in the MS2 survival fraction occurred in either of the control studies for the free chlorine of for the chloramination disinfection experiments. The slight changes in MS2 survival fraction that were observed in the control studies were well within the limits of experimental error for microbial inactivation studies.



Note: Each data point represents the average of six individual points
Bars on data points indicate 95 percent confidence intervals
* Indicates values less than sensitivity of the experimental assay

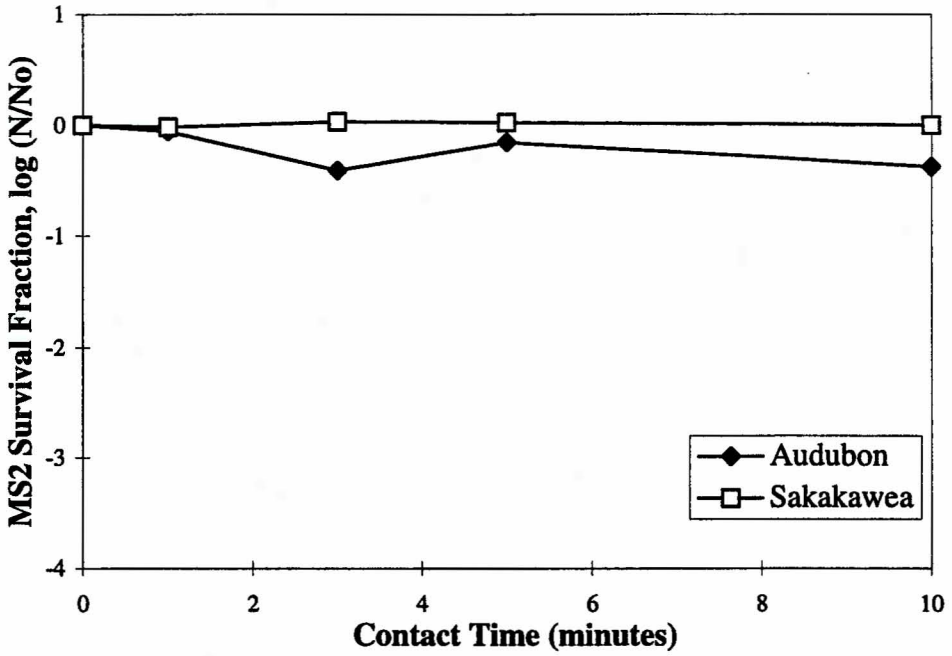
Figure 4.1 - Inactivation of MS2 By Free Chlorine at pH 8.4 and 4°C



Note: Each data point represents the average of six individual points
Bars on data points indicate 95 percent confidence intervals

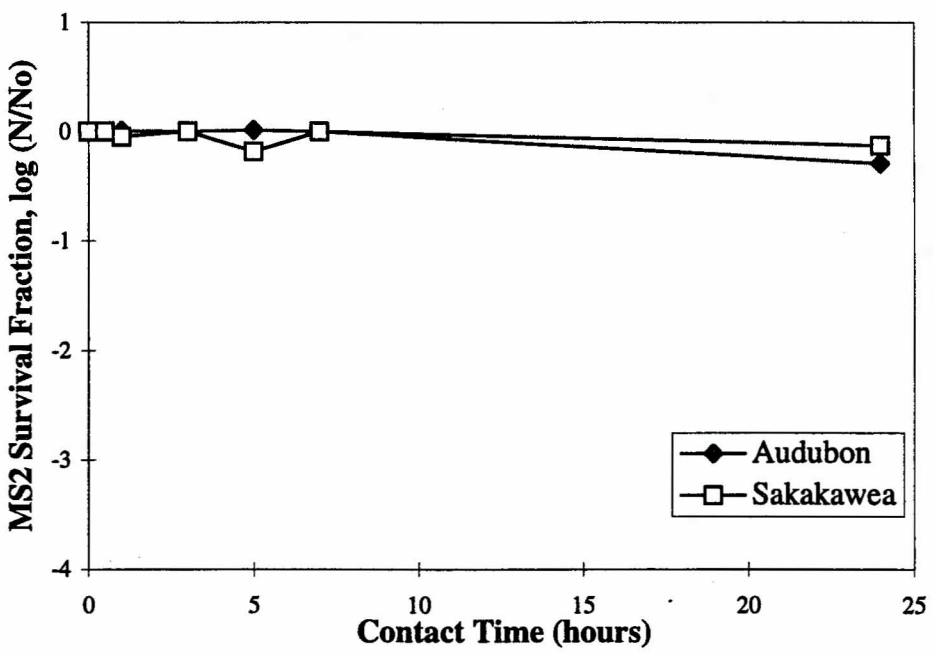
Figure 4.2 - Inactivation of MS2 By Preformed Chloramines at pH 8.4 and 4°C

Free Chlorine Control Experiments



Note: Each data point represents the average of four trials
(with the exception of the ten minute time point which represents one trial)

Chloramination Control Experiments



Note: Each data point represents the average of three trials

Figure 4.3 - Inactivation/Removal of MS2 bacteriophage during Control Experiments with Lake Audubon and Lake Sakakawea waters at 4°C

Figure 4.1 shows that approximately 6 logs of MS2 inactivation were achieved in less than 50 seconds of free chlorine contact time with a disinfectant dose of 4 mg/L and at conditions of pH 8.4 and 4°C. When no free chlorine contact time was allowed (Figure 4.2), however, approximately 2 logs or less of MS2 inactivation was observed after 24 hours of contact time for the same disinfectant dose at the same conditions of pH and temperature. The data underscore the need to have a period of free chlorine contact time (at least one minute) for MS2 virus inactivation. Ninety-five percent confidence intervals are presented with each data point. Overlap of these intervals indicates that there was no statistical difference (at 95 percent confidence) in inactivation when comparing the two waters. Although 6 logs of the virus were inactivated in less than one minute, a free chlorine contact time of 5 minutes was employed for the *Giardia* inactivation experiments as a margin of safety. At this free chlorine contact time, DBP concentrations over the three Phase III seasonal sampling events were on average 28 mg/L and 21 mg/L for THMs and HAAs, respectively. These levels are substantially below those discussed during the USEPA regulatory negotiations.

GIARDIA INACTIVATION EXPERIMENTS

Monochloramination

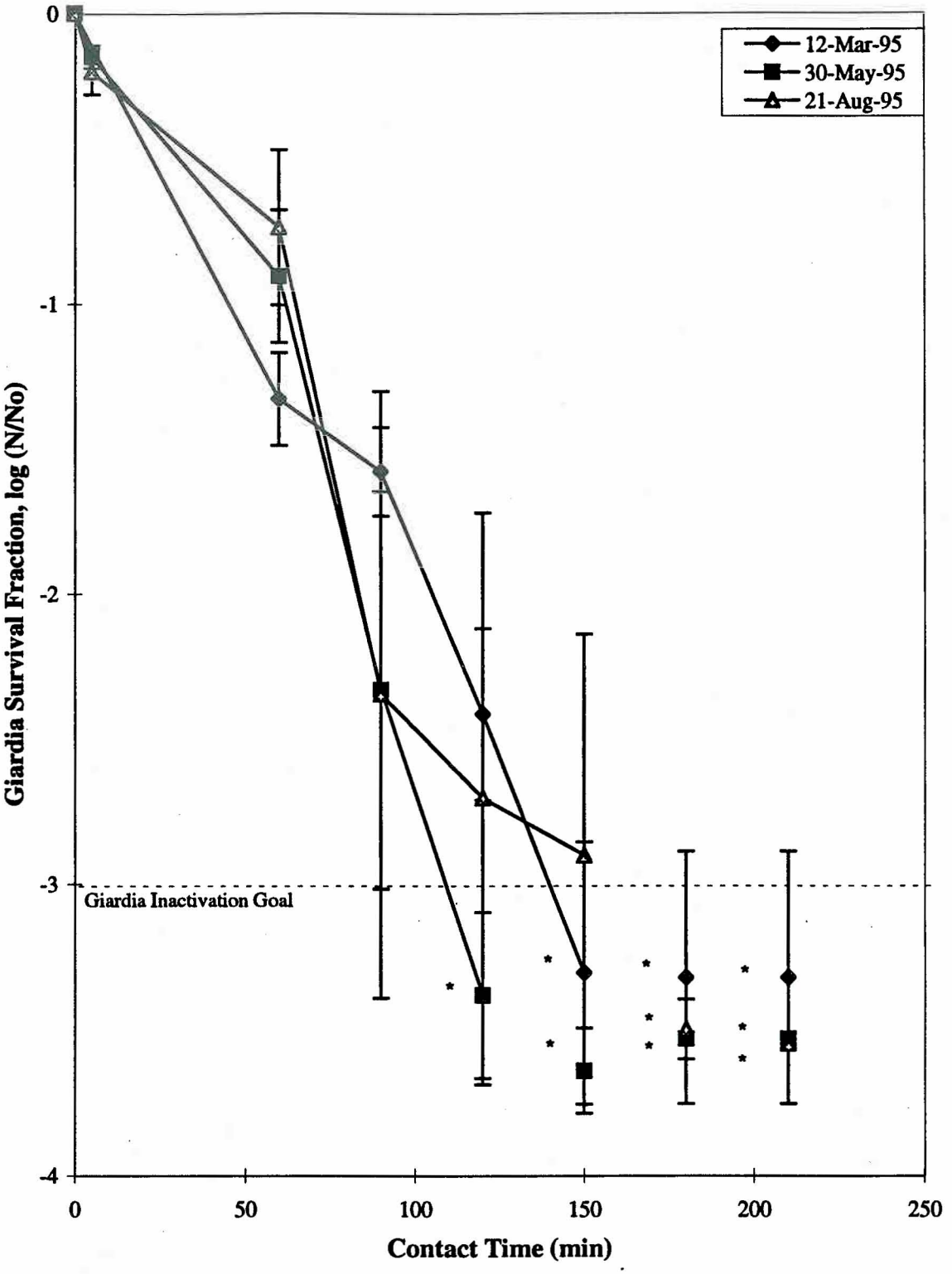
Control experiments were conducted to determine whether *Giardia* cysts adhered to the surfaces of the reactors and whether cyst viability remained stable over the duration of experiments. These control experiments were conducted in parallel with both the chloramination and the ozonation disinfection trials. For all the inactivation experiments conducted with *Giardia* cysts, little or no change in cyst number was observed in the control reactor vessels over the duration of experiments (Table 4.1); moreover, no trend indicating any loss in cyst viability was detected.

Table 4.1
Inactivation/Removal of *Giardia* during Control Experiments with Lake Audubon water at 4°C

	Giardia Survival Fraction, Log (N/No)	
Sampling Date	Chloramination	Ozonation
March 12, 1995	0.007	-0.005
May 30, 1995	0.020	-0.002
August 21, 1995	0.025	0.007

The results of the *Giardia* inactivation experiments using monochloramine are shown for Lake Audubon water in Figure 4.4. The data are presented as the log of the *Giardia* survival fraction, in the same manner as the MS2 virus inactivation studies. The *Giardia* inactivation experiments with monochloramine were conducted using a free chlorine contact time of 5 minutes and a monochloramine dose of approximately 4.5 mg/L. The results of chloramine residual measurements during the *Giardia* inactivation studies are provided in Appendix A.

Figure 4.4 shows that approximately 3 logs of *Giardia* inactivation were achieved for all seasonal sampling events in less than 3 hours of chloramine contact time after the initial 5 minutes of free chlorine contact time. The data points with stars next to them indicate that the *Giardia* cysts were inactivated



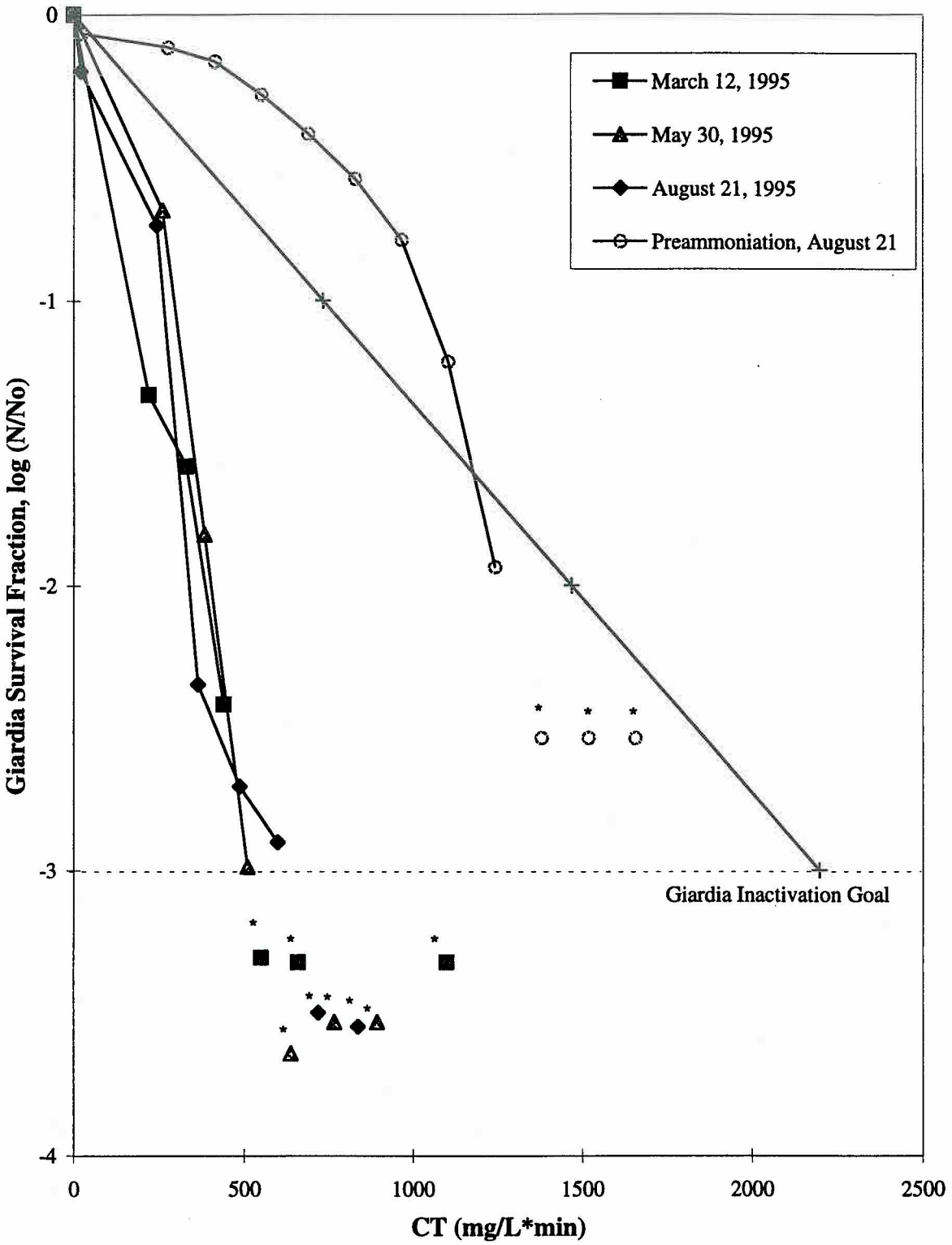
Note: Each data point represents the average of six individual points;
bars represent 95% confidence intervals

* Indicates values less than sensitivity of the experimental assay

Figure 4.4 - Inactivation of *Giardia* with Monochloramine dose of 4.5 mg/L at pH 8.2 and 4°C (Free chlorine contact time of 5 minutes) Lake Audubon

to levels below the sensitivity limit of the *Giardia* assay. It is convention not to connect lines to points that are below the sensitivity limit of the *Giardia* assay. The inactivation results from monochloramine experiments are presented in terms of CT in Figure 4.5. In this Figure, the USEPA values for CT at 5°C are presented for comparison. In all cases, inactivation of *Giardia* was experimentally measured at CT values far lower than indicated by the CT regulations from the USEPA Guidance Manual. It should be noted, however, that the Guidance Manual CT values were developed with preformed chloramines; the cysts were not exposed at any time to free chlorine. Nonetheless, the findings from this study suggest that the USEPA CT values for disinfection by monochloramine are conservative when a short period of free chlorine contact time is employed for the Lake Audubon water tested under these conditions of water quality.

Results from preammoniation inactivation studies are also shown in Figure 4.5. For these experiments, no free chlorine contact time was included, and instead ammonia was added to the reaction vessels prior to the addition of chlorine (chlorine to ammonia ratio of 4:1). For preammoniation experiments conducted in May and August 1995, log inactivations of *Giardia* are achieved at significantly longer contact times (larger CT values) than were required in the monochloramine experiments with the 5 minutes of free chlorine contact time. The results indicate that log inactivation from preammoniation (no free chlorine contact time) was not as effective as inactivation studies which included a free chlorine contact time of 5 minutes.



Note: Each data point for seasonal monochloramine studies (5 minute free chlorine contact time) represents the average of six individual points.

* Indicates values less than sensitivity limit of assay.

Preammoniation studies were conducted with no free chlorine contact time.

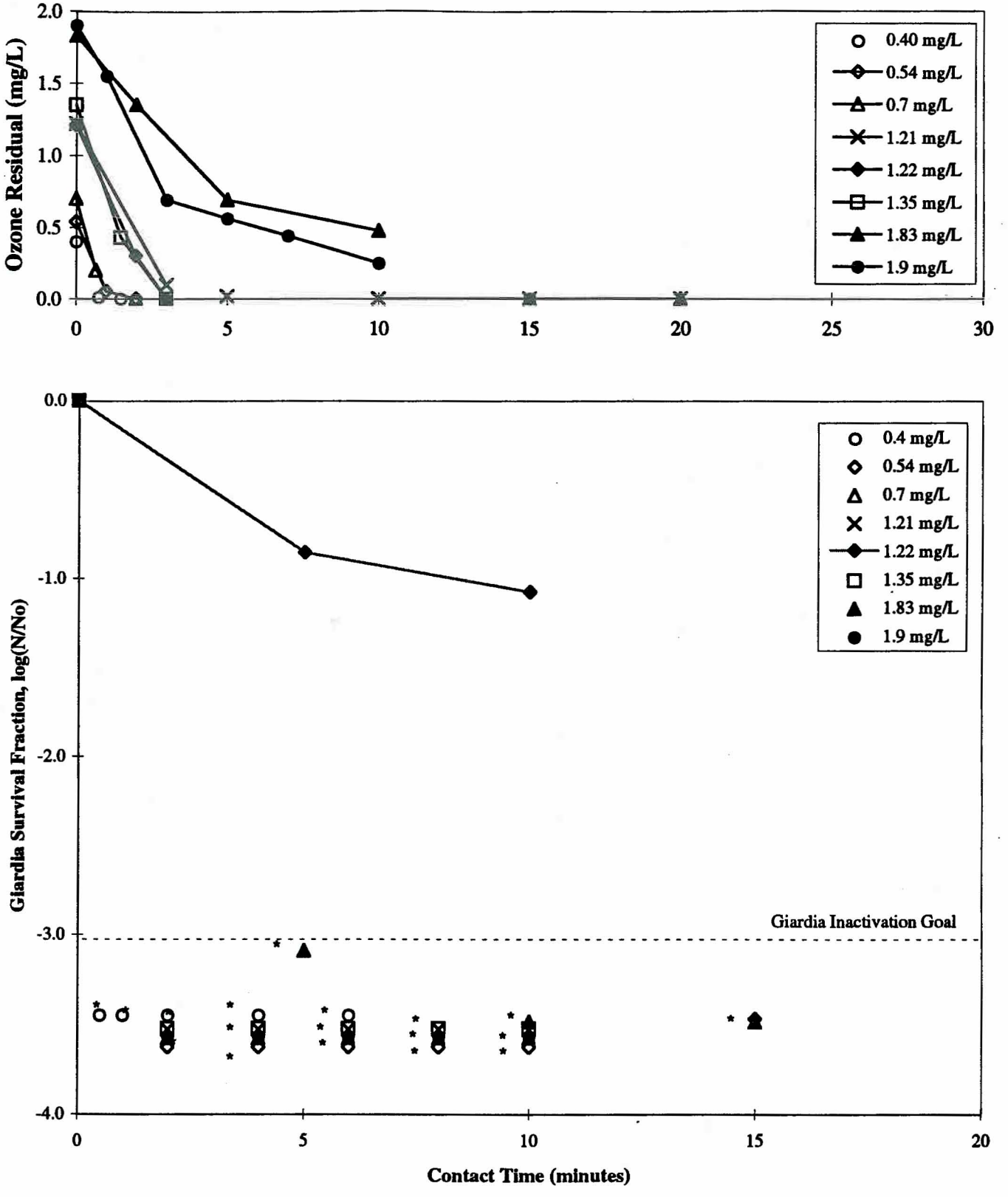
Figure 4.5 - Inactivation of Giardia by Monochloramine at pH 8.2 and 4°C at Lake Audubon

Ozonation

Results from control experiments to determine *Giardia* cyst viability were presented in Table 4.1 for the ozonation inactivation studies. No trend indicating loss in cyst viability was detected in these control experiments.

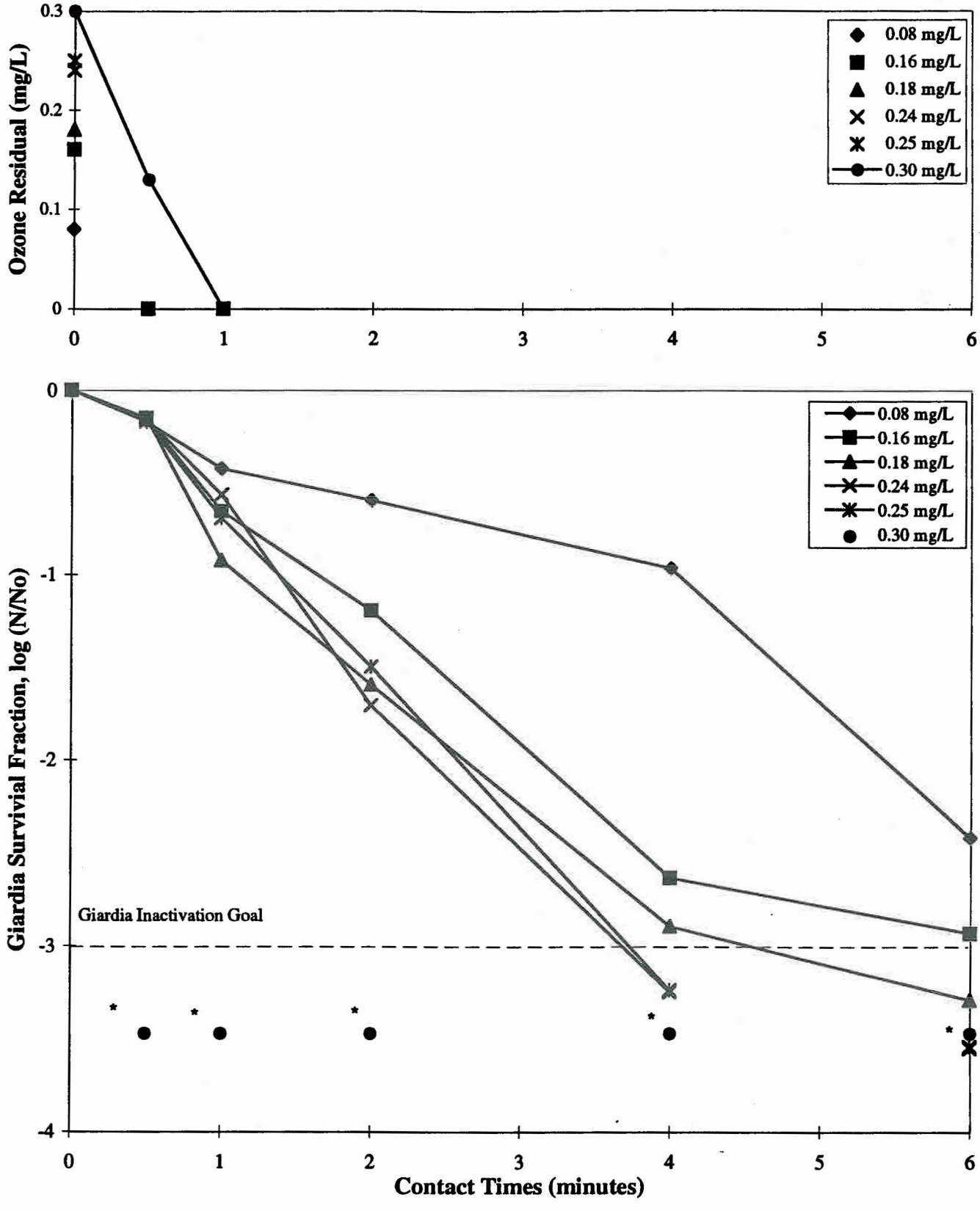
The results of *Giardia* inactivation experiments conducted using ozone are shown by season in Figures 4.6, 4.7 and 4.8. Each plot presents the log inactivation of the protozoan cysts at various doses and the residual concentration of ozone over time. All experiments were conducted at 4°C to provide a conservative estimate of the doses of ozone required to achieve 3 logs inactivation. Water obtained from the Winter sampling of Lake Audubon was the first water tested for inactivation of *Giardia*. The range of ozone doses used in the Winter sampling period was higher than the range employed during the other seasons. With the exception of one experimental trial at an ozone dose of 1.22 mg/L, greater than three logs inactivation of *Giardia* cysts were achieved for all the ozone doses applied (0.4 to 1.9 mg/L). Moreover, all of the samples were below the sensitivity limit of the *Giardia* excystation assay with the exception of the two data points shown for the 1.22 mg/L dose (Figure 4.6).

A lower range of ozone doses (between 0.08 and 0.3 mg/L) was employed for the Spring and Summer seasonal sampling periods. The inactivation and disinfectant residual curves for these seasons are shown Figures 4.7 and 4.8. The inactivation of *Giardia* achieved by ozone during the Spring and Summer sampling periods was quite similar for the range of ozone doses tested. Approximately 3 logs inactivation were achieved in approximately 4 minutes at ozone doses greater than 0.2 mg/L for both seasons. At a dose of 0.3 mg/L, greater log inactivation of cysts was achieved, exceeding the sensitivity limit of the assay (approximately 3.5 logs). The similarity of the curves for these two seasons suggests that seasonal differences in water quality did not have a substantial impact upon the inactivation kinetics. It is important to note that ozone is considerably less stable in natural waters than chloramines. The applied ozone in this study was consumed (in the Spring and Summer samples) in less than 60 seconds by ozone-demanding materials in the raw water. Although ozone residuals were not measured after this



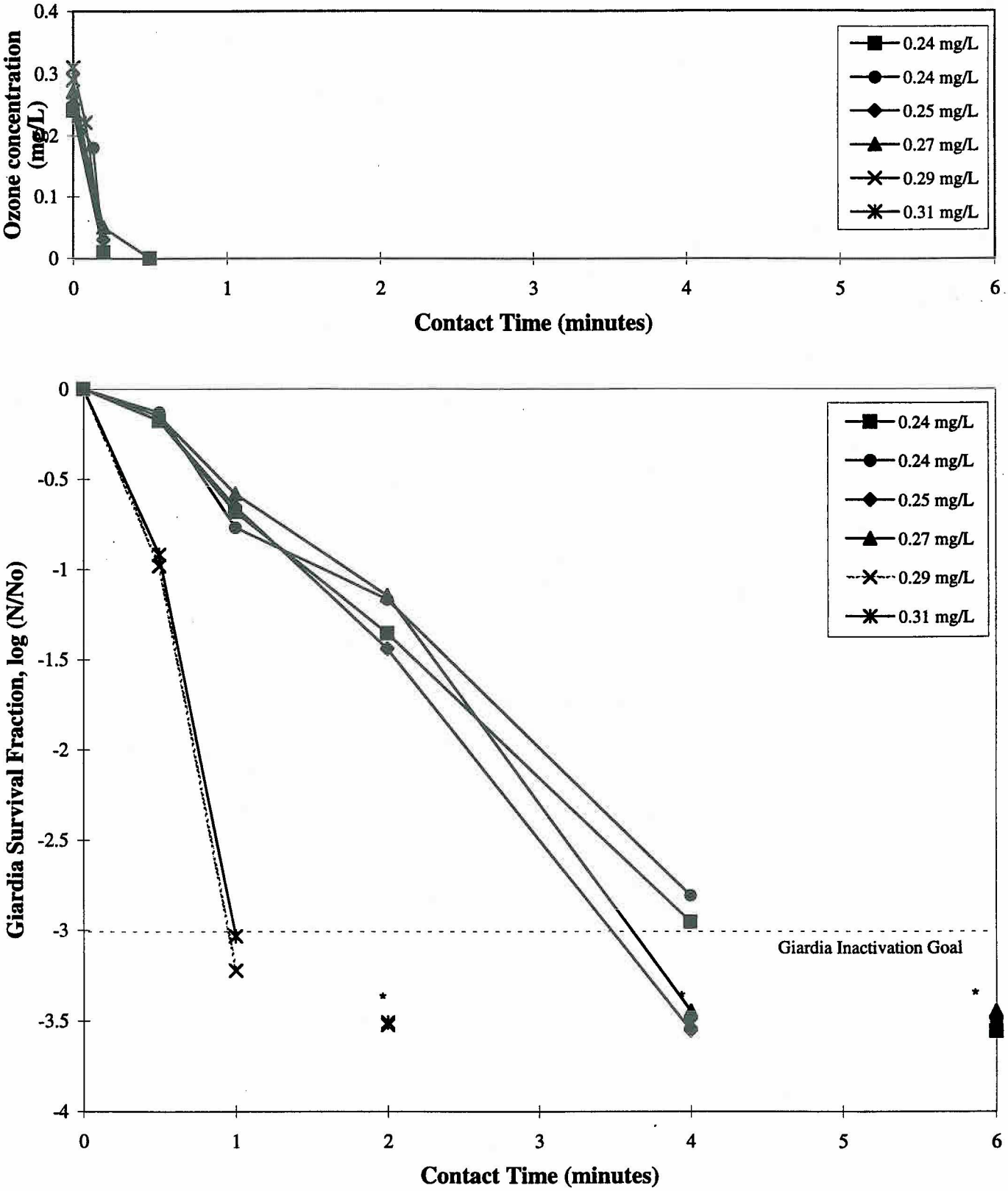
* Indicates values below the sensitivity of the experimental assay.

Figure 4.6 - Inactivation of *Giardia* in Lake Audubon Water by various ozone concentrations at pH 8.2 and 4°C
March 12, 1995



* Indicates values less than sensitivity of the experimental assay.

Figure 4.7 - Inactivation of *Giardia* in Lake Audubon Water by various ozone concentrations at pH 8.2 and 4°C
May 30, 1995



* Indicates values less than sensitivity of the experimental assay.

Figure 4.8 - Inactivation of *Giardia* in Lake Audubon Water by various ozone concentrations at pH 8.2 and 4°C
August 21, 1995

time period, inactivation of *Giardia* cysts continued. This may have been due to the continued oxidation brought about by formation of hydroxyl radicals or other ozonation by-products. Since inactivation of *Giardia* continued without the presence of a measurable ozone residual, it is difficult to directly apply a CT concept. However, the data do demonstrate that as long as a dose of 0.3 mg/L is applied to the water at 4°C or above, greater than 3 logs of inactivation of *Giardia* should be achieved.

Section 5 Summary

This study focused on the use of chloramines and ozone for disinfection of Lake Audubon water. Each was assessed in light of meeting SWTR requirements for unfiltered supplies, i.e., 3 logs inactivation of *Giardia* and 4 logs inactivation of viruses. The microbial inactivation studies and DBP formation studies were conducted at conservative temperatures (4° and 20°C, respectively).

This study demonstrated that chloramines could be employed for disinfection of Lake Audubon water. Four logs of MS2 virus were inactivated in less than 30 seconds of free chlorine contact time with a residual between 3.5 mg/L and 4.0 mg/L. However, 5 minutes of free chlorine contact time are recommended as a margin of safety. *Giardia* inactivation experiments showed that with a dose of 4.5 mg/L and 5 minutes of free chlorine contact time followed by ammonia addition to form chloramines, greater than 3 logs of inactivation were achieved in less than 180 minutes. Under these conditions, the contact time for inactivation is approximately one half of the residence time in the pipeline to the divide (5.9 hours) corresponding to the peak daily flow of 28 MGD. The CT required for 3 logs inactivation was conservatively estimated at 700 mg/L•min, which is considerably less than that stipulated by the Guidance Manual to the SWTR for 3 logs inactivation (2,200 mg/L•min at 5°C without any free chlorine contact time). However, when only preammoniation was employed in experiments, the rate of inactivation decreased considerably. These results underscore the necessity of employing a five minute free chlorine contact time if this disinfection scenario is ultimately employed.

All inactivation experiments with MS2 bacteriophage and *Giardia* were conducted at 4°C ± 1.0 °C. Because the temperature in Lake Audubon may at times decrease to as low as 1°C, safety factors to accommodate this difference would be incorporated into the design of a full scale system. For example, greater than 4 logs of virus were inactivated in less than 30 seconds by the dose of free chlorine employed. However, 5 minutes of free chlorine contact time were employed in Phase II of the study to provide a safety factor greater than 5. It also appears that even longer free chlorine contact times could be designed if necessary, since the DBP levels formed under the conditions

studied were less than those discussed for Stage 2 of the D/DBP rule during the USEPA regulatory negotiations. In the case of *Giardia*, the results demonstrated that greater than 3 logs of the protozoa were inactivated in less than 3 hours at the chloramine disinfectant doses and free chlorine contact time employed. With the pipeline residence time of 5.9 hours to the drainage divide (maximum day demand), a safety factor of approximately 2 would be achieved under this disinfection scenario for *Giardia* inactivation.

Turbidity has been demonstrated, in some cases, to affect disinfection either through expression of a disinfectant demand or through shielding of microorganisms. For Lake Audubon, raw water turbidities during the seasonal sampling ranged from 0.4 to 6.4 NTU. These seasonal changes in raw water turbidity did not however, appear to impact the inactivation of either the virus or the protozoa. Disinfectant residuals were carefully monitored throughout the experiments and any additional demand exerted by the turbidity was met by increasing the disinfectant dose. A full scale system would operate in a similar manner, i.e., the chlorination/ammonia system would be designed to accommodate additional demand due to turbidity so that the target residual could be achieved.

Because of the low concentrations of viruses and protozoa in natural waters, the inactivation experiments of this study were conducted by seeding microorganisms into a batch reactor and then adding disinfectant. No attempt was made to artificially associate the virus or cysts with particles contributing to turbidity in order to simulate a "shielding or protective" effect which may or may not be present in natural systems. In examination of this protective effect, Hoff (1978) compared the inactivation rate of poliovirus 1 (using free chlorine at pH 6 and 5°C) adsorbed to inorganic turbidity to the inactivation rate of poliovirus that was not associated with turbidity. He showed that turbidity ranging from 4 to 7 NTU (bentonite clay and precipitated aluminum phosphate) had little or no effect on the rate of virus inactivation. These results suggest that under the conditions tested in this study, seasonal changes in inorganic raw water turbidity would not substantially affect disinfection efficacy of Lake Audubon water.

With regard to formation of DBPs, concentrations less than 35 µg/L of THMs and 20 µg/L of HAAs were formed by a five minute free chlorine contact time (chlorine dose of 4.5 mg/L) followed by ammonia addition. These concentrations are less than the Stage 1 MCLs of the proposed D/DBP Rule, which are 80 and 60 µg/L for THMs and HAAs, respectively. They are also lower than those levels discussed at the USEPA regulatory negotiations for Stage 2 (40 and 30 µg /L for THMs and HAAs, respectively). However, additional formation of DBPs will occur through the Minot treatment plant as a result of meeting disinfection requirements. These levels need to be assessed through the treatment process.

This study also demonstrated that ozone could be employed for disinfection of Lake Audubon water. Greater than 3 logs of *Giardia* inactivation were achieved in approximately 4 minutes at doses greater than or equal to 0.3 mg/L ozone at 4°C. Inactivation continued to occur despite the consumption of ozone residual. Although inactivation of viruses by ozone was not investigated at bench-scale, viruses are more sensitive to ozone than protozoan cysts; therefore virus inactivation requirements would be met if 3 logs of *Giardia* inactivation is achieved by ozone treatment.

Aldehydes were the major DBPs formed as a result of disinfecting with ozone. Concentrations of total aldehydes ranging from 39 to 54 µg/L were formed as a result of ozonation of Lake Audubon water. There are currently no MCLs for aldehydes. Moreover, it is anticipated that substantial concentrations of these compounds would be removed if biological filtration were employed as part of the treatment process after the water arrived at Minot. No bromate was detected in the ozonated raw waters; this oxidation by-product will be regulated at 10 µg/L under Stage I of the proposed D/DBP regulations.

Ozone demand and decays were conducted on raw waters from both Lake Audubon and Lake Sakakawea. In general, ozone demands at 20°C for Lake Audubon and Lake Sakakawea ranged from 2.4 to 3.1 mg/L. The ozone demand and decay results may be useful for design purposes.

Section 6 References

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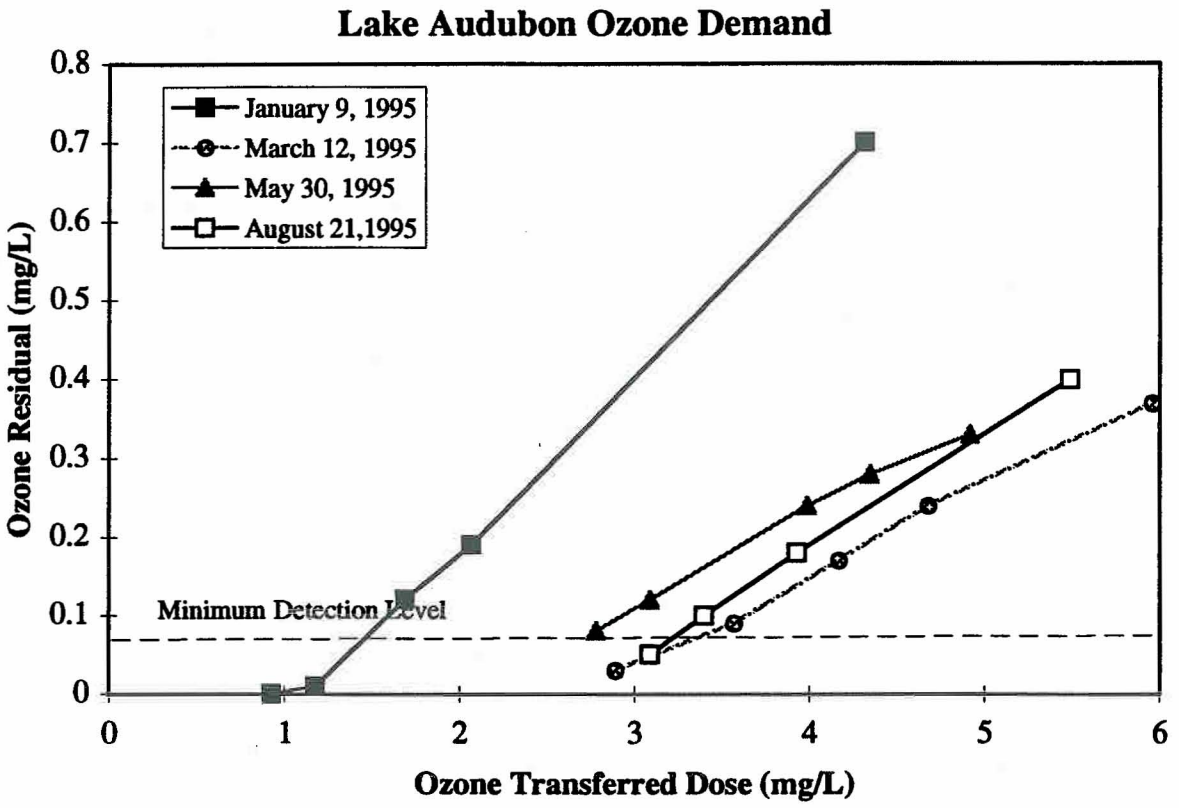
Appendix A

HOUSTON ENGINEERING
AMERICAN ENGINEERING
MONTGOMERY WATSON



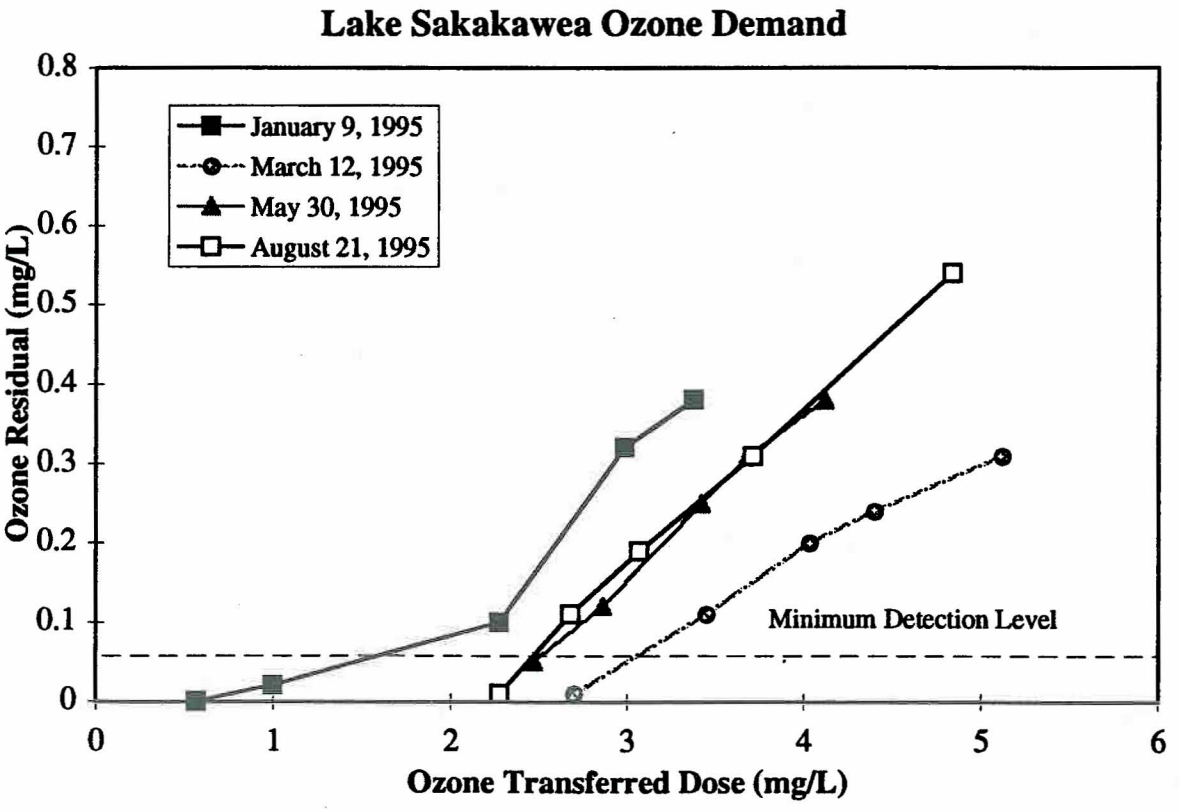
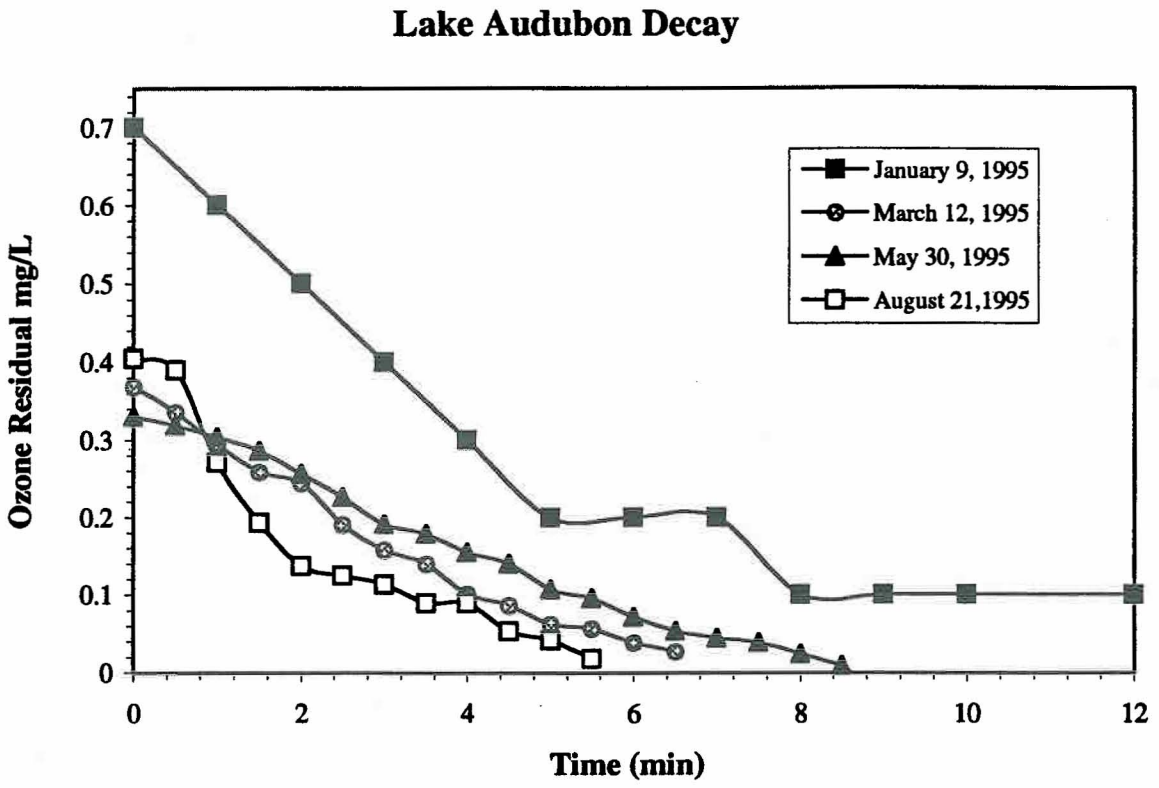


Figure A-1 - Ozone Demand for Lake Audubon and Lake Sakakawea



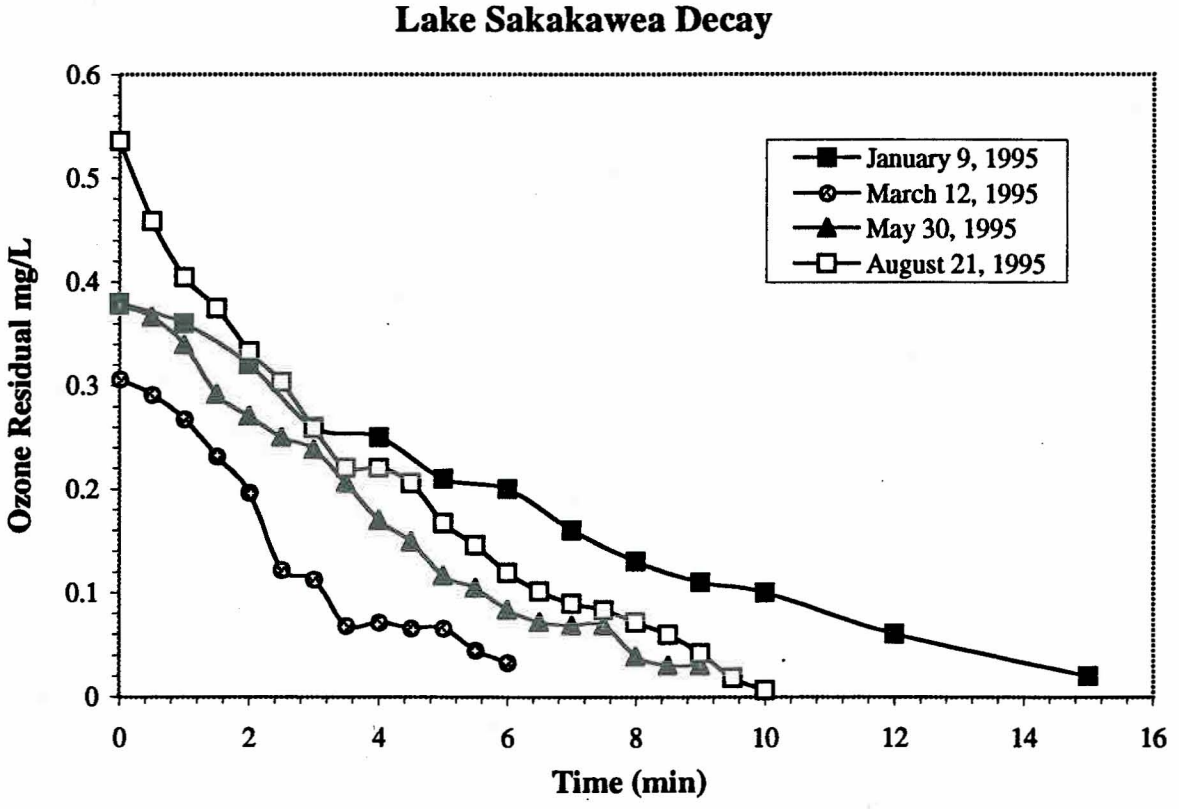


Figure A-2 - Ozone Decay in Lake Audubon and Lake Sakakawea Waters

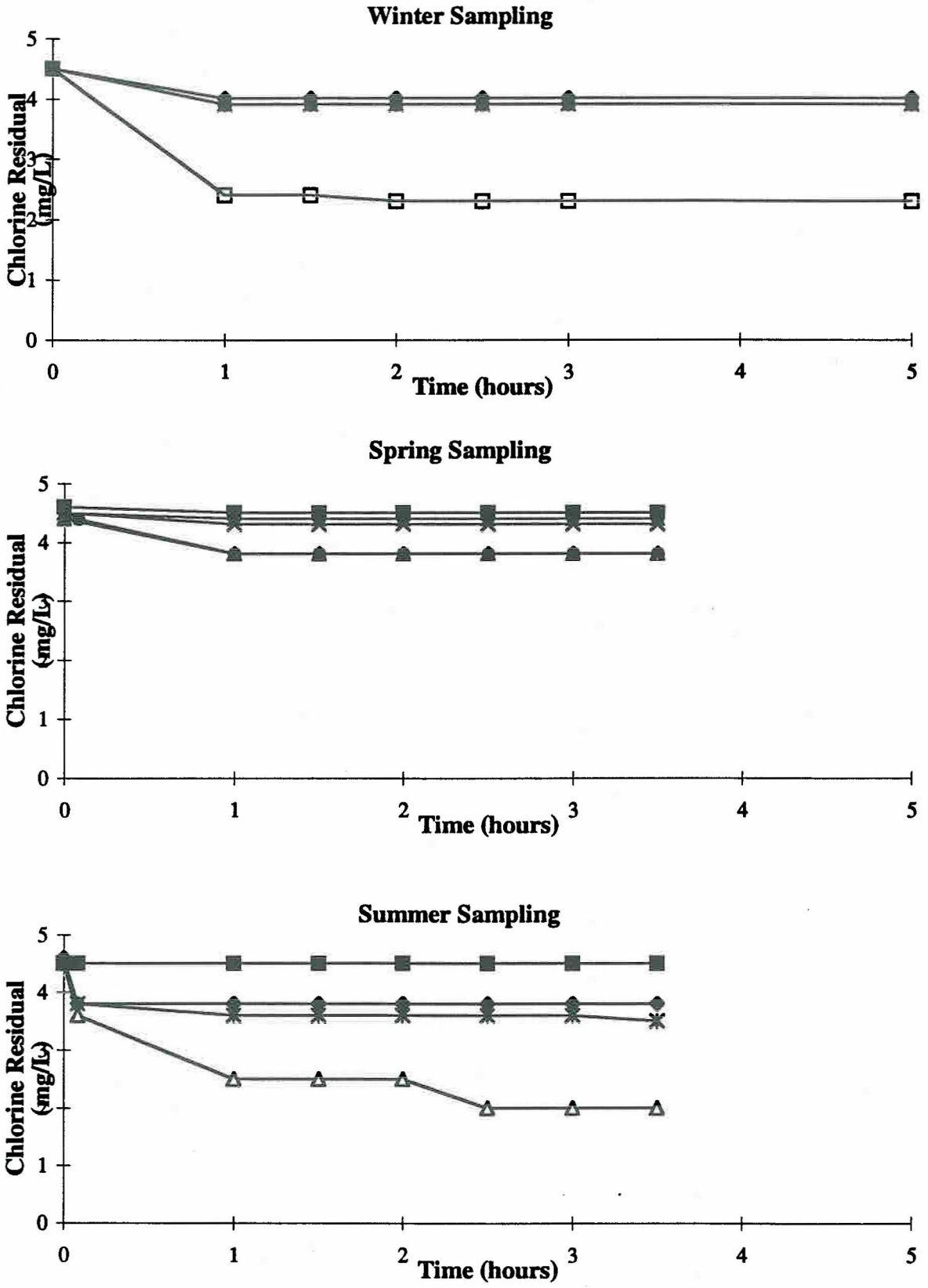


Figure A-3 -Chlorine Residuals for *Giarida* Inactivation Studies in Lake Audubon water at 4°C

PHASE II - November 7, 1994
NAWS CHLORAMINE CHALLENGE STUDY
DBP FORMATION EXPERIMENTAL RESULTS

Lake Audubon
Raw Water TOC = 5.6 mg/L
Temperature = 4°C

Free Chlorine Contact Time (min)	Chlorine Dose (mg/L)	Ammonia Dose (mg/L)	Total Chlorine Residual			pH at 48 hours	DBP Formation (16.5 hour)	
			0 hour (mg/L)	16.5 hour (mg/L)	48 hour (mg/L)		TTHM (µg/L)	HAA5 (µg/L)
1	4.3	1.0	4.10	3.95	3.95	8.5	7.7	9
5	4.5	1.0	3.90	3.70	3.70	8.5	16.3	12
10	4.7	1.0	4.00	3.75	3.70	8.5	21.8	13
30	5.0	1.0	4.00	3.85	3.80	8.4	34.2	15
60	5.2	1.0	4.00	3.85	3.80	8.4	32.7	16

Lake Sakakawea
Raw Water TOC = 4.1 mg/L
Temperature = 4°C

Free Chlorine Contact Time (min)	Chlorine Dose (mg/L)	Ammonia Dose (mg/L)	Total Chlorine Residual			pH at 48 hours	DBP Formation (16.5 hour)	
			0 hour (mg/L)	16.5 hour (mg/L)	48 hour (mg/L)		TTHM (µg/L)	HAA5 (µg/L)
1	4.2	1.0	4.05	3.90	3.80	8.4	6.9	12
5	4.3	1.0	3.95	3.75	3.75	8.4	15.0	15
10	4.5	1.0	4.00	3.80	3.80	8.4	20.6	18
30	4.7	1.0	4.00	3.80	3.80	8.4	25.8	20
60	4.9	1.0	4.05	3.80	3.80	8.4	30.3	24

PHASE II - November 7, 1994
NAWS CHLORAMINE CHALLENGE STUDY
CT VALUES AND INACTIVATION ACHIEVED

Lake Audubon
Raw Water TOC = 5.6 mg/L
Temperature = 4°C

Free Chlorine Contact Time (min)	CT Achieved		Estimated Removal of Giardia*			DBP Formation (16.5 hour)	
	Free Chlorine (mg/L - min)	Chloramines (mg/L - min)	Free Chlorine (logs)	Chloramines (logs)	Total (logs)	TTHM (µg/L)	HAA5 (µg/L)
1	4	3857	0.0	3.0	3.1	7.7	9
5	20	3694	0.1	2.9	3.0	16.3	12
10	40	3724	0.2	2.9	3.2	21.8	13
30	120	3648	0.7	2.9	3.7	34.2	15
60	243	3534	1.5	2.8	4.3	32.7	16

* Based on extrapolation of USEPA Guidance Manual values at 0.5°C

Lake Sakakawea
Raw Water TOC = 4.1 mg/L
Temperature = 4°C

Free Chlorine Contact Time (min)	CT Achieved		Estimated Removal of Giardia*			DBP Formation (16.5 hour)	
	Free Chlorine (mg/L - min)	Chloramines (mg/L - min)	Free Chlorine (logs)	Chloramines (logs)	Total (logs)	TTHM (µg/L)	HAA5 (µg/L)
1	4	3907	0.0	3.1	3.1	6.9	12
5	20	3645	0.1	2.9	3.0	15.0	15
10	40	3675	0.2	2.9	3.1	20.6	18
30	120	3696	0.7	2.9	3.6	25.8	20
60	240	3581	1.5	2.8	4.3	30.3	24

* Based on extrapolation of USEPA Guidance Manual values at 0.5°C

PHASE II - January 9,1995
NAWS CHLORAMINE CHALLENGE STUDY
DBP FORMATION

Lake Audubon
Raw Water TOC = 6.4 mg/L
Temperature = 20 °C

Free Chlorine Contact Time (min)	Chlorine Dose (mg/L)	Ammonia Dose (mg/L)	Total Chlorine Residual		pH at 16.5 hours	DBP Formation (16.5 hour)	
			0 hour (mg/L)	16.5 hour (mg/L)		TTHM (µg/L)	HAA5 (µg/L)
1	4.7	1.0	4.00	3.75	8.4	12.3	15
5	5.2	1.0	3.95	3.70	8.4	22.1	17
10	5.4	1.0	4.00	3.70	8.4	27.1	20
30	5.7	1.0	4.00	-	-	38.0	21
60	6	1.0	3.95	3.65	8.4	37.7	25

Lake Sakakawea
Raw Water TOC = 4.2 mg/L
Temperature = 20 °C

Free Chlorine Contact Time (min)	Chlorine Dose (mg/L)	Ammonia Dose (mg/L)	Total Chlorine Residual		pH at 16.5 hours	DBP Formation (16.5 hour)	
			0 hour (mg/L)	16.5 hour (mg/L)		TTHM (µg/L)	HAA5 (µg/L)
1	4.7	1.0	4.35	4.10	8.4	8.2	16
5	4.8	1.0	4.20	3.90	8.4	14.8	19
10	5	1.0	4.25	4.05	8.4	20.0	21
30	5.3	1.0	4.25	4.10	8.3	25.1	25
60	5.4	1.0	4.35	4.10	8.3	29.4	30

PHASE II - January 9, 1995
NAWS CHLORAMINE CHALLENGE STUDY
CT VALUES AND INACTIVATION ACHIEVED

Lake Audubon
Raw Water TOC = 6.4 mg/L
Temperature = 20°C

Free Chlorine Contact Time (min)	CT Achieved		Estimated Removal of Giardia*			DBP Formation (16.5 hour)	
	Free Chlorine (mg/L - min)	Chloramines (mg/L - min)	Free Chlorine (logs)	Chloramines (logs)	Total (logs)	TTHM (µg/L)	HAA5 (µg/L)
1	4	3709	0.0	2.9	3.0	12.3	15
5	20	3645	0.1	2.9	3.0	22.1	17
10	40	3626	0.2	2.9	3.1	27.1	20
30	120	-	0.7	-	-	38.0	21
60	237	3395	1.4	2.7	4.1	37.7	25

* Based on extrapolation of USEPA Guidance Manual values at 0.5°C

Lake Sakakawea
Raw Water TOC = 4.2 mg/L

Free Chlorine Contact Time (min)	CT Achieved		Estimated Removal of Giardia*			DBP Formation (16.5 hour)	
	Free Chlorine (mg/L - min)	Chloramines (mg/L - min)	Free Chlorine (logs)	Chloramines (logs)	Total (logs)	TTHM (µg/L)	HAA5 (µg/L)
1	4	4055	0.0	3.2	3.2	8.2	16
5	21	3842	0.1	3.0	3.2	14.8	19
10	43	3969	0.3	3.1	3.4	20.0	21
30	128	3936	0.8	3.1	3.9	25.1	25
60	261	3813	1.6	3.0	4.6	29.4	30

* Based on extrapolation of USEPA Guidance Manual values at 0.5°C

Phase III SPRING - March 12, 1995
NAWS CHLORAMINE CHALLENGE STUDY
DBP FORMATION EXPERIMENTAL RESULTS

Lake Audubon
Raw Water TOC = 5.3 mg/L
Temp 20°C

Free Chlorine Contact Time (min)	Chlorine Dose (mg/L)	Chlorine Stock (mL)	Ammonia Dose (mg/L)	Ammonia Stock (mL)	Total Chlorine Residual		pH at 16 hours	DBP Formation (16.5 hour)	
					0 hour (mg/L)	16.5 hour (mg/L)		TTHM (µg/L)	HAA5 (µg/L)
1	4.70	3.8	1.00	1.00	4.20	4.00	8.05	18.9	13
5	5.00	4.1	1.00	1.00	4.40	4.05	8.16	33.9	17
10	5.10	4.2	1.00	1.00	4.30	3.90	8.24	39.4	18
30	5.50	5.0*	0.96	0.87†	4.40	3.73	8.13	47.4	23
60	5.60	4.6	1.00	1.00	4.20	4.00	8.26	51.9	26

Lake Sakakawea
Raw Water TOC = 3.6 mg/L
Temp 20°C

Free Chlorine Contact Time (min)	Chlorine Dose (mg/L)	Chlorine Stock (mL)	Ammonia Dose (mg/L)	Ammonia Stock (mL)	Total Chlorine Residual		pH at 16 hours	DBP Formation (16.5 hour)	
					0 hour (mg/L)	16.5 hour (mg/L)		TTHM (µg/L)	HAA5 (µg/L)
1	4.60	3.8	1.00	1.00	4.15	4.05	8.13	15.1	14
5	4.80	3.9	1.00	1.00	4.20	3.85	8.14	25.9	20
10	5.10	4.2	1.05	1.05	4.35	4.05	8.14	31.1	22
30	5.40	4.9*	1.01	0.91†	4.45	4.05	8.24	40.1	28
60	5.50	4.5	1.05	1.05	4.30	4.00	8.15	45.3	33

Cl2 stock [ ]: 1225 mg/L
Ammonia stock [ ]: 1000 mg/L
*Cl2 stock [ ]:1100 mg/L
†Ammonia stock addition based on a 900 mL sample volume

Phase III SPRING - May 30, 1995
NAWS CHLORAMINE CHALLENGE STUDY
DBP FORMATION EXPERIMENTAL RESULTS

Lake Audubon
Raw Water TOC = 5.3 mg/L
Temp 20°C

Free Chlorine Contact Time (min)	Chlorine Dose (mg/L)	Chlorine Stock (mL)	Ammonia Dose (mg/L)	Ammonia Stock (mL)	Total Chlorine Residual		pH at 16 hours	DBP Formation (16.5 hour)	
					0 hour (mg/L)	16.5 hour (mg/L)		TTHM (µg/L)	HAA5 (µg/L)
1	4.70	3.8	1.00	1.00	4.20	4.00	8.05	12.9	14
5	5.00	4.1	1.00	1.00	4.40	4.05	8.16	23.7	16
10	5.10	4.2	1.00	1.00	4.30	3.90	8.24	32.3	17
30	5.50	5.0*	0.96	0.87†	4.40	3.73	8.13	42.4	22
60	5.60	4.6	1.00	1.00	4.20	4.00	8.26	44.5	24

Lake Sakakawea
Raw Water TOC = 3.6 mg/L
Temp 20°C

Free Chlorine Contact Time (min)	Chlorine Dose (mg/L)	Chlorine Stock (mL)	Ammonia Dose (mg/L)	Ammonia Stock (mL)	Total Chlorine Residual		pH at 16 hours	DBP Formation (16.5 hour)	
					0 hour (mg/L)	16.5 hour (mg/L)		TTHM (µg/L)	HAA5 (µg/L)
1	4.60	3.8	1.00	1.00	4.15	4.05	8.13	14.2	17
5	4.80	3.9	1.00	1.00	4.20	3.85	8.14	24.8	20
10	5.10	4.2	1.05	1.05	4.35	4.05	8.14	31.4	23
30	5.40	4.9*	1.01	0.91†	4.45	4.05	8.24	35.8	17
60	5.50	4.5	1.05	1.05	4.30	4.00	8.15	40.6	30

Cl2 stock [ ]: 1225 mg/L
Ammonia stock [ ]: 1000 mg/L
*Cl2 stock [ ]:1100 mg/L
†Ammonia stock addition based on a 900 mL sample volume

PHASE III SUMMER - AUGUST 21, 1995
NAWS CHLORAMINE CHALLENGE STUDY
DBP FORMATION EXPERIMENTAL RESULTS

Lake Audubon
Raw Water TOC = 5.8 mg/L
Temp 20°C

Free Chlorine Contact Time (min)	Chlorine Dose (mg/L)	Chlorine Stock (mL)	Ammonia Dose (mg/L)	Ammonia Stock (mL)	Total Chlorine Residual						DBP Formation (16.5 hour)	
					0 hour (mg/L)	Free, Calc'd based on 8/29 demand Used for NH3 add'n	free chlorine to ammonia ratio	16.5 hour (mg/L)	16.5 hr demand	pH at 16 hours	TTHM (µg/L)	HAA5 (µg/L)
1	4.50	3.8	0.97	0.97	4.05	3.86	4.18	3.85	0.20	8.3	13.5	14
5	5.00	4.2	0.95	0.95	4.15	3.79	4.37	3.90	0.25	8.3	26.5	16
10	5.10	4.3	0.91	0.91	4.05	3.66	4.45	3.70	0.35	8.3	34.2	18
30	5.50	4.60	0.95	0.95	3.95	3.80	4.16	3.65	0.30	8.3	42.6	22
60	5.80	4.8	0.97	0.97	4.00	3.87	4.12	3.70	0.30	8.3	53.1	27

Lake Sakakawea
Raw Water TOC = 4.0 mg/L
Temp 20°C

Free Chlorine Contact Time (min)	Chlorine Dose (mg/L)	Chlorine Stock (mL)	Ammonia Dose (mg/L)	Ammonia Stock (mL)	Total Chlorine Residual						DBP Formation (16.5 hour)	
					0 hour (mg/L)	Free, Calc'd based on 8/29 demand Used for NH3 add'n	free chlorine to ammonia ratio	16.5 hour (mg/L)	16.5 hr demand	pH at 16 hours	TTHM (µg/L)	HAA5 (µg/L)
1	4.50	3.8	0.98	0.98	4.15	3.90	4.23	3.95	0.20	8.2	13.9	15
5	4.60	3.9	0.96	0.96	4.15	3.83	4.32	3.90	0.25	8.2	22.8	19
10	4.80	4.0	0.99	0.99	4.15	3.96	4.19	3.95	0.20	8.2	30.8	23
30	4.90	4.1	0.98	0.98	3.95	3.92	4.03	3.80	0.15	8.2	36.0	27
60	5.20	4.3	1.0	1.0	4.15	3.94	4.19	3.90	0.25	8.2	46.1	31

Cl2 stock [ ]: 1200 mg/L
Ammonia stock [ ]: 1000 mg/L

Appendix B

HOUSTON ENGINEERING
AMERICAN ENGINEERING
MONTGOMERY WATSON

APPENDIX B

PROTOCOL FOR MICROBIAL INACTIVATION STUDIES

EXPERIMENTAL PROTOCOL

The experimental protocol for microbial inactivation using chloramine and ozone is presented in the following sections. The chloramine protocol will include both *Giardia* and MS2 Bacteriophage inactivation experiments. The ozone protocol is developed for *Giardia* inactivation, only. The equipment to be used in the studies is described in the following.

Experimental Apparatus

The experimental apparatus employed during the chloramine disinfection experiments will consist of two glass reactors, one serving as a disinfection reactor and one as a control reactor, a temperature controlling circulating water bath, and magnetic stirring devices to maintain completely mixed conditions within the reactors.

Disinfection Reactors. Three-liter glass reaction vessels will be used to conduct the chloramine disinfection experiments. They will be equipped with two side arms that will be used for dosing the reactor and withdrawing samples. **Figure 1** shows a schematic of the disinfection reactors.

The large screw-capped top will be used for cleaning and filling the reactor and spiking the reactor with Giardia cysts and virus. The second, smaller screw-capped opening will be used for adding disinfectant to the reactor.

A special adapter will be fitted to the second sidearm which consists of a glass tube running into the reactor connected to a glass stopcock outside the reactor; this assembly will be used to control the flow of water from the reactor when withdrawing samples. The reactors will be maintained under a positive pressure of 1 to 2 pounds-per-square-inch (psi) during disinfection experiments; this will provide the driving force for sample withdrawal, as well as reduce the volatilization of the disinfectant. Air from a compressed air cylinder will be introduced through a special fitting attached to the side arm that contains the sample withdrawal tube. When the sample stopcock is opened, the reactor contents will flow from the reactor.

The reactor will be equipped with indentations that provide baffling and eliminate vortexing of the reactor contents during mixing. Mixing will be provided by a Teflon™ coated magnetic stir bar driven by a large magnetic stirring device, on top of which the reactor will be placed.

Temperature Control. The chloramine and ozone disinfection experiments will be conducted at 4.0°C ± 0.5°C to represent worst case conditions for biological inactivation.

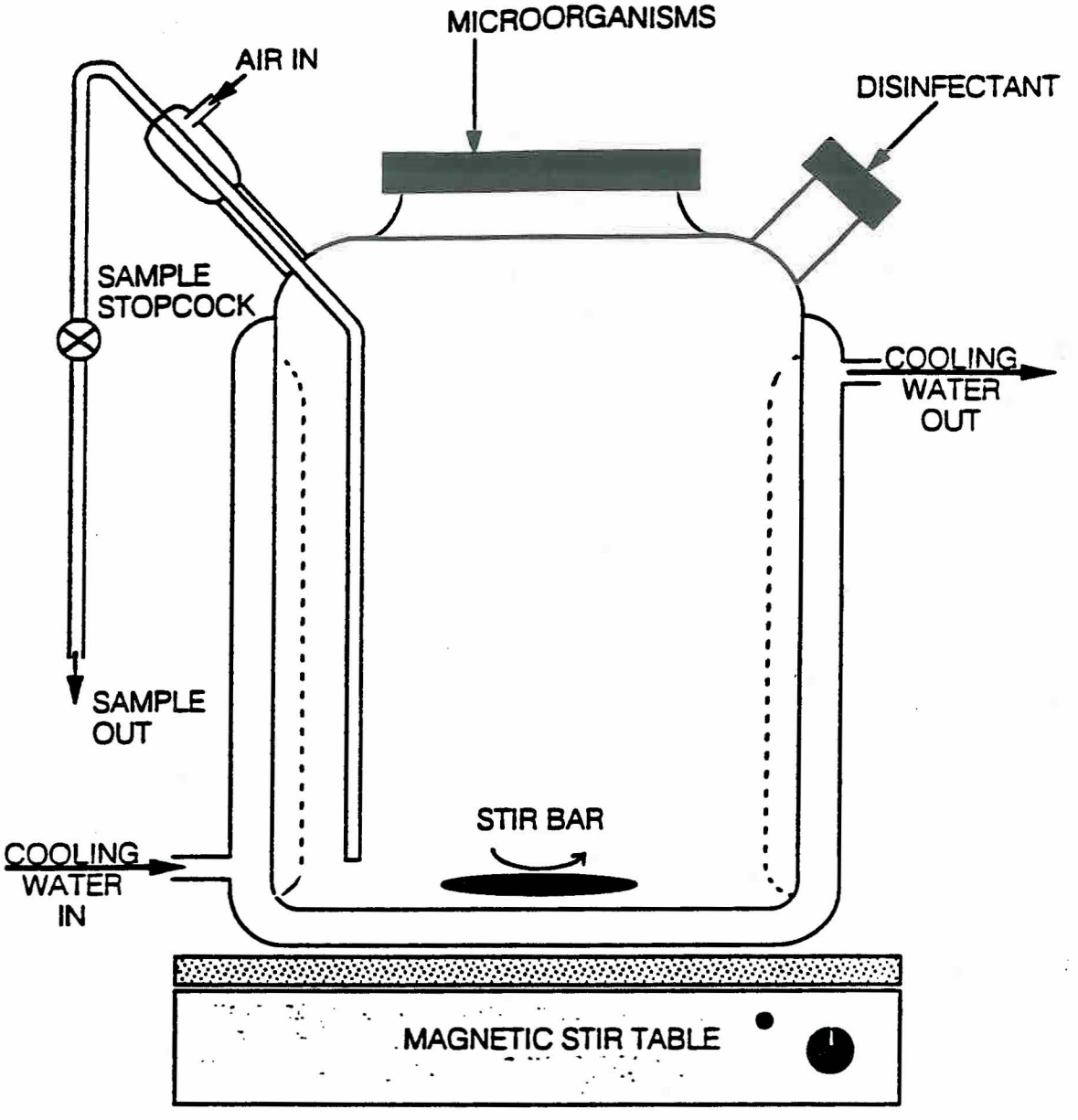


Figure 1. Chloramine disinfection reactor

Temperature will be controlled by pumping cooling water through the water jackets of the disinfection reactors. Cooling water will be provided from a temperature controlled circulating water bath.

CHLORAMINE DISINFECTION EXPERIMENTS

Chloramine disinfection experiments will be conducted in temperature-controlled batch reactors. The time of microorganism exposure to the disinfectants will be controlled by varying the time of sample collection.

Giardia muris Inactivation Experiments

Giardia inactivation experiments will be conduced by Dr. Charles Haas of Drexel University. The experimental method is described in a publication by Haas et al, 1994 (included at the end of this Appendix). Two reactors will be utilized for each Giardia inactivation experiment, one for disinfection and the other for a control reactor. Two thousand mL of raw water will be added to each reactor and the temperature will be allowed to equilibrate at 4.0°C. A magnetic stirring device, operated at a slow rotational speed, will be used to provide completely mixed conditions within each reactor. Once the temperature of both reactors reach 4.0 °C, *Giardia* cysts will be added.

Cyst Spiking. A concentrated preparation of *Giardia muris* cysts will be added to each reactor. The targeted final cyst concentration within each reactor will be 10^3 to 10^4 cysts/mL. At this point, an initial sample will be taken from each reactor and analyzed for pH, total cyst concentration and cyst viability.

Disinfectant Dosing. One chlorine dose will be utilized throughout the chloramine disinfection experiments. The chlorine to ammonia ratio will be 4:1. After initial samples are withdrawn from the disinfection and control reactors, a timer will be set to zero and the chlorine working solution will added to the disinfection reactor. Timing of the experiment will begin at the instant the chlorine is added. After a specified amount of free chlorine contact time (as determined in Phase I), an ammonia solution will be added to the disinfection reactor, and the reactor will be sealed to allow the internal pressure to build to 1 to 2 psi. A volume of de-ionized water, equivalent to that used in disinfection experiments (generally 50 to 75 mL), will be added to the control reactor.

Sample Collection. An initial sample will be taken from the disinfection reactor for chlorine residual analysis 15 to 30 minutes after the start of the experiment to verify that the desired chlorine dose has been achieved. Sample times used for calculating Ct products will correspond to the time at which samples are withdrawn from the reactor for cyst counting and viability. Ct is the product of the concentration of the residual disinfectant and the contact time. Sample times will be designed to target 1, 2, and 3 logs of *Giardia* inactivation. As more disinfection runs are completed, sample times will be adjusted to target more closely the desired levels of inactivation.

***Giardia* Control Experiments.** The objectives of conducting parallel *Giardia* control experiments will be two-fold: first, to determine whether *Giardia* cysts adhere to the surfaces of the reactor; and second, to determine the viability of *Giardia* cysts over the time course of the experiment in the absence of any disinfectant.

A control experiment will be conducted for each disinfection experiment. The control reactor will be run in an identical manner as the disinfection reactor except that no disinfectant will be added. Samples will be collected from the control reactor at the same times as the disinfection reactor and subjected to the same analyses except for residual chlorine concentration.

MS2 Bacteriophage Inactivation Experiments

Bacteriophage inactivation experiments will be conducted in a manner similar to the *Giardia* inactivation experiments according to the method by Adams and the USEPA (Adams, M.H., Bacteriophages, Interscience publication, NY, 1959), with some modifications. The method is outlined in detail in an article by Jacangelo et al which is included at the end of this Appendix. Raw water will be added to each of two reactors, the temperature will be allowed to equilibrate to 4°C, and the bacteriophage will introduced into the reactors. One of the reactors served as the disinfection reactor, while the second will serve as a control.

MS2 Bacteriophage Spiking. MS2 bacteriophage will be added to the disinfection and control reactors containing 2,000 mL of raw water equilibrated at 4°C, in a manner similar to the *Giardia* inactivation experiments. The bacteriophage spike will be prepared immediately prior to each inactivation experiment by adding 0.5 mL of a concentrated phage stock solution to 500 mL of sterile de-ionized water to provide an approximate density of 10^9 plaque forming units (PFU)/mL. The disinfection and control reactors will each be spiked with 2 mL of this working stock, resulting in a final bacteriophage density of approximately 10^6 PFU/mL within the reactors.

Sample Collection, Timing, and Analysis. The methods of sample collection, timing and analysis employed during the bacteriophage inactivation experiments will be similar to those used for the *Giardia* inactivation experiments. However, due to the rapid rate of bacteriophage inactivation, chlorine, temperature, and pH measurements will be made when sample time intervals permit.

Bacteriophage Control Experiments. Bacteriophage control experiments will be conducted in a manner similar to those conducted during the Giardia inactivation experiments. The purpose of the control experiments will be to evaluate the viability of the virus over the time course of the experiments.

OZONE DISINFECTION EXPERIMENTS

Ozone disinfection experiments will be conducted in temperature-controlled batch reactors. The time of microorganism exposure to the disinfectants will be controlled by

varying the time of sample collection. Ozone inactivation studies will be conducted at various ozone doses and at various disinfectant contact times.

***Giardia muris* Inactivation Experiments.** *Giardia* inactivation experiments using ozone will be conduced by Dr. Charles Haas of Drexel University. The experimental method is described in the paper by Haas et al., 1994. Three reactors will be utilized for each *Giardia* inactivation experiment, as mentioned previously. The first reactor (survival measurement) will be used to determine the inactivation caused by the disinfectant. The second reactor (residual measurement) will be used to determine the loss of disinfectant (ozone decay) over the length of the experiment. The third reactor (control) will be used to check the concentration of *Giardia* in the absence of disinfectant. Two thousand mL of raw water will be added to each reactor and the temperature will be allowed to equilibrate at 4.0°C. A magnetic stirring device, operated at a slow rotational speed, will be used to provide completely mixed conditions within each reactor. Once the temperature of both reactors reach 4.0 °C, *Giardia* cysts will be added.

Cyst Spiking. A concentrated preparation of *Giardia* muris cysts will be prepared and mixed to a homogeneous solution. The homogeneous solution will then be divided into three volumes (1 L each) and added to each reactor. The targeted final cyst concentration within each reactor will be 10^3 to 10^4 cysts/mL. At this point, an initial sample will be taken from each reactor and analyzed for pH, total cyst concentration and cyst viability.

Disinfectant Dosing. After the initial (disinfectant-free) samples are withdrawn from the disinfection and control reactors, a timer will be set to zero and the ozone working solution will added to the disinfection and the residual measurement reactors. Stock ozone solution was prepared in the manner described in the enclosed publication (Hass et.al., 1994). The control reactor will receive the same volume of disinfectant-demand-free buffer solution. The volume of disinfectant dosing solution will be as close as possible to 5% of the *Giardia* suspension volume. Timing of the experiment will begin at the instant the ozone is added. Ozone inactivation studies will be conducted at various ozone doses and at various disinfectant contact times. The range of ozone doses used will be based upon results from ozone demand and decay studies conducted in earlier phases of the research. After a pre-determined contact times, samples will be withdrawn from all three reactors, and the disinfectant residuals will be immediately quenched with sterile sodium thiosulfate (0.1 mL of a 10 percent solution). This sampling process will be repeated at several different sampling time points in order to determine the rate of *Giardia* inactivation using ozone as disinfectant.

Sample Collection. An initial sample will be taken from the ozone disinfection reactor as soon as possible (30 sec) after the start of the experiment to verify that the desired ozone dose has been achieved and to detect any initial inactivation by ozone. Sample times and ozone concentrations used for calculating CT products will correspond to the time at which samples are withdrawn from the reactor for cyst counting and viability; however, owing to the rapid ozone decay predicted in the batch reactor vessels, it is likely that calculation of CT values for ozone disinfection will not be possible. CT is the product of the concentration of the residual disinfectant and the contact time. Ozone

dosages and sample times will be designed to target 3 logs of *Giardia* inactivation. As more disinfection runs are completed, sample times will be adjusted to target more closely the desired levels of inactivation.

Residual Ozone Experiments. The objectives of conducting parallel ozone residual experiments will be to determine the rate of ozone decay within the disinfection reactor. An ozone residual experiment will be conducted in parallel with each disinfection experiment. The ozone residual reactor will be run in an identical manner as the disinfection reactor except that analysis will be performed on the residual disinfectant contained in the sample solution. Samples will be collected from the residual measurement reactor at the same times as the disinfection reactor and subjected to analysis for residual ozone.

***Giardia* Control Experiments.** The objectives of conducting parallel *Giardia* control experiments will be two-fold: first, to determine whether *Giardia* cysts adhere to the surfaces of the reactor; and second, to determine the viability of *Giardia* cysts over the time course of the experiment in the absence of any disinfectant.

A control experiment will be conducted for each disinfection experiment. The control reactor will be run in an identical manner as the disinfection reactor except that no disinfectant will be added. Samples will be collected from the control reactor at the same times as the disinfection reactor and subjected to the same analyses except for residual ozone concentration.

PROJECT QUALITY ASSURANCE

This section prices an overview of the quality assurance and quality control (QA/QC) program for the proposed study. The objectives of the project's QA/QC program are to assure that verification, validation, precision, accuracy, completeness, representativeness, and comparability of the data are known and documented.

Quality assurance (QA) for the analytical chemistry aspects of the project will be the responsibility of Montgomery Watson and Drexel University. Quality assurance (QA) is maintained through the use of a program to verify that methods used for analytical measurements operate within acceptable statistical limits. Through the use of this QA program, measurement errors are reduced to agreed upon (tolerable) limits.

The QA program consists of the following three major elements: (1) quality control (QC), which includes sampling procedures, sample chain of custody, measurement of data, calibration procedures and frequency, data reduction, validation, and reporting; (2) quality assessment, which includes performance and system audits, and corrective action; and (3) reporting. Overall, the objectives of the project's QA program will be to assure that the verification, validation, precision, accuracy, completeness, representativeness, and comparability of the data are know and documented. Presented below is an overview of the project's proposed QA program.

Quality Control (QC)

Project QC is maintained to control analytical measurement errors and includes the following elements: sampling procedures, sample chain of custody, development and strict adherence to principles of good laboratory practice (using qualified staff and reliable, well maintained equipment), verified and specified analytical methods for measurements (including sensitivity, selectivity, precision, accuracy), and consistent use of standard instrument operation procedures (including calibration and standardization procedures, minimum reporting limits (MRLs) and/or method detection limits (MDLs), and frequency). Project QC, when properly executed, results in a measurement system operating in a state of statistical control whereby errors are reduced to acceptable levels.

Quality Assessment

Through the use of control charts, quality assessment is used to verify that analytical methods are operating within acceptable limits, i.e., within statistical control. For control charts, data generated from the measurement of homogeneous and stable blank (control) samples or calibration standards in a planned repetitive process are plotted in a manner to indicate whether the measurement system is in a state of statistical control. At least one control measurement is made such that all aspects of an analytical method are simulated, including sampling, matrix, and measurement. Either the result of a single sample measurement, the difference between duplicate measurements, or both may be plotted sequentially. The first mode is an indicator of both precision and bias (systematic error), while the second monitors precision only.

Inorganic and Organic Compounds Analyses. The methodology for all organic and inorganic analyses will follow Standards Methods according to the following table. Maintenance logs and calibration logs are kept for each analysis.

Reporting

Each project status report will contain the analytical methods for those phases discussed in the report. The detailed project QA program will be published in the final project report.

Data Handling and Presentation

The data management system involves the use of computer spreadsheets as well as a protocol for the entry, verification, and presentation of the data in appropriate tabular and graphical forms. Field data (lab evaluations and field sampling) will be recorded by hand on specially prepared data sheets for all operational and water quality measurements and/or observations. These data will be entered into the appropriate spreadsheet. Following entry, the spreadsheet will be printed out and the hard copy checked against the handwritten data sheet. Any corrections will be noted on the hard copy; corrected on the screen and saved followed by a print out of the updated spreadsheet. Each step of the verification process will be initialed by the person entering the data. Data will be

exported directly from the spreadsheets for use in commercial graphics, statistics and water quality models.

ANALYTICAL METHODS TO BE EMPLOYED

Parameter	Analysis Method
Ammonia	Standard Method 417E
Alkalinity	Standard Method 403
Chlorine Demand	Standard Method 409A
Chlorine Residual	Standard Method 408E
Dissolved Oxygen	Standard Method 421F
Giardia	Haas, et. al.*
Hardness	Standard Method 314B
MS2 Bacteriophage	Jacangelo, et. al.**
Ozone Residual	Standard Method 422
pH	Standard Method 423
Temperature	Standard Method 212
Total Organic Carbon	Standard Method 505

Note: Standard Methods from *Standard Methods for the Examination of Water and Wastewater*, 16th ed., APHA, AWWA, WPCF, 1985.
*Hass, et. al., Journal AWWA, February 1994, pp. 115-120.
**Jacangelo, et. al., Journal AWWA, September, 1991, pp. 97-106.

RAW WATER SAMPLING PROTOCOL

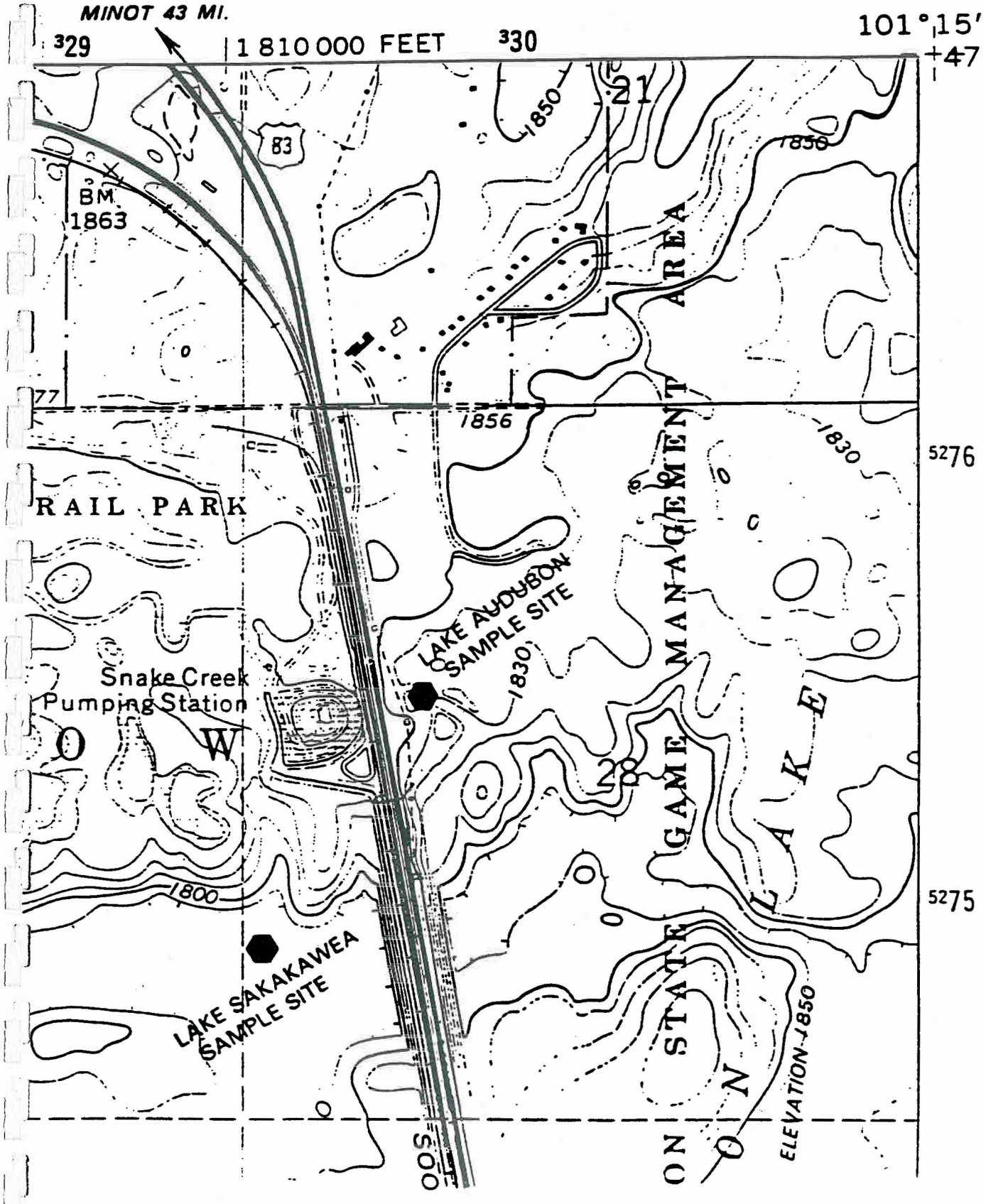
Water samples will be taken from Lakes Sakakawea and Audubon at the locations shown on the attached map. Both of the sample sites are in Section 28, T148N-R83W. The weather conditions at the time of sampling will be noted on the shipping labels of the five-gallon (18.9 liter) samples which will be taken at each site and packaged for shipping. A Kemmer sampler will be used to take the samples which will be packed in blue ice when sampling is completed. The sample from Lake Audubon will be taken at elevation 1830 msl or from a water depth of 17 feet. The Lake Sakakawea sample will be taken at elevation 1775 msl or from a water depth of 65 feet. The parameters which will be measured at the time of sampling are:

1. Water temperature of sample
2. pH
3. Dissolved oxygen (DO)
4. Turbidity

The samples will be shipped to the testing laboratory the same day via next day Federal Express.

RIVERDALE NORTH QUADRANGLE
NORTH DAKOTA
7.5 MINUTE SERIES (TOPOGRAPHIC)
SE/4 GARRISON 15' QUADRANGLE

5779 II NW
(COLEHARBOR



A volumetric method for assessing Giardia inactivation

Based on these tests, the results of the proposed volumetric method—which has the advantage of reducing labor requirements—were indistinguishable from those of the standard method.

Charles N. Haas, Joel C. Hornberger, Uma Anmangandla, Mark Heath, and Joseph G. Jacangelo

Because the standard method of determining *Giardia* inactivation in disinfection tests—which involves counting dead and living organisms—can be extremely tedious, an alternative method, whereby the concentration of only viable trophozoites is estimated, is being sought. This article describes the development of such a volumetric method and its verification by comparing the results obtained with the standard and volumetric methods. This work demonstrates that the two methods yield equivalent results—at least for the disinfectants and waters examined. Whether this equivalence operates for larger-scale concentration techniques and for different waters, disinfectants, and organisms remains open to further research.

Because *Giardia* cysts are among the most resistant organisms to disinfection during water treatment, the Surface Water Treatment Rule (SWTR) specifies $C \times T$ (concentration times time) values for inactivation of *Giardia* cysts. However, utilities have the option of demonstrating, by experimental studies, that their system achieves a certain level of inactivation of *Giardia* cysts, rather than relying on the SWTR default tables. This option may be particularly attractive to utilities practicing chloramination or investigating novel processes such as the use of advanced oxidation.

The most widely used method for determining cyst viability in disinfection experiments is in vitro excystation,[1–9] during which the viable cysts

excyst into trophozoites (two per cyst). Some partially excysted forms are also generally noted, as well as complete or unexcysted forms; the latter are considered nonviable.

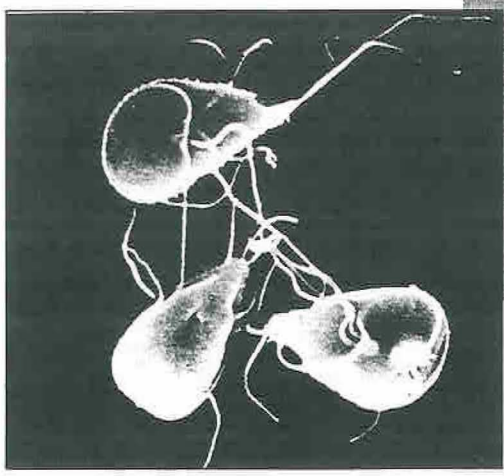
Although slightly different conventions have emerged for scoring viability in *Giardia muris* and *Giardia lamblia* excystation experiments, the general procedure is to consider the disinfection survival ratio as being the ratio of the fraction of excysted organisms in an experimental system to the fraction excysted in an unexposed control population. In other words, if *TR, PET, EC,* and *CC* are trophozoites, partially excysted trophozoites, empty cysts (totally excysted shells), and complete (unexcysted) cysts, and the subscripts *T* and 0 are measured at a given exposure time and in an unexposed population, respectively, then the survival ratio (N/N_0) is defined by Eqs 1 and 2.

Although some authors have indicated that it is easier to count empty cysts than trophozoites of *G. muris* and so have preferred Eq 2, the two scoring procedures are regarded as equivalent and imply a relatively constant excystation number of two per cyst.

$$\frac{N}{N_0} = \frac{\left[\dfrac{\left(\dfrac{TR}{2}\right)_T + PET_T}{\left(\dfrac{TR}{2}\right)_T + PET_T + FCC_T}\right]}{\left[\dfrac{\left(\dfrac{TR}{2}\right)_0 + PET_0}{\left(\dfrac{TR}{2}\right)_0 + PET_0 + FCC_0}\right]} \qquad (1)$$

$$= \frac{\left[\dfrac{EC_T + PET_T}{EC_T + PET_T + CC_T}\right]}{\left[\dfrac{EC_0 + PET_0}{EC_0 + PET_0 + CC_0}\right]} \qquad (2)$$

$$\frac{N}{N_0} = \frac{\left(\dfrac{TR_T}{V_T}\right)}{\left(\dfrac{TR_0}{V_0}\right)} \qquad (3)$$



A new way to determine Giardia inactivation is being sought because the standard method is tedious and time-consuming.

A similar finding has been reported with respect to counting viable *Cryptosporidium* by excystation.[10,11]

This standard method has a serious disadvantage. In an experiment in which 99 percent inactivation is obtained, the numerator would be 1 percent of the denominator. To reliably determine this level of inactivation, therefore, in excess of 100 total cysts would need to be scored to obtain at least one viable cyst equivalent. Clearly, the determination of higher levels of inactivation, e.g., 99.9 percent, by this method is extremely tedious.

In essence, using this method, it is necessary to count both the dead and the living organisms. This is in extreme contrast to procedures used in the disinfection testing of other organisms, in which only the density (concentration) of viable organisms is determined as a function of time. The objective of this work was to ascertain whether such an alternative procedure could be used in disinfection testing with *Giardia muris*, thus facilitating studies requiring an assessment of high degrees of inactivation.

Materials and methods

Glassware preparation. All glassware was cleaned by using laboratory glassware cleaner* and rinsing with distilled water. Sample bottles were sterilized by autoclaving. Chlorine-demand-free glassware was prepared by exposing glassware to disinfectant-demand-free buffer containing at least 10 mg/L free chlorine for at least 3 h, rinsing with chlorine-demand-free buffer, and then drying at 110°C overnight. Ozone-demand-free glassware was prepared by exposing glassware to at least 2 mg/L ozone in disinfectant-demand-free buffer for at least 1 h and then drying at 110°C overnight.

Stock oxidant solutions. Stock chlorine solution was prepared to a concentration ≥150 ± 10 mg/L by bubbling reagent-grade chlorine gas into a weak alkaline solution prepared by adding sufficient sodium hydroxide to distilled water to bring it to a final pH of at least 8.0. The stock chlorine solution was stored in dark refrigerated conditions until the concentration of chlorine was determined to be <150 ± 10 mg/L.

Preformed monochloramine was prepared daily as needed by mixing equal volumes of chlorine and

*Sigma Clean, St. Louis, Mo.

ammonium chloride solutions at a 3:1 (Cl_2-to-N) weight ratio, yielding a 150 ± 10-mg/L (as chlorine) solution. Each solution was prepared in a 0.01 *M* pH 8.0 phosphate buffer (Na_2HPO_4 and KH_2PO_4). This solution yielded approximately a 0.6:1 molar ratio (Cl_2 to N).

Stock ozone solution was prepared on the day required using an ozone generator* fed by 2.6-grade oxygen at a pressure of 8 psig, a flow of 0.4 L/min, and a voltage of 115 V. Oxygen carrier gas containing approximately 5 percent ozone was bubbled for a minimum of 20 min at 20°C through 400 mL of buffered reagent-grade water in a 500-mL gas absorption flask.[12] Effluent gas was neutralized by passage through a solution containing 132 g/L sodium thiosulfate and 3 g/L potassium iodide. The stock solution was maintained by constant bubbling of ozone through it.

TABLE 1 **Water quality characteristics of Bull Run and Willamette River waters**

Characteristic	Bull Run	Willamette River
Total organic carbon (TOC)—*mg/L*	1.0–1.7	0.8–7.1
True color—*unit*	<5	NA*
Ammonia—*mg/L as N*	<0.02	NA
pH	7.0–7.2	5.0–8.5
Total hardness—*mg/L as $CaCO_3$*	7–15	NA
Total alkalinity—*mg/L as $CaCO_3$*	5–11	14–36
Turbidity—*ntu*	0.26–1.48	0.7–50
Temperature (annual range)—*°C*	4–16	4–24

*NA—not available

TABLE 2 **Summary of experimental runs**

Water	Disinfectant	Dose—*mg/L*	pH	Experiments *number*
Buffered demand-free	Free chlorine	0.5	6.9	1
		1	6.9	2
		2	6.9	1
	Monochloramine	0.5	6.9	1
		1	6.9	2
		2	6.9	1
	Ozone	0.25	6.9	2
		0.75	6.9	1
Bull Run	Free chlorine	1	6.52–6.54	2
		2	6.93	1
	Monochloramine	1	6.37–6.55	2
		2	6.70	1
	Ozone	0.25	6.53	1
		0.4	6.53–6.93	2
Willamette River	Free chlorine	2	7.30–7.34	2
		3	7.17	1
	Monochloramine	1	7.41	1
		2	7.15–7.54	2
	Ozone	0.5	7.37–7.54	2
		0.75	7.44	1

Waters. Three types of water were used for the inactivation experiments: (1) buffered disinfectant-demand-free, (2) Bull Run, and (3) Willamette River. Pure water† was determined to have no disinfectant demand and was used to make buffered disinfectant-demand-free water by adding Na_2HPO_4 and KH_2PO_4 to produce a 0.01 molar buffer at pH 6.9. Bull Run and Willamette River waters are natural waters obtained from Portland, Ore. (Table 1). These waters were shipped by overnight mail and stored for a period of no longer than three weeks under refrigerated conditions (3–5°C).

Control solutions. The three types of control solutions used were source water in the absence of disinfectant, disinfectant-demand-free buffer at the desired pH and ionic strength in the absence of disinfectant, and source or disinfectant-demand-free buffer in the absence of microorganisms. Buffered demand-free water was prepared using a 0.01 molar phosphate buffer (Na_2HPO_4 and KH_2PO_4) in disinfectant-demand-free water.

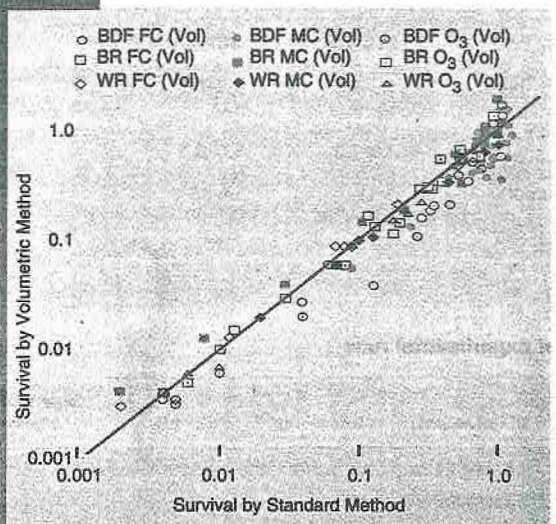
Cyst preparation. All *G. muris* cysts were obtained from Ernest Meyer at the Oregon Health Sciences University, Portland, Ore. They had been cultured in specific-pathogen-free mice. In order to produce highly purified cysts, the feces were collected from the host, and the cysts were isolated from the fecal material by a sucrose gradient technique. The cyst suspension was washed two to four times by centrifugation using disinfectant-demand-free buffer. The final cyst preparation was then resuspended to the desired concentration (determined by hemocytometer‡ count) in disinfectant-demand-free water and shipped by overnight mail in a cooled container to Drexel University, Philadelphia, Pa. These cysts were used within 14 days.

The cysts were washed three times in disinfectant-demand-free buffer immediately prior to experimental use and then resuspended in 10 mL of disinfectant-demand-free buffer at the desired cyst concentration, determined by hemocytometer count, for inoculation into reactor vessels.

Equipment setup. Three 2-L heat-resistant glass beakers were used as reaction vessels, which were covered with aluminum foil to minimize photodecomposition and volatilization of disinfectant. Each reactor vessel was mixed by a submersible magnetic stirrer§ and a large star-shaped stir bar (58 × 15 mm).

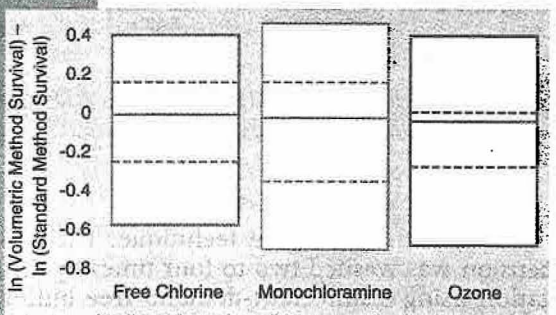
*T-408, Polymetrics, San Jose, Calif.
†Milli-Q, Millipore Corp., Bedford, Mass.
‡No. 3556, Hausser Scientific
§Model 700, Troemner Inc., Philadelphia, Pa.

FIGURE 1 **Comparison of survival ratios measured by standard and volumetric methods**



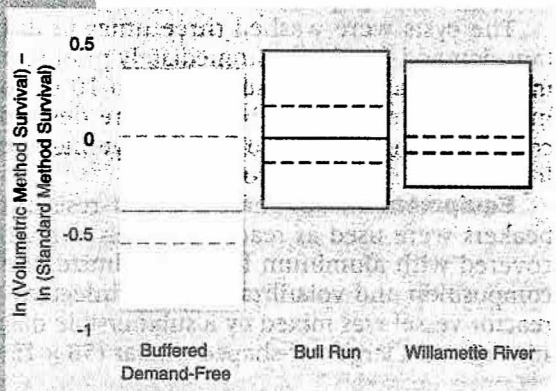
Line of perfect agreement is shown for comparison. BDF—buffered demand-free water; BR—Bull Run water; WR—Willamette River water; FC—free chlorine; MC—monochloramine; O_3—ozone

FIGURE 2 **Distribution of differences in methods segregated by disinfectant**



Medians shown by solid line, quartiles by dashes; boxes enclose 90 percent of observations.

FIGURE 3 **Distribution of differences in methods segregated by water**



Medians shown by solid line, quartiles by dashed; boxes enclose 90 percent of observations.

Samples were removed from the reactor vessels by means of a 60-mL presterilized high-density polyethylene syringe,* which allowed samples to be withdrawn at a minimum time difference of 5 s. The temperature of the reaction vessels was held constant at 18 ± 1°C in a circulating cooled water bath.

Experiments. Three reactor vessels were run in parallel. The first reactor (control) was used to check the presence of *Giardia* decay in the absence of disinfectant. The second reactor (residual measurement) was used to determine the loss of disinfectant during the experiment. The third reactor (survival measurement) was used to determine the inactivation caused by the disinfectant. To initiate the experiment, a suspension of *Giardia* at the desired density ($4 \times 10^4 \pm 5 \times 10^3$/mL) in disinfectant-demand-free buffer was added to a 3-L beaker with source water or demand-free buffer and was mixed to a homogeneous concentration. The homogeneous solution was then divided into three equal volumes of 1 L for each of the reactor vessels.

At zero time (start of the experiment), a solution of disinfectant was added to reactors 2 and 3 in a volume as close as possible to 5 percent of the *Giardia* suspension volume. The first reactor received the same volume of a disinfectant-demand-free buffer solution in the absence of disinfectant. At predetermined times, samples were withdrawn from the control and survival reactors, and disinfectant residuals were immediately quenched with excess sterile sodium thiosulfate (0.1 mL of a 10 percent solution).

Table 2 summarizes the experimental conditions used in this testing. A total of 34 tests was conducted, with some duplicates, as noted. Each run consisted of several exposure times. Each line in the table represents an experiment performed on a given day. In general, one batch of cysts was not used for more than two or three experiments, so that some level of batch-to-batch variability existed as well.

Excystation. A 10-mL sample was concentrated to 1 mL by centrifugation using a clinical centrifuge† at 500 × G (setting 5) for 5 min in a conical-bottom centrifuge tube.[3] In vitro excystations were accomplished by a modified procedure.[13] Ten millilitres of reducing solution (Hank's balanced salt solution, supplemented with 32 m*M* glutathione and 57 m*M* L-cysteine HCl) and 10 mL of 0.1 sodium bicarbonate were added to the cyst suspension, yielding a final pH of 4.7. The suspension was vortexed and incubated in a heated water bath at 37°C for 30 min. The cysts were then removed and centrifuged at 650 × G (setting 6) for 2 min, and washed once in 20 mL of excystation medium by centrifugation at 650 × G (setting 6) for 2 min. The cysts were then suspended in 1 mL of 0.5 percent of prewarmed proteose peptone in PBS [0.8 g NaCl, 0.2 g KH_2PO_4, 2.9 g Na_2HPO_4 (12 H_2O), 0.2 g KCl, 1,000 mL final volume] at pH 7.2 and incubated at 37°C for 30 min in a heated water bath. The

*2-oz. Catheter-tip syringe, no. 9664, B&D, Franklin Lakes, N.J.
†Rotor #215, IEC, Needham Heights, Mass.

5 percent (w/v) stock proteose peptone was prepared in distilled water, gently boiled for 10 min to destroy any remaining enzymes, filter sterilized (0.1-µm pore size), and stored under refrigerated conditions (3–5°C). Excystation medium was prepared fresh by adding 10 mL of 5 percent (w/v) stock proteose peptone solution and 10 mL of 10× PBS [80 g NaCl, 2 g KH_2PO_4, 29 g Na_2HPO_4 (12 H_2O), 2 g KCl, 1,000 mL final volume] to 80 mL of distilled water.[13] This process resulted in a concentration factor of 10 (final sample relative to initial water).

Enumeration by standard method. After incubation the number of trophozoites (*TR*), partially excysted cysts (*EC*), and complete cysts (*CC*) were counted under a hemocytometer using phase contrast microscopy (400×). The volume of concentrated sample counted was equal to that required for an observed two-log inactivation (approximately 100–300 complete cysts or equivalent). The data were analyzed using the standard approach (Eq 1), based on trophozoites, partially excysted cysts, and complete cysts. Samples from the control (nondisinfected) reactor were taken at the same exposure time as from the treated reactor to assess survival ratio using the standard method. From 2 to 5 µL of concentrate were generally counted under the hemocytometer.

Enumeration by volumetric method. In the volumetric method, the number of trophozoites (*TR*) in the disinfected sample was counted, and the equivalent volume of sample from which those trophozoites were enumerated (*V*) was determined. The latter must be computed, correcting for volume changes arising during cyst concentration and excystation incubation steps. The survival ratio can be computed from this information (Eq 3). Note that the factor of 2 for trophozoite yield is unnecessary, because it is canceled in the computation.

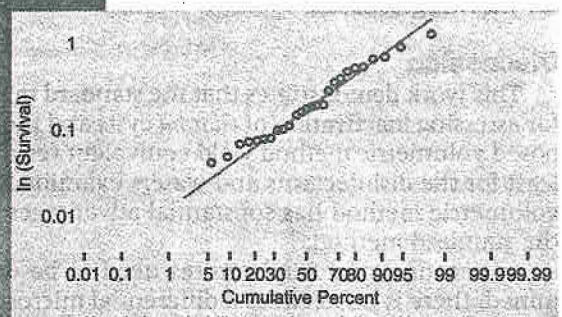
The volumetric method and standard method scorings were performed using the same excystation samples and the same microscopic fields. Note that the volumetric method simply requires a subset of data from the standard method, as well as the equivalent volume of sample used.

This method does not score partially excysted trophozoites. The authors found that the number of partially excysted organisms relative to trophozoites was small; however, further work may be needed to ascertain whether it is desirable to modify Eq 3 in some circumstances to include such organisms.

Results

Figure 1 summarizes the survival ratio obtained by the standard method and by the volumetric method. The diagonal line of perfect agreement is shown. Qualitatively, there is good agreement between the two methods over the entire range of inactivations observed, in all three waters, and with all three disinfectants.

FIGURE 4 **Distribution of standard deviations in lognormal survival (standard method) measured in replicate experiments**



X-axis is probability transformed; line of best fit is shown for comparison.

To explore the results in more detail, it is useful to look at the survival ratio with the volumetric method divided by that with the standard method. This quotient would be expected to equal unity if the two methods were equivalent (or equivalently, the difference in their logarithms would be expected to equal zero). A quotient >1.0 indicates greater survival (poorer disinfection) measured by the volumetric method, and a quotient <1.0 indicates poorer survival (more efficient disinfection).

When the difference in logarithms is tested, no statistically significant difference is found between the log survivals as determined by the two methods (Wilcoxson signed rank test). Figures 2 and 3 show the distribution of differences in the two methods segregated by disinfectant and by water, respectively.

The volumetric method has substantial advantages over the standard method.

The boxes enclose 90 percent of the data, and the dashed lines indicate the quartile boundaries. The magnitude of these differences should be considered in the context of the error between duplicated experiments. Certain experimental conditions were duplicated on different days (Table 2). Figure 4 shows the distribution of replication standard deviations (standard method) among these duplicated experiments—this can be regarded as a pure error term. Note that the median standard deviation is approximately 0.3, with an upper confidence limit of 1.0. An upper confidence limit of 1.0 indicates that replicate experiments provide survival ratios that generally differ by a factor of about 2.7—this is typical in the experience of the authors.

By comparing Figures 2 and 3 with the intrinsic experimental error, it is concluded that the between-method difference (volumetric versus standard) is generally well under that of the pure error term.

Thus, it is concluded that within the intrinsic variability of performing disinfection studies, the volumetric and standard methods produce results that are indistinguishable.

Discussion

This work demonstrates that the standard method for assessing inactivation of *Giardia* cysts and the proposed volumetric method yield equivalent results, at least for the disinfectants and waters examined. The volumetric method has substantial advantages over the standard method:

- because only trophozoites need to be determined, there is no need for a differential microscopic count and the labor is diminished; and
- because it is not necessary to score a large number of dead cysts to determine high levels of inactivation, it becomes more feasible to determine >2 logs inactivation by a direct analysis (perhaps only 20 trophozoites need to be counted to get a fairly precise estimate of inactivation, rather than 1,000+ total cyst equivalents to get to 3 logs inactivation).

This work should be verified in different waters, particularly using centrifugation or filtration to concentrate cysts from larger volumes of water than used in this study. In this work, relatively small volumes of sample were required in order to obtain the necessary cyst counts. Whether the method equivalence operates with larger-scale concentration techniques remains open to further research.

Conclusions

This study has demonstrated the equivalence of a volumetric method to the standard method for scoring disinfection experiments with *Giardia* cysts. There is great potential for labor savings by use of the proposed method. It would be of interest to determine whether this type of volumetric method could produce similar efficiencies in the investigation of disinfection kinetics of other organisms, such as *Naegleria* and *Cryptosporidium*, as well as in systems in which other disinfectants, such as chlorine dioxide or UV light, are employed.

Acknowledgment

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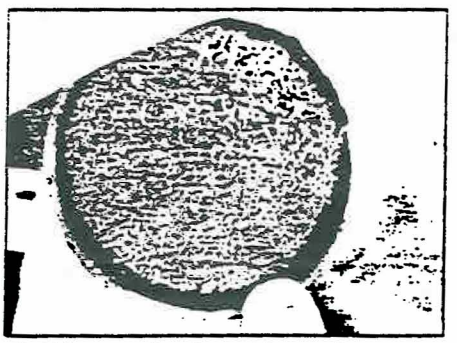
Low-Pressure Membrane Filtration for Removing Giardia and Microbial Indicators

Joseph G. Jacangelo, Jean-Michel Lainé, Keith E. Carns, Edward W. Cummings, and Joël Mallevialle

As part of a project on the use of ultrafiltration (UF) for particle removal, studies were performed to evaluate the use of low-pressure hollow-fiber UF as an alternative for complying with Surface Water Treatment Rule (SWTR) requirements for microbial removal and/or inactivation. Pilot studies were conducted on four different untreated source waters, two from northern California and two from Boise, Idaho. Process efficacy was assessed by conducting MS2 virus, total coliform bacteria, and *Giardia muris* seeding studies, as well as monitoring for naturally occurring bacteria. The study showed that UF was capable of meeting SWTR requirements for alternative filtration technologies without the use of chemical disinfection. Four or more logs of *Giardia* and more than 6.5 logs of virus were removed from each of the untreated source waters. Differences in water quality or changes in operating parameters did not appear to affect removal capabilities of the process. Maintenance of membrane integrity was critical to assuring process efficacy. When module integrity was compromised, as in fiber breakage, both MS2 virus and *G. muris* were detected in the permeate. Changes in membrane integrity were not necessarily reflected by changes in permeate turbidity; however, particle counting was an effective method for detecting a compromised membrane module.

The use of membrane processes in water treatment has traditionally focused on the removal of salts or organic materials. Over the past several years, however, there has been an increasing interest in employing membrane technology for removal of particles and microorganisms from untreated drinking water supplies. The Surface Water Treatment Rule (SWTR) has been the impetus for examining the application of this technology to drinking water treatment in the United States.[1] Under the SWTR, utilities are required to provide 99.9 percent or 3 logs removal/inactivation of *Giardia* and 99.99 percent or 4 logs removal/inactivation of virus. In addition, conventional and direct filtration plants must achieve a filtered water turbidity of ≤0.5 ntu in 95 percent of the samples collected each month with no samples having a turbidity >5 ntu.

Ultrafiltration (UF) is a membrane process that has been used in a variety of industries for liquid–solid separation. It has recently been the focus of increasing research oriented toward application in drinking water treatment. There are currently no major full-scale UF plants in the United States, although several, ranging in capacity from 0.07 to 1.0 mgd (240 to 3,600 m^3/d), are now on line in Europe[2] and Southeast Asia.



Bubbles escaping from this membrane demonstrate the single broken fiber within the module.

The efficacy of UF in removing particles from untreated water supplies has been well documented. Several researchers[3–6] have shown that UF is capable of consistently reducing turbidities to less than 0.1 ntu, regardless of the influent turbidity level. Previous work by the authors[7] showed that UF was effective in removing particles in the 1- to 120-µm size range. Little information is available, however, on the removal of microorganisms from untreated drinking water sources by this membrane process, particularly for those organisms that are targeted by the SWTR. Most of the early investigations and much of the current work on membrane filtration of viruses have focused on isolation techniques.[10–12] Viral detection by UF has been accomplished by various methods that have been shown to be effective for a variety of water qualities.[13–14] Additionally, hollow-fiber membranes, similar to those used in this and other studies, have been employed for virus concentration, in which separation of viruses from water was probably based on virus size, not surface characteristics.[15] As with virus filtration, investigations on membrane filtration of *Giardia* have involved detection and enumeration,[16–18] rather than re-

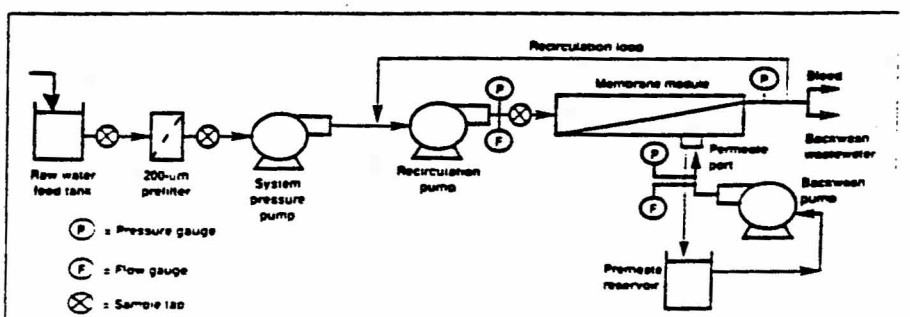
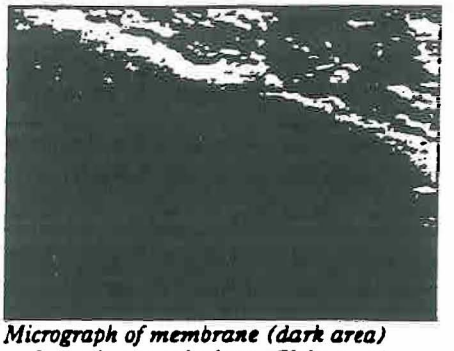


Figure 1. UF pilot-plant schematic

moval from water supplies for disinfection purposes.

Table 1 presents a compilation of data from various sources regarding the removal of bacteria and viruses for disinfection purposes by low-pressure membrane processes employed primarily for liquid–solid separation. The efficacy of UF and microfiltration (MF) is shown for various types of waters: wastewaters are included because little information on drinking water was available. The table shows that the two membrane processes were effective in removal of total coliform bacteria: only one study,[6] which tested a hollow-fiber MF membrane, reported a large percentage of positive-coliform samples (16 percent). The median density of total coliform bacteria in the permeate for all studies, however, was less than the detection limit of the assay employed. For heterotrophic plate count (HPC) bacteria, positive samples were reported in all studies. It was often unclear, however, whether the microorganisms were penetrating the membrane. Detection of bacteria in the permeate may be due to a number of factors, including imperfections in the membrane surface,[19,20] bacterial adhesion to the membrane followed by biofilm formation and sloughing,[21,22] degradation of the membrane by bacterial enzymes or other materials,[23] inferior packaging or installation of membranes modules or elements, and/or introduction of bacteria into the permeate from sources exterior to the membrane module. The removal of viruses by various membrane processes is also shown in Table 1. Several log removals were reported in the studies identified, although complete rejection of viruses was not always observed.



Micrograph of membrane (dark area) and membrane cake layer (light area) from filtration of Boise River collector-water (magnification 2,000 ×).

Study objectives

Low-pressure UF, which employs applied pressures ranging from 10 to 40 psi (0.7 to 2.8 bars), has the potential to provide an alternative method for primary disinfection of untreated water supplies. As shown in Table 1, however, little information is available on the removal of viruses, and none was found on the removal of *Giardia*. To evaluate the efficacy of UF in removing microorganisms, pilot-scale studies, focusing on removal of indicator bacteria, MS2 virus, and *Giardia muris*, were conducted. The specific objective was to determine whether UF was capable of meeting SWTR requirements as an alternative filtration process for removing microorganisms from drinking water. Four raw water sources were used to evaluate microbial removal under conditions of varying water quality.

Experimental methods

Source waters. Four untreated raw water sources were used in this study, two from Boise, Idaho, and two from northern California. The Idaho waters were from the Boise River and from collectors, which draw water from the Boise River through a system of laterals located under the river. The California waters were drawn from the Mokelumne River and the Sacramento–San Joaquin Delta, which serve as the primary raw water supplies for East Bay Municipal Utility District and the Contra Costa Water District, respectively. Testing of these waters allowed for comparison of UF on sources that varied considerably in water quality. Selected parameters of raw water

TABLE 1

Removal of various microorganisms for disinfection purposes by low-pressure membrane filtration processes in recently reported studies

Membrane Process	Nominal Size Exclusion	Source Water	Influent Microbial Density Range	Microorganisms Detected in Permeate: Number of Samples	Percent Positive	Median Density	Reference
HPC bacteria—*cfu/mL*							
MF	0.2 µm	Groundwater	1–370	60	88	7	6
UF	0.01 µm	Surface	1–4,500	17	59	1	9
UF	0.01 µm	Surface	4,400–10,400	NR*	100	10	8
UF	<0.01 µm	Groundwater	1–370	60	78	10	6
Total coliform bacteria—*cfu/100 mL*							
MF	0.1 µm	Surface	32†	NR	NR	<2.2†	39
MF	0.2 µm	Groundwater	<1–600	57	16	<1	6
MF	0.2 µm	Waste	2.6×10^3–5.0×10^6 cfu/mL	19	0	< 1 cfu/mL	40
MF	0.2 µm	Surface	10–480	21	0	<1	30
MF	0.2 µm	Waste	2.8×10^5–2.2×10^6	19	5	<1	31
UF	0.01 µm	Surface	<2.2–490†	3	0	<2.2†	9
UF	0.01 µm	Surface	100–1,750	NR	0	<1	8
UF	<0.01 µm	Groundwater	<1–600	59	2	<1	6
Other bacteria							
MF	0.2 µm	Wastewater‡	4.0×10^2–6.2×10^5/mL	70	0	<1/mL	40
MF	0.2 µm	Wastewater§	1.5×10^3–2.6×10^5/100 mL	9	0	<1/100 mL	31
MF	0.2 µm	Wastewater**	2.7×10^2–6.2×10^3/100 mL	9	0	<1/100 mL	31
Viruses							
MF	0.2 µm	Wastewater††	10^2–10^6 $TCID_{50}$‡‡	70	0	<1 $TCID_{50}$	40
UF	0.001 µm	Synthetic solution§§	3.7×10^4–6.8×10^8 pfu/L	5	80	3.2 pfu/L	41

*NR—Not reported
†Reported as most probable number
‡Fecal streptococci
§Fecal coliform
**Enterococci
††Enterovirus
‡‡Tissue culture inoculation dose—50 percent
§§T2 virus

quality for the four waters are given in Table 2. The waters varied to an appreciable extent in pH (6.5–9.0), turbidity (0.49–9.0 ntu) and TOC (0.9–3.6 mg/L). There was also a 2.5-log variation in density of HPC bacteria.

Pilot-plant description and operation. The pilot plants for this study were located at the Boise River collector chlorination building in Boise and at the Bollman Water Treatment Plant in Concord, Calif. Figure 1 is a schematic of the pilot plants; a detailed description of the plants is provided elsewhere.[9] In general, raw water was pumped to the plant and prefiltered using a 200-μm nylon bag filter. A multistage centrifugal pump then delivered water from the tank to the pilot system. The prefiltered water entered a recirculation loop where a second pump sustained the recirculation flow rate. Water that did not pass through the membrane as permeate was recirculated as concentrate and blended with additional feedwater. The pilot plant maintained a constant flux by linking a flow meter, located in the permeate water line, to a feedback and control loop that regulated the system pressure pump. Temperature corrections to 20°C for transmembrane flux and determination of transmembrane pressure have been described elsewhere.[9] Normal operating transmembrane pressures ranged from 5 to 15 psi (0.4 to 1.0 bars), and instantaneous transmembrane flux rates from 60 to 100 gallons per day per square foot of membrane area (gpd/sq ft) (102 to 170 litres per hour per square metre of membrane area, $L/h/m^2$).

Backwashing was employed for controlling membrane fouling. A turbine backwash pump delivered permeate from the water reservoir through the membrane module, from the outside to the inner lumen of the hollow fibers. Sodium hypochlorite was applied to the backwash water to obtain a final concentration of 3 mg/L as free chlorine. Effective backwash pressure was 36 psi (2.5 bars) with a flow rate of approximately 9 to 12 gpm (34 to 45 L/min). The membranes were backwashed for 45 s every 30 to 60 min, depending on raw water quality. Between 5 and 10 percent of the permeate produced was used for backwashing; total feedwater recoveries ranged from 85 to 92 percent.

Hollow-fiber membrane modules were used in this study. The membranes were composed of a cellulosic derivative and had an effective filtration area of 77.5 sq ft (7.2 m^2) and a molecular-weight cutoff of approximately 100,000. To determine whether membrane integrity was compromised, a bubble point test, as described by Meltzer and Meyers[19] was performed on the membrane module. The membrane was submerged longitudinally in water while air was pumped through the permeate port at approxi-

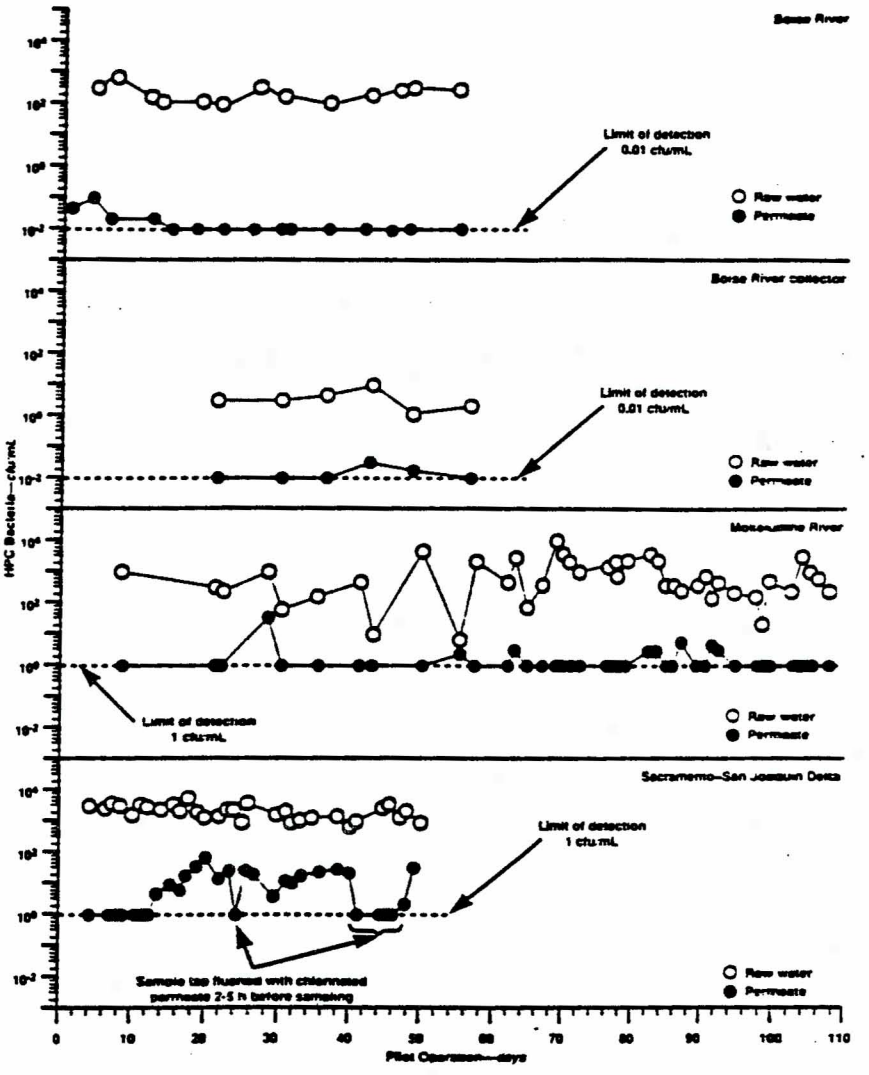


Figure 2. Removal of HPC bacteria from various waters by ultrafiltration

TABLE 2

Arithmetic means of selected raw water quality parameters for the four waters tested during UF membrane study

Parameter	Boise River	Boise River Collector	Mokelumne River	Sacramento—San Joaquin Delta
pH	7.7	6.5	9.0	8.1
Total dissolved solids—*mg/L*	61	88	ND*	ND
Alkalinity—*mg as $CaCO_3$/L*	ND	ND	23	71
Hardness—*mg as $CaCO_3$/L*	ND	ND	22	106
Turbidity—*ntu*	4.9	0.46	0.49	9
Total organic carbon—*mg/L*	3.2	0.9	1.7	3.6
Temperature—*°C*	12.6	13	17	17
Particle density—*× 10^3/mL*	26.3**	0.5**	5.4++	111++
Total coliform bacteria+	30‡	1.7‡	<2.8§	86§
HPC bacteria+—*cfu/mL*	148	4	141	1,300

*ND—not determined
+Geometric mean
‡Reported as cfu/mL
§Reported as MPN/mL
**2 to 120 μm
++1 to 120 μm

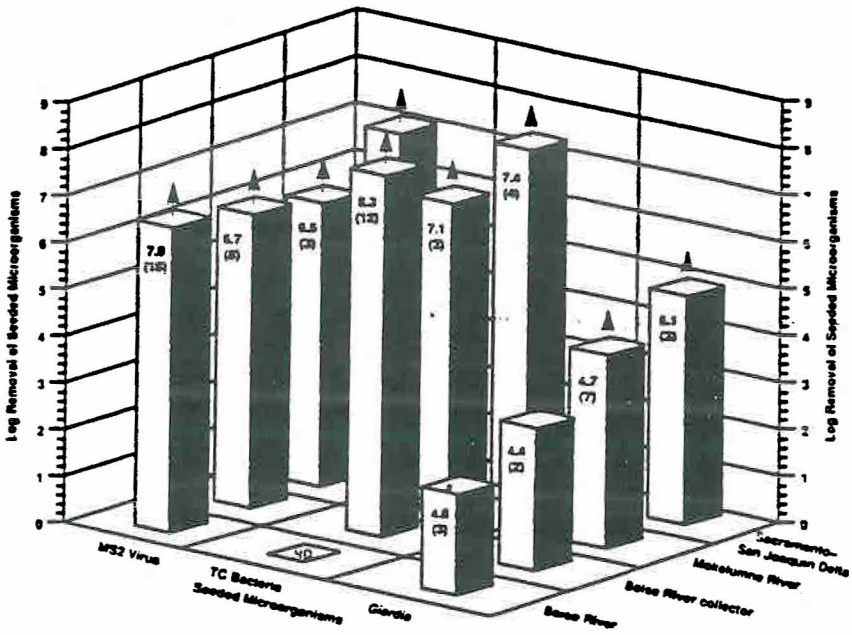


Figure 3. Removal of selected seeded microorganisms from various waters by UF *(numbers in parentheses represent number of samples assayed during batch seeding studies: ND—no data; ↑ indicates removal greater than value shown; * one cyst detected in one of the three seeding experiments)*

TABLE 3
Removal of total coliform bacteria in four source waters by UF

Source Water	Sampling Period	n*	Total Coliform Bacterial Density: Raw Water Mean+	Raw Water Range	Prefiltered Raw Water Mean	Prefiltered Raw Water Range	Permeate Mean
Mokelumne—*MPN/100 mL*	98 days	24	NA‡	NA	<3.8	<2.2–170	<2.2
Delta—*MPN/100 mL*	49 days	14	NA	NA	62	2.2–1,600	<2.2
Boise River collector—*cfu/100 mL*	47 days	6	<1.7	<1–4	<2.0	<1–9	<1
Boise River—*cfu/100 mL*	67 days	15	48	8–16	54	14–189	<1

*Number of samples
+Mean reported as geometric mean; < symbols used when bacteria were not detected in one or more samples
‡NA—not analyzed

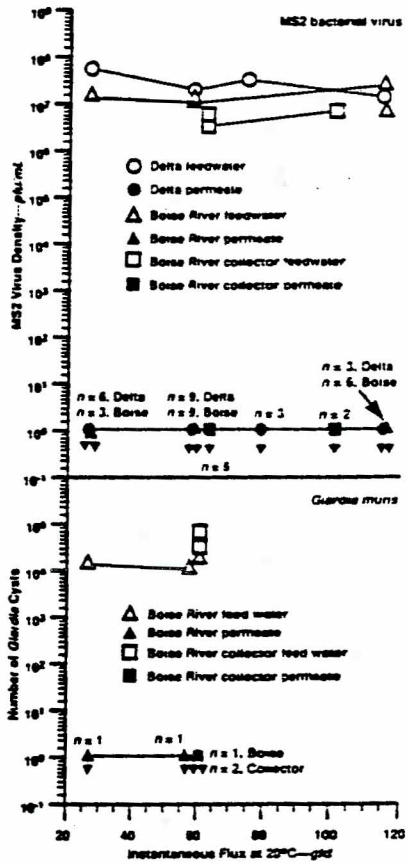


Figure 4. Effect of instantaneous flux on removal of selected seeded microorganisms *(↓ = below detection limit; n= number of samples collected)*

mately 1 psi (0.1 bar). Holes or intrusions in the membrane allow air to rapidly penetrate from the outside of the hollow fiber to the inner lumen. Thus, loss of membrane integrity was immediately detected by the presence of air bubbles at the membrane module inlet or outlet.

Turbidity. Turbidities of the raw and permeate waters in Boise were determined on site using three turbidimeters.* Feed and permeate turbidities were recorded on line. The turbidimeters were calibrated with formazin standards of 0.1 to 100 ntu according to *Standard Methods* (214A).[24] The 0.1-ntu standard was provided by the manufacturer. For the northern California pilot plants, turbidities were determined using on-line turbidimeters;+ 2.1 L of sample volume were used for analysis. The turbidimeters were calibrated with a standard curve developed from a benchtop unit,‡ and the standardization of this turbidimeter was performed according to *Standard Methods* (214 A).[24]

Direct particle counting. Because the study was conducted at two different locations, two identical particle counters from the same manufacturer were employed.§ These counters measured raw, prefiltered, and permeate waters. The particle counters were set at a flow rate of 50 mL/min with a 30-s counting time. A total volume of 25 mL was counted. For the Idaho studies, the instrument was set to count particles in six discrete size ranges: 2–3 µm, 3–5 µm, 5–15 µm, 32–64 µm, and 64–120 µm. For the California studies, the ranges were set to 1–2 µm, 2–4 µm, 4–7 µm, 7–15 µm, 15–32 µm, and

*HF-Scientific DRT-200B, Fort Meyers, Fla.
+Hach 1720C, Hach Co., Loveland, Colo.
‡Hach 2100, Hach Co., Loveland, Colo.
§Met One Model 210 Counting Systems with a Model 211 sensors and Model 214 laser counters, Grants Pass, Ore.

TABLE 4

Approximate sizes of microorganisms employed in microbial challenge studies

Organism	Model	Approximate Size—μm
Virus	MS2 bacteriophage	0.025
Coliform bacteria	*Escherichia coli*	1–2
Protozoa	*Giardia muris* cyst	7–14

TABLE 5

Microbial mass balance during batch-mode seeding studies

Microorganism	Raw Water*	Sample Points: Feedwater	Sample Points: Wastewater	Sample Points: Permeate Water
		Log Number of Microorganisms†		
MS2 virus	Boise River	12.5 ± 0.3	12.0 ± 1.5	ND‡
	Mokelumne River	11.6§	11.4§	ND
	Sacramento–San Joaquin Delta	12.4 ± 0.3	12.3 ± 0.2	ND
Giardia muris	Boise River	4.8 ± 0.1	4.6 ± 0.1	**
	Boise River collector	5.2 ± 1.9	5.2 ± 1.3	ND
	Mokelumne River	5.2 ± 0.9	5.2 ± 0.3	ND
	Sacramento–San Joaquin Delta	5.1 ± 0.3	5.2 ± 0.2	ND

*No data were available on Boise River collector virus because waste was chlorinated.
†Average and 95 percent confidence interval of log numbers for each sample location
‡ND—none detected
§No confidence interval shown because mass balance performed on one batch seeding
**One *Giardia muris* cyst detected in one 5-gal sample

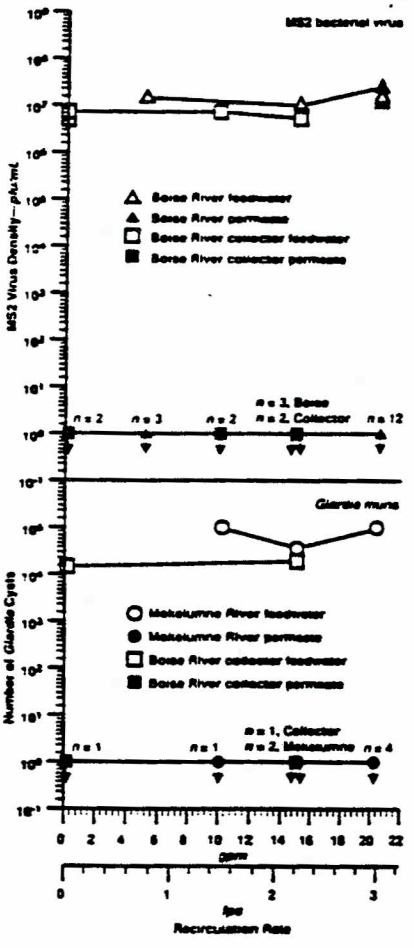


Figure 5. Effect of recirculation rate on removal of selected seeded microorganisms (↓ = *below detection limit;* n= *number of samples collected)*

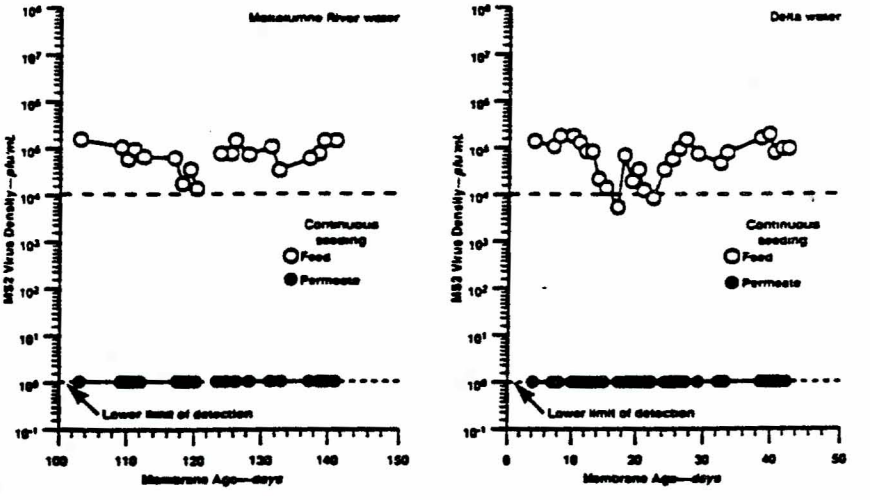


Figure 6. Removal of continually seeded MS2 bacterial virus by UF of Mokelumne and Delta waters

32–120 μm. When both raw and prefiltered particle counts were more than 10,500/mL, the samples were diluted with pilot-plant permeate water. All the analyses were performed on site.

Bacterial enumeration. Raw and permeate waters were collected in sterile 100-mL bottles with 0.4 mL of 10 percent sodium thiosulfate and stored at 4°C until assayed. For the Idaho studies, coliform and HPC bacteria were enumerated according to methods 9222B[24] and 9215D,[24] respectively. Coliform densities were reported as most probable number (MPN) per 100 mL, and HPC densities were reported as colony forming units per millilitre (cfu/mL). Methods 9221B[24] and 9215C[24] were used to enumerate coliform and HPC densities, respectively, in the California studies. Coliform densities were reported as MPN per 100 mL, and HPC bacterial densities were reported as colony forming units per millilitre. *Escherichia coli** was employed as the model for the total coliform-seeding studies. Densities of *E. coli* were reported as total coliform bacteria (cfu/mL).[24]

MS2 virus propagation and enumeration. MS2 bacterial virus† was employed as the model virus for the microbial challenge studies. MS2 virus was propagated by inoculating a flask containing 100 mL of viral host bacteria, *E. coli*,‡ to which 1 mL of 0.1 *M* sterile calcium chloride ($CaCl_2$) was added. Bacteria were grown in a flask that was incubated in a shaking water bath at 37°C. When the density of bacteria reached approxi-

*American Type Culture Collection, catalog number 13706, Rockville, Md.
†American Type Culture Collection, catalog number 15597-B1, Rockville, Md.
‡American Type Culture Collection, catalog number 15597, Rockville, Md.

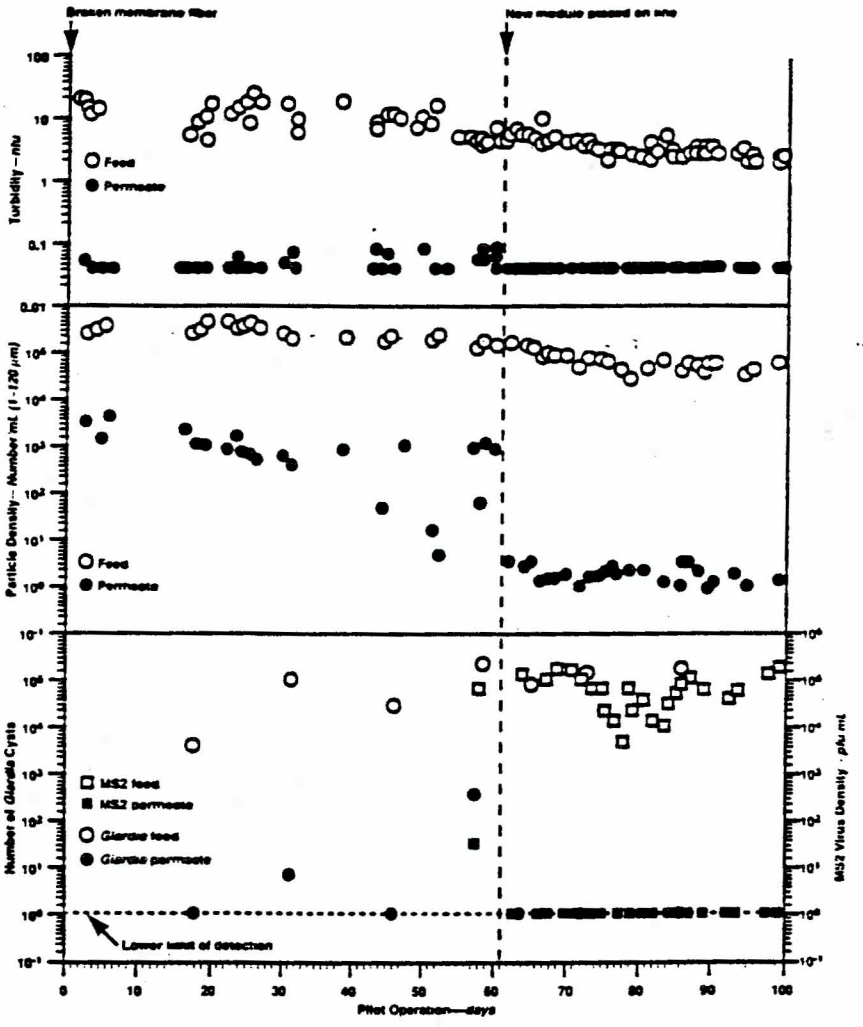


Figure 7. Effect of membrane loss of integrity on selected water quality parameters for Delta water

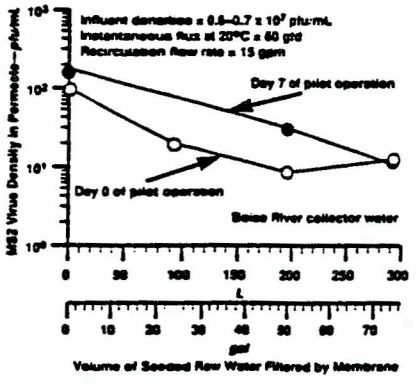


Figure 8. Densities of MS2 virus detected in samples collected after filtration of various volumes of seeded raw water with an UF module with one broken fiber

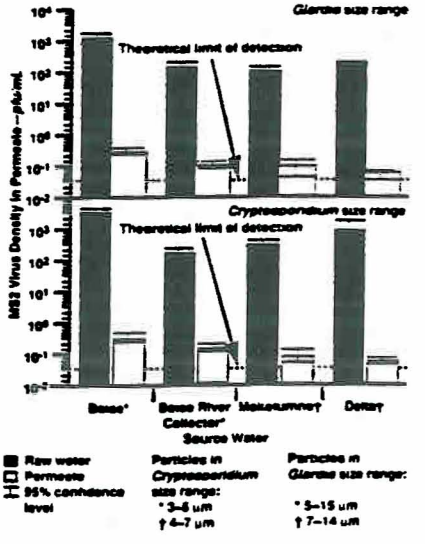


Figure 9. Removal of particles in the *Cryptosporidium* and *Giardia* size ranges by ultrafiltration of four untreated raw waters

mately 1×10^7 cfu/mL, an aliquot of the virus stock (approximately 10^{12} plaque forming units per millilitre [pfu/mL]) was added to provide a multiplicity of infection (MOI) of 0.1. This MOI was employed to assure two rounds of virus replication. Incubation of the bacterial culture continued until the host cells lysed, after which 0.01 g of crystallized lysozyme and 3 mL of sterile 0.2 *M* EDTA were added, followed by additional incubation for 1 h in a shaking water bath at 37°C. The propagated virus and cellular debris were then centrifuged for 20 min at $3,000 \times G$, filter-sterilized, and refrigerated at 4°C until needed. Transmission electron microscopy showed that a large percentage of the bacteriophage existed as single virions in the feedwaters.

MS2 was assayed by the agar overlay technique described by Adams[25] with some modifications. Host cultures of *E. coli* were grown on the day of the assay in trypticase yeast extract (TYE) broth at 37°C under aerated conditions for 5 to 6 h and dispensed in 20-mL aliquots in sterile dropper bottles. Just prior to use, 1.0 mL of 0.1 *M* sterile $CaCl_2$ solution was added to the dropper bottle. After the MS2 samples were serially diluted in 0.001 *M* phosphate saline buffer (PBS), 0.1 mL was added to 2 mL of TYE soft agar, which was maintained at 46–48°C. Three to four drops of the host *E. coli* were added, and then the soft agar was mixed gently and poured on a TYE hard agar petri dish. After the soft agar solidified, the petri dishes were incubated at 37°C for 24 h, after which the plaques, which are clearings in the bacterial lawn, were counted. All dilutions were plated in duplicate. Results were expressed in plaque forming units per millilitre.

Giardia muris enumeration. *G. muris* cysts and polyclonal antibodies were obtained from a private firm.* Samples for *Giardia* were collected from the influent water, permeate, and waste. One gallon was a sufficient volume for enumerating *Giardia* cysts in the feed and waste because the pilot-plant feed tank was seeded with approximately 10^6 to 10^7 cysts. Permeate samples were usually filtered on site: because they were expected to have very low densities of *Giardia* cysts, 20–115 L were usually processed. Samples were filtered under pressure using a variable-speed peristaltic pump,† set at a flow rate of 1 L/min. For these samples, 5.0-μm polycarbonate filters‡ (142 mm diameter) in plexiglass housings were employed. The filters were then washed with 200 mL of 0.002 percent of a surfactant and processed as described.

Feed and waste samples were placed in a stainless-steel pressure vessel and fil-

*Waterborne Inc., New Orleans, La.
†MasterFlex, Chicago, Ill.
‡Nuclepore, Pleasanton, Calif.

tered under 10–20 psi (0.7–1.4 bars) through a 5.0-μm, 142-mm polycarbonate membrane filter.* The carboy was then rinsed with 5 L of distilled, deionized water, and the procedure was repeated. After filtration, the filters were removed from the housing and rinsed with 200 mL of 0.002 percent of a surfactant. For feed and waste samples, washings were observed directly after staining and were centrifuged only if cyst concentrations were not high enough for enumeration. For permeate samples, the washings were placed in four 50-mL centrifuge tubes, and centrifuged at 3,000 × *G* for 10 min. The supernatant was then aspirated, and the pellets in the four tubes were combined.

Giardia cysts were then enumerated in a manner similar to that described by Rose.[18] The pellet volume was split into two aliquots, filtered through a 13-mm cellulose acetate filter* housed in a stainless-steel filter holder, and stained with polyclonal fluorescent antibodies.† After 30 min of incubation at room temperature, the filters were rinsed with PBS. Next, a secondary antibody was applied to the filters, allowed to incubate for another 30 min, and then rinsed in a manner similar to that described previously. Filters were mounted on glass slides using glycerol with 5 percent 0.2 *M* PBS. Cover slips were applied, and *Giardia* cysts were enumerated on the entire filter by epifluorescent microscopy under 20× magnification. When necessary, counts were confirmed under 40× magnification, using a fluorescent microscope equipped with a mercury lamp.‡ *Giardia* recovery efficiencies were evaluated for permeate, feed, and waste samples. For this study, recoveries ranged from 47 to 66 percent.

Microbial seeding protocols. Raw water feed tanks were seeded with the appropriate microorganisms at the beginning of the experiments, and the transmembrane flux and recirculation rates to be evaluated were set. Samples were collected from the feed tank, the permeate, and, in some cases, the waste. The volumes filtered by the membrane during any particular challenge run ranged from 97 to 290 L. For the bacterial and virus studies, 100-mL grab samples were collected at the beginning, middle, and end of the runs. For the *Giardia* seedings, larger volumes were necessary for analysis, as described earlier. In addition to batch studies, continual virus seedings were conducted to determine the efficacy of the membrane process in removing the organism when continually challenged. MS2 bacterial virus (between 10^7 and 10^8 pfu/mL) were injected into the feed stream by a peristaltic pump at flow rates that provided a final concentration in the feed ranging from approximately 10^4 to 10^5 pfu/mL. The virus pumping flow rate was adjusted according to the transmembrane flux being studied. Samples were collected from the feed and the permeate to determine the removal efficiency of the membrane.

Results

Removal of naturally occurring microorganisms. Table 3 presents the removal of total coliform bacteria in the four test waters by UF. Prefiltered raw water densities of total coliform bacteria ranged from <1 to 1,600/100 mL, depending on the source water. UF reduced the coliform densities, however, to less than detectable levels (<2.2 coliform bacteria/100 mL for Mokelumne and Delta waters and <1 cfu/100 mL for Boise River collector and Boise River waters). These results are consistent with those reported previously.[9]

The removals of HPC bacteria from the four waters tested are shown in Figure 2. The influent densities varied from 1 to approximately 10^4 cfu/mL, depending on the raw water tested. The HPC bacteria detected in the permeate were always lower than 0.2 cfu/mL for Boise River and Boise River collector waters, representing more than 3 logs and 2 logs removal, respectively, as a result of UF treatment. For Mokelumne water, the densities of HPC bacteria were always reduced to less than 5 cfu/mL, except for one sample.

Figure 2 also shows that the densities of HPC bacteria in Delta water were usually greater than 10^3 cfu/mL. For the first 50 days of pilot operation after a new membrane was placed on line, the observed densities in the permeate were inconsistent, ranging from <1 to 75 cfu/mL. Experiments were conducted to determine whether the HPC bacteria detected in the permeate were penetrating the membrane or were caused by colonization of the permeate side of the membrane or piping between the membrane module and the sample tap. As noted in the "Experimental methods" section, approximately 3 mg/L of free chlorine were applied to the permeate used for backwashing the membrane. The chlorinated backwash water did not, however, contact the short piece of piping between the sample tap and the pipe that directs water from the permeate tank to the membrane module during backwashing. Figure 2 shows that on day 24 and from day 40 to day 46 of pilot operation, the permeate sample tap was left open during backwashing so that chlorinated water flushed the tap and associated piping. After it was assured that no residual chlorine continued to be present, samples were collected during regular UF operating cycles. The figure shows that during these periods, the HPC densities were reduced to <1 cfu/mL. However, in subsequent days when the tap was not flushed, increased HPC densities were again observed. These data suggest that the HPC bacteria found in the permeate samples were due to colonization of a section of the sample tap piping rather than to penetration of bacteria through the membrane.

Removal of seeded microorganisms. To evaluate the efficacy of the UF membrane tested in removing microorganisms from Boise River and Boise River collector waters, the membranes were challenged with three different organisms: protozoa, coliform bacteria, and bacterial virus. The microbial models employed were *G. muris*, *E. coli* B, and MS2 bacteriophage (bacterial virus). Table 4 shows each microorganism and its approximate size. The smallest organism employed in the challenge studies was MS2 bacteriophage (~0.025 μm), which is similar in size, shape (icosahedron), and nucleic acid (RNA) to poliovirus and hepatitis A virus. The icosahedron virus contains a single strand of RNA with 3,569 nucleotides[25] and infects only male *E. coli* bacteria by injection of its RNA and A protein.[27,28] It was important in this study to investigate virus removal because the nominal pore size of the membrane, which is 0.01 μm, was only 2.5 times smaller than the bacteriophage. Table 4 also shows that MS2 bacteriophage is 40 to 80 times smaller than the bacterial seed and several hundred times smaller than cysts of *G. muris*. Thus, by removing the virus, both bacteria and protozoa should theoretically be removed.

Figure 3 summarizes the results obtained from the microbial challenge studies. The data are presented by microbial model and water tested. For the virus-seeding experiments, more than 6.5 logs of MS2 bacterial virus and 7 logs of total coliform bacteria were observed for all of the waters tested. In these experiments, neither virus nor bacteria were detected in any of the permeate samples, the log removals being limited by the density of seed organisms. It should be noted that differences in water qualities did not appear to affect the microbial removal efficiency of the membrane for these microorganisms.

In the *G. muris* challenge studies, 4.0–5.1 logs of *Giardia* cysts were removed by UF. One cyst was detected in the permeate during the first batch seeding on Boise River water. Viruses were not, however, detected in the permeate of a virus seeding conducted immediately after the *Giardia* test, suggesting that the cyst detected may have been an artifact, possibly because of contamination of the permeate sample during collection or analysis.

Because no microorganisms, except for one *G. muris* cyst, were recovered in the permeate of the four test waters when

*Nuclepore, Pleasanton, Calif
†Waterborne Inc., New Orleans, La
‡Olympus BH2 RFCA, Japan

the membrane was intact, the waste from the seeding studies was assayed for several of the trial runs. After any particular run was completed, the membrane was backwashed with approximately 60 to 100 L of permeate. All of the concentrate and the backwash water was then collected and the volume measured. Table 5 presents the log number of virus and *G. muris* at various sample points. Log numbers of virus and *Giardia* cysts recovered in the feed and waste were similar and statistically indistinguishable from each other at 95 percent confidence. That the MS2 virus densities were similar at these sample points indicates the organisms were being physically removed rather than inactivated during UF. Table 5 also shows that comparable levels of *G. muris* cysts were detected in the feed and waste of the various test waters.

Effect of transmembrane flux and recirculation rate. Two of the most important operating parameters in employing hollow fiber UF are transmembrane flux and recirculation rate (linear crossflow velocity). Thus, when possible, the effects of these parameters on microbial removal were investigated on the test waters. The impact of transmembrane flux on removal of MS2 virus and *G. muris* is shown in Figure 4. The data from these studies, which were conducted in a batch mode, show that transmembrane flux did not affect the removal of either organism; it appeared that removal was independent of flux rate over the range tested. More than 6 logs of seeded MS2 virus were removed from Boise River, Boise River collector, and Delta waters at transmembrane flux rates and transmembrane pressures as high as 112 gpd/sq ft (190 L/h/m^2) at 20°C and 25 psi (1.7 bars), respectively. Figure 4 also shows that approximately 4 to 5 logs of *G. muris* cysts were removed from Boise River water at flux rates ranging from 27 to 60 gpd/sq ft (46 to 102 L/h/m^2) at 20°C.

Recirculation (or crossflow fiber velocity) was necessary to maintain the inorganic and organic materials in suspension in the bulk concentrate so that membrane fouling was minimized. The effect of recirculation rate on virus and *G. muris* removal is shown in Figure 5. Despite viral seed densities in Boise River and Boise River collector waters ranging from 3×10^6 to 2×10^7 pfu/mL, no viruses were detected in any of the samples, regardless of the recirculation rate. Four or more logs removal were also observed for *G. muris* at recirculation rates ranging from 0 to 20 gpm (0 to 76 L/min). It is important to note that even when the membrane was operated with no recirculation (dead-end mode) on Boise River collector water, viral densities and the total number of *Giardia* cysts were reduced to nondetectable levels.

Effect of continuous microbial exposure on removal efficiency. To determine the capability of UF to remove microorganisms under constant-exposure conditions, continuous-seeding experiments were conducted. MS2 bacterial virus was chosen as the model for these experiments because it is the organism that is closest in size to the nominal pore size of the membrane (0.01 µm), as shown in Table 4, and could be assayed rapidly. The continuous seeding was conducted over a period of 37 days on Mokelumne water and 38 days on Delta water. At the start of the seeding experiments, the membranes had already been operational on Mokelumne and Delta waters for 103 and 4 days, respectively. Approximately 100 mL of the feed and the permeate samples were collected for analysis over the test period. Figure 6 shows that the initial virus feed density was approximately 1×10^4 pfu/mL or greater. Over the course of the experiment, however, no viruses were recovered in any of the permeate samples, thus demonstrating a consistent removal of 4 logs.

Impact of membrane integrity on microbial removal. The effect of a loss of membrane integrity (fiber breakage) on microbial removal was demonstrated when the membrane was subjected to transmembrane pressures exceeding the maximum operating pressure recommended by the manufacturer (29 psi [2.0 bars]). The integrity of the membrane was evaluated by monitoring turbidity and particle counts. Bubble point tests[19] were also performed on membranes that were suspected of being broken. Seeding studies, as described previously, were conducted on membranes in which loss of membrane fiber integrity was observed.

Loss of membrane fiber integrity: Delta water. Figure 7 presents data from a compromised UF membrane that was evaluated using Delta water. During the entire testing period, the permeate turbidity was consistently less than 0.08 ntu. Influent particle densities ranged from 3.0×10^4 to 3.3×10^5/mL in the 1- to 120-µm size range. However, during the initial 61 days of operation, particle densities as high as 3.8×10^3/mL were observed in the permeate.

Both *G. muris* and bacterial virus seedings were conducted during this period. Figure 7 shows that in two of the seedings, *Giardia* cysts were detected in the permeate: in one sample, six cysts were detected; in another, 353 cysts were detected. Additional evidence for a compromised membrane was demonstrated by the MS2 virus seeding conducted on day 58 of pilot operation. The pilot feed tank was seeded with 6.4×10^4 pfu/mL; 3.1×10^1 pfu/mL were recovered in the permeate. A bubble test on day 61 of operation confirmed that two membrane fibers had been broken. Although fiber integrity was compromised, at least 3 logs of *G. muris* and MS2 virus were still removed.

After day 61 of pilot operation, a new UF membrane was placed on line. Figure 7 shows that particle densities (1- to 120-µm size range) were reduced to less than 3/mL in the permeate. Additionally, in subsequent seedings of *G. muris* cysts and MS2 virus, neither of the microorganisms was detected in any of the permeate samples analyzed.

Loss of membrane fiber integrity: Boise River collector water. The photograph on page 98 shows a compromised or broken fiber that was detected in a new membrane module used for treatment of Ranney collector water. Air bubbles were detected protruding from one fiber at the outer edge of the membrane module. It was undetermined whether the break in the fiber occurred during the membrane manufacturing process, shipping of the modules, or initial module startup.

The initial seed density was 7×10^6 pfu/mL. Figure 8 presents MS2 virus densities recovered in the permeate as a function of the volume of seeded feedwater filtered by the compromised membrane. Each point in the figure represents a virus sample collected after a known volume of seeded water was filtered by the membrane. For the virus seedings at day zero, which represents the time when the new membrane was placed on line, approximately 1×10^2 pfu/mL were detected in the permeate after approximately 1 L of seeded feedwater was filtered. After 290 L were filtered, however, this level decreased to approximately 1×10^1 pfu/mL. On day seven, a hydraulic cleaning with an extended backwash was performed before the viral challenge to clean the membrane of any cake or gel layer formed. Results similar to those obtained on day zero were observed. A 1-log decrease in viral penetration from the beginning to the end of the trial was demonstrated. The trend observed in the data suggests virus removal by this membrane may not be strictly a function of pore size, but may be related to other factors such as gel or cake formation on the membrane surface. An example of the cake layer at the surface of a membrane used on Boise River collector water is shown in the electron micrograph on page 97. It should be noted, however, that despite the broken fiber, approximately 5 logs of virus were still removed.

Removal of particles. Particle counting was conducted to determine the removal of particles in size ranges similar to the size of *Giardia* cysts. Figure 9 presents the removal of particles in the 5–15-µm range for Boise and Boise River collector waters and in the 7–14-µm range for Mokelumne and Delta waters. The size ranges that approximate *Cryptosporidium* cysts are also shown. For each of the four test waters, the particles in the *Giardia* size ranges were always reduced to less than 0.3 mL. The log removals,

which ranged between approximately 3 and 4, were a function of the initial density of particles in that size range in the raw water. High removals were also observed for particles in the *Cryptosporidium* size range for each water. Particles in the 3–5-µm and 4–7-µm size ranges were always reduced to less than 1/mL. Moreover, another study[32] suggested that particles detected in the permeate probably did not pass through the membrane but were introduced on the permeate side of the membrane by the backwashing procedure.

Discussion

Hollow-fiber UF was effective in reducing naturally occurring microorganisms, such as total coliform and HPC bacteria. No coliforms were detected in any of the permeate samples collected. Removals of these organisms to detection limits are consistent with results of a previous study[5] and by other investigators[6,30,31] studying low-pressure membrane filtration. Although HPC bacteria are larger than the pore size of the membrane tested (0.01 µm), these bacteria were detected in the permeate of some samples. Other researchers have observed similar phenomena in studies on membrane filtration.[5,6,32–35] However, that none was detected after the sample tap was thoroughly flushed with chlorinated permeate suggests that the bacteria found in the permeate samples were due to colonization of a section of the system piping not exposed to the disinfectant during backwash. That no total coliform bacteria were detected in any of the permeate samples also suggests that bacterial penetration was not occurring. In order to provide additional evidence that the membrane provided a barrier to feedwater microorganisms, continual-seeding studies with MS2 bacterial virus were conducted while regular HPC sampling was performed. The data (Figure 6) showed that no virus penetrated the membrane. MS2 virus is approximately 4 to 400 times smaller than HPC bacteria and was seeded at higher densities than the HPC bacteria enumerated in the feedwater. Thus, if bacteria had passed through the membrane, it is probable the virus would also have been detected in the permeate.

In a review of the literature published on virus removal in full-scale plants, Hurst[36] calculated the removal efficiencies for various unit processes of conventional treatment. He reported that virus removal efficiencies by coagulation, sedimentation, and filtration (but not disinfection) ranged from 64.3 to 98.6 percent, not including one negative value. In comparison, this study showed that low-pressure hollow-fiber UF, based on the studies using four different waters, was capable of removing more than 99.99997 percent of the seeded virus. That no virus was detected in the permeate suggests that UF functioned as an absolute filtration process for microbial removal, as opposed to a relative filtration process sometimes observed in conventional treatment. It should be noted, however, that only one type of virus was employed in the seeding experiments. Other types of viruses may have behaved differently. In theory, though, complete disinfection with any virus can be achieved by a membrane process if the largest pore of the membrane pore size distribution is smaller than the microorganisms of concern. The nominal pore size of the UF membranes tested was 0.01 µm, and the size of the virus tested was approximately 0.025 µm. Thus, if the pore size distribution of the membranes was less than this diameter, removal to microbial detection limits should be expected. With this reasoning, it was expected that no *Giardia* would penetrate the membrane barrier. Figure 3 shows that more than 99.99 percent of the seeded *G. muris* in all four waters tested were removed as long as the membrane remained intact. The SWTR allows filtration technologies other than conventional, direct, slow sand, and diatomaceous earth provided that when combined with disinfection, it is demonstrated to the primacy agency that the filtration technology achieves at least a 3-log removal/inactivation of *Giardia* and a 4-log inactivation/removal of viruses (turbidity performance criteria for slow sand filtration must also be met). This study showed that in four waters, more than 6 logs of MS2 virus and 4 logs of *G. muris* were removed. High removals of *Giardia* were also in agreement with particle counting, in which particles in the approximate cyst size range of the organism were reduced close to the theoretical detection limits of the analysis. Instantaneous flux rate and recirculation rate did not appear to affect the removal efficiency of the membrane. Even under conditions of membrane stress, i.e., low crossflow fiber velocity and high transmembrane flux, no viruses were detected in the permeate as long as membrane integrity was not compromised.

The water qualities of the four test waters were significantly different from one another, particularly with respect to turbidity, particle densities, and TOC. This study showed that the differences in water quality did not appear to affect the microbial removal efficiencies of the membrane. The data support the premise that the mechanism of removal of microorganisms by the membrane tested in this study was, at least in part, a physical straining based on pore size distribution and size of the organism. In one case, however, in which a membrane fiber was broken during testing on Ranney collector water, MS2 virus was detected in the permeate. The density of virus appeared to decrease over time as the seeded-virus feedwater was filtered. The decrease suggests that in addition to a physical removal of the organism by the membrane, other factors such as gel or cake formation on the membrane surface may also play a role in microbial removal.

One of the most important findings of this study was that membrane integrity was of paramount importance to providing an absolute barrier to microorganisms. By applying pressures greater than those specified by the manufacturer, it was shown that membrane integrity was compromised. For cases in which membrane fibers were broken, as confirmed by a bubble point test, MS2 virus and, in one case, *G. muris* were detected in the permeate during seeding experiments. It should be noted, though, that at least a 3-log removal of both organisms was still demonstrated. When a loss of membrane integrity was observed for Delta water, there was no readily discernable increase in turbidity. The loss of membrane integrity was detected, however, by particle counting (and detection of virus and *Giardia* in the permeate). Therefore, using turbidity as the sole method of monitoring for membrane integrity may not provide adequate sensitivity for detection of small pinholes in membrane fibers. This observation is consistent with the observations of other researchers[37,38] who reported that particle counting yielded more useful water quality information than turbidity when the turbidity was approximately 0.1 ntu or less. In addition to showing the need for adequate monitoring of UF product water, the membrane integrity studies also showed the necessity of residual disinfection as an additional microbial barrier for distributed water.

Conclusions

Low-pressure hollow-fiber UF was effective in removing *G. muris*, MS2 virus, and total coliform and HPC bacteria. The data demonstrated that UF was capable of meeting SWTR requirements for alternative filtration technologies without the use of chemical disinfection. Four or more logs of *Giardia* and more than 6.5 logs of virus were removed from four different untreated source waters. Differences in water quality did not appear to affect removal capabilities of the membrane. Additionally, performance was not affected by changes in operating parameters such as transmembrane flux or recirculation rate, even under conditions of membrane stress.

Maintenance of membrane integrity was critical to assuring process efficacy. This study showed that when the module integrity was compromised, as in fiber breakage, both MS2 virus and *G. muris* were detected in the permeate. Consequently, the multiple barrier approach to water treatment, by employing residual disinfection in addition to UF, remains a

prudent treatment practice. Changes in membrane integrity were not necessarily reflected by changes in permeate turbidity; however, particle counting was an effective method for detecting fiber breakage.

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