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Characterizing the Effect of Chlorine and Chloramines on the Formation of Biofilm in a Simulated Drinking Water Distribution System

by

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E. Timothy Oppelt, Director
National Risk Management Research Laboratory

Abstract

Drinking water treatment in the United States has played a major role in protecting public health through the reduction of waterborne disease. However, carcinogenic and toxic contaminants continue to threaten the quality of surface and ground water in the United States. The passage of the Safe Drinking Water Act of 1974 and the subsequent amendments reflect this concern.

The Safe Drinking Water Act and its Amendments have been interpreted as meaning that some Maximum Contaminant Levels (MCLs) promulgated under the Act shall be met at the consumers tap, which in turn, has forced the inclusion of entire distribution system when considering compliance with a number of the Act's MCLs, Rules and Regulations. The Surface Water Treatment Rule which was promulgated under the Act requires that a detectable disinfectant be maintained at representative locations in the distribution system to provide protection from microbial contamination and to maintain water quality in the distribution system.

One aspect of maintaining water quality in drinking water distribution systems is controlling biofilm on distribution system pipe walls. Investigators have demonstrated the occurrence of high concentrations of bacteria in tubercles that exist in water mains, especially unlined cast iron mains, and on various types of pipe surfaces.

A study was conducted jointly by the U.S. Environmental Protection Agency and the University of Nancy in France to examine the control of microorganisms in treated water and at the pipe wall. A special pilot facility was constructed in which finished water from parallel water treatment pilot plants was discharged into pipe loops that contained sample tap locations to facilitate biofilm sampling. The facility was utilized to compare the effects of post-chlorination and post-chloramination on the concentration of microorganisms in the bulk phase and at the pipe wall.

The analysis utilized in this study characterizes these effects as measured by direct count epifluorescence, and cultural techniques. It found that chlorine is as effective or more effective in reducing the concentration of microorganisms in the bulk phase and in controlling biofilms at the pipe wall.

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1. INTRODUCTION

Drinking water treatment in the United States (U.S.) has played a major role in protecting public health through the reduction of waterborne disease. For example, in the 1880s for one year, the typhoid death rate was 158 deaths per 100,000 in Pittsburgh, Pennsylvania but by 1935 the typhoid death rate had declined to 5 per 100,000. The reduction in waterborne disease outbreaks was brought about by the use of sand filtration, disinfection and the application of drinking water standards (Clark et al., 1991a).

Concern over waterborne disease and uncontrolled water pollution resulted in a dramatic increase in Federal water quality legislation between 1890 and 1970. Even though significant advances were made in elimination of waterborne disease outbreaks during that time period, other concerns began to emerge. By the 1970s, more than 12,000 chemical compounds were known to be in commercial use with many more being added each year. Many of these chemicals cause contamination of ground and surface water and are known to be carcinogenic and/or toxic. The passage of the Safe Drinking Water Act of 1974 was a reflection of this concern.

The Safe Drinking Water Act of 1974 and its Amendments of 1986 (SDWAA) requires that the U.S. Environmental Protection Agency (U.S. EPA) establish maximum contaminant level goals (MCLGs) for each contaminant which may have an adverse effect on the health of persons. Each goal is required to be set at a level at which no known or anticipated adverse effects on health occur, allowing for an adequate margin of safety (Clark et al., 1987). Maximum Contaminant Levels (MCLs) must be set as close to MCLGs as feasible. The Safe Drinking Water Act was amended again in 1996.

Most of the regulations established under the SDWAA have been promulgated with little consideration of the effect that the distribution system can have on water quality. However, the SDWAA has been interpreted as meaning that some MCLs shall be met at the consumer's tap, which in turn, has forced the inclusion of the entire distribution system when considering compliance with a number of the SDWAA MCLs, Rules and Regulations.

Distribution systems are frequently designed to insure hydraulic reliability, which includes adequate water quantity and pressure for fire flow as well as domestic and industrial demand. In order to meet these goals, large amounts of storage are usually incorporated into system design, resulting in long residence times, which in turn may contribute to water quality deterioration. In addition, many water distribution systems in this country are approaching 100 years old and an estimated 26 percent of the distribution system pipe is unlined cast iron and steel and is in poor condition. At current replacement rates for distribution system components, a utility will replace a pipe every 200 years (Kirmeyer et al., 1994).

SDWAA regulations that emphasize system monitoring include the Surface Water Treatment Rule (SWTR), the Total Coliform Rule (TCR), the Lead and Copper Rule and the Total Trihalomethane Regulation. Both the SWTR and the TCR specify treatment and monitoring requirements that must be met by all public water suppliers.

The SWTR requires that a detectable disinfectant residual be maintained at representative locations in the distribution system to provide protection from microbial contamination. The TCR regulates coliform bacteria which are used to indicate the potential presence of enteric pathogens, as well as efficiency of disinfection. However, some total coliforms may grow in biofilm under the right conditions and, therefore, do not reasonably indicate recent contamination in the distribution system. Monitoring for compliance with the Lead and Copper Rule is based entirely on samples taken at the consumer's tap. The current standard for trihalomethanes (THMs) is 0.1 mg/L for systems serving more than 10,000 people but the recently promulgated Disinfectant and Disinfection By-Products (D-DBP) rule will impose a reduced THM level on large systems. This regulation also requires monitoring and compliance at selected monitoring points in the distribution system. Some of these regulations may, however, provide contradictory guidance. For example, the SWTR and TCR recommend the use of chlorine to minimize risk from microbiological contamination. However, chlorine or other disinfectants interact with natural organic matter in treated water to form disinfection by-products. Raising the pH of treated water may assist in controlling corrosion but may also increase the formation of trihalomethanes (Clark and Sivaganesan, 1998).

One aspect of maintaining water quality in drinking water distribution systems is controlling biofilm that forms on distribution system pipe walls. A bacterial biofilm can be defined as a structured community of microorganisms (including protozoa) enclosed in a self-produced polymeric matrix and adherent to an inert or living surface (Costerton et al., 1999). There is strong evidence that microorganisms colonize pipe surfaces in drinking water distribution systems. Investigators have demonstrated the occurrence of high concentrations of bacteria in tubercles that exist in water mains, especially unlined cast iron mains, and on various types of pipe surfaces (LeChevallier et al., 1987).

This report characterizes the effect of chlorine and chloramine on the concentration of biofilm at the pipewall and the concentration of microbes in the bulk phase as measured by epiflourescence direct count , and cultural techniques. These results are based on data from a series of experiments conducted in a pilot simulated distribution system located in Nancy, France.

2. WATER QUALITY DETERIORATION IN DISTRIBUTION SYSTEMS

There are many opportunities for water quality to change as it moves between the treatment plant and the consumer. Figures 1 and 2 illustrate some of the transformations that take place in the bulk phase and at the pipe wall. Cross connections, treatment barrier failures, and transformations in the bulk phase can all degrade water quality. Corrosion, leaching of pipe material, and biofilm formation and hydraulic scour can occur at the pipe wall to degrade water quality.

Many investigators have undertaken studies in an attempt to understand the possible deterioration of water quality once it enters a distribution system. It has been documented that microbiological changes in water quality may cause aesthetic problems involving taste and

odor development, discolored water, slime growths, and economic problems including corrosion of pipes and biodeterioration of materials (Water Research Centre, 1976). Bacterial numbers tend to increase during distribution and are influenced by a number of factors including the microbiological quality of the finished water entering the system, temperature, residence time, presence or absence of a disinfectant residual, construction materials, and availability of nutrients for growth (Geldreich et al., 1972; LeChevallier et al., 1987; Maul et al., 1985a,b).

The relationship of microbiological quality to turbidity and particle counts in distribution water was studied by McCoy and Olson (1986). An upstream and a downstream sampling site in each of three distribution systems (two surface water supplies and a ground water supply) were sampled twice per month over a one year period. Turbidity was found to be related in a linear manner to total particle concentration, but not to the number of bacterial cells. Degradation of microbiological water quality was shown to be the result of unpredictable intermittent events that occurred within the system.

LeChevallier et al. (1987) conducted a study on the effect of distribution system biofilms on water quality at a drinking water utility which experienced continuous microbiological problems. The treatment plant effluent contained concentrations of coliform at <1/100 mL, but, based on the total number of gallons produced, it was clear that some total coliforms were entering the system from the plant. A monitoring program showed increased coliform densities as the water moved further out into the distribution system. Maintenance of a 1.0 mg/L free chlorine residual was insufficient to control coliform occurrence. This was considered to be a problem because coliform bacteria growing in distribution system biofilms may mask the presence of other indicators that might indicate a breakdown in the treatment barrier.

3.0 EXPERIMENTAL PILOT FACILITY

The pilot facility utilized to generate the data in this paper consisted of two parallel pilot plants and two sets of three pipe loops in series (Clark et al., 1994b). Each set of loops received treated water from a pilot plant. The source of water for the pilot plants was a non-disinfected raw surface water.

The capacity of the 'control' pilot plant was approximately 1 m^3/h. Basic operation of the control consisted of chlorination followed by coagulation with ferric chloride at a rate of 30-50 mg/L depending on influent turbidity. After flocculation and sedimentation, the water was filtered using European-style sand with a grain diameter of 0.5 mm and a filtration rate of 6 m/h. Back-washing of the sand filter was accomplished by a three-step procedure consisting of air, air and water, and air for 4-5 min approximately every 18 h depending on head loss. Post disinfection was accomplished with chlorine or chloramine at concentrations selected to maintain a free chlorine residual of 0.2-0.5 mg/L or a monochloramine residual of 1 mg/L after the first 24-h residence time in the experimental distribution system (pipe loops). Chloramines were generated using an in-line mixer that contained HOCl and NH_4Cl to obtain a chloramine solution with a ratio of $N:Cl_2$ of 1:5.

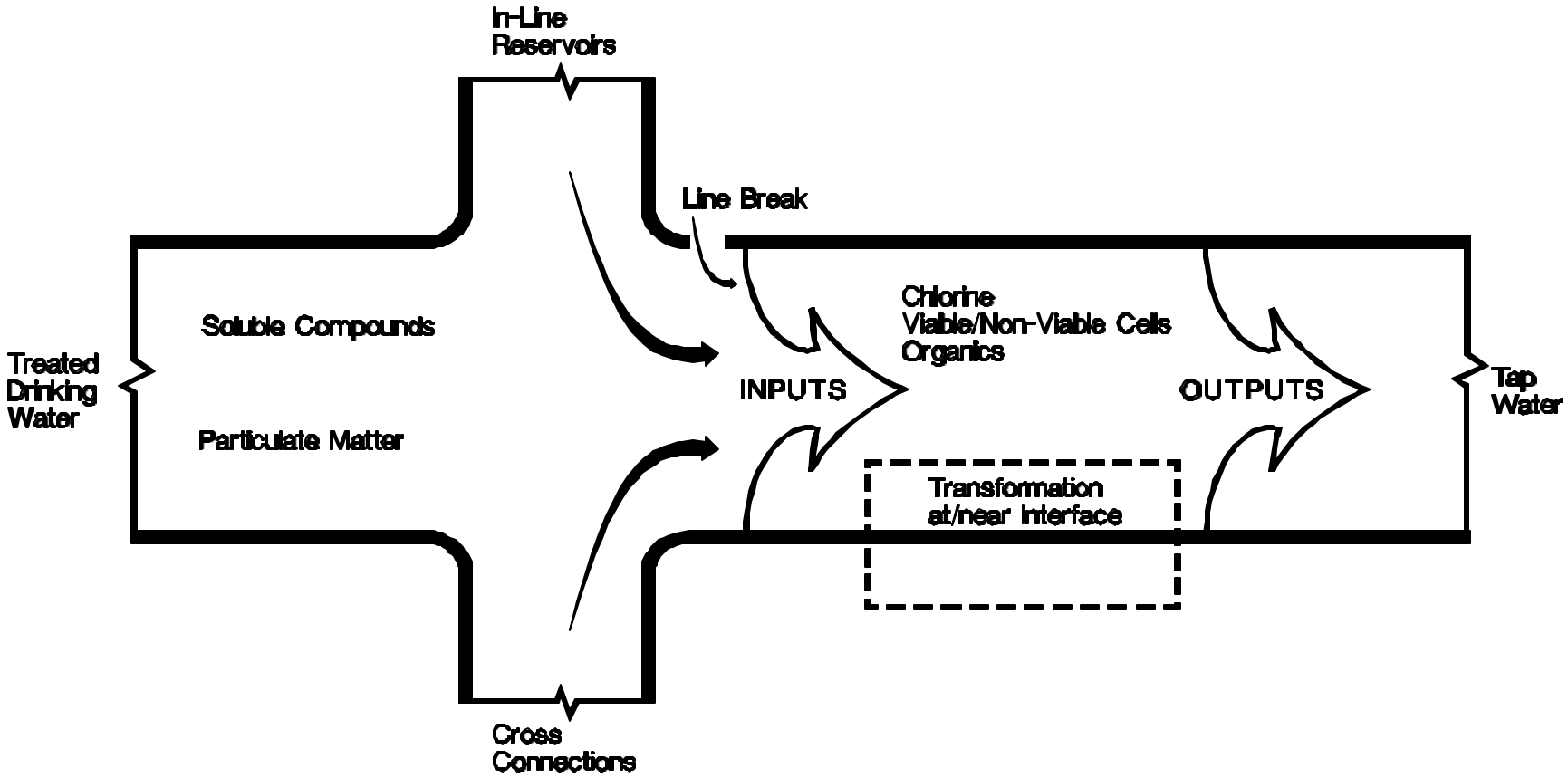

Figure 1. Schematic of Chemical and Microbiological Transformations in Drinking Water

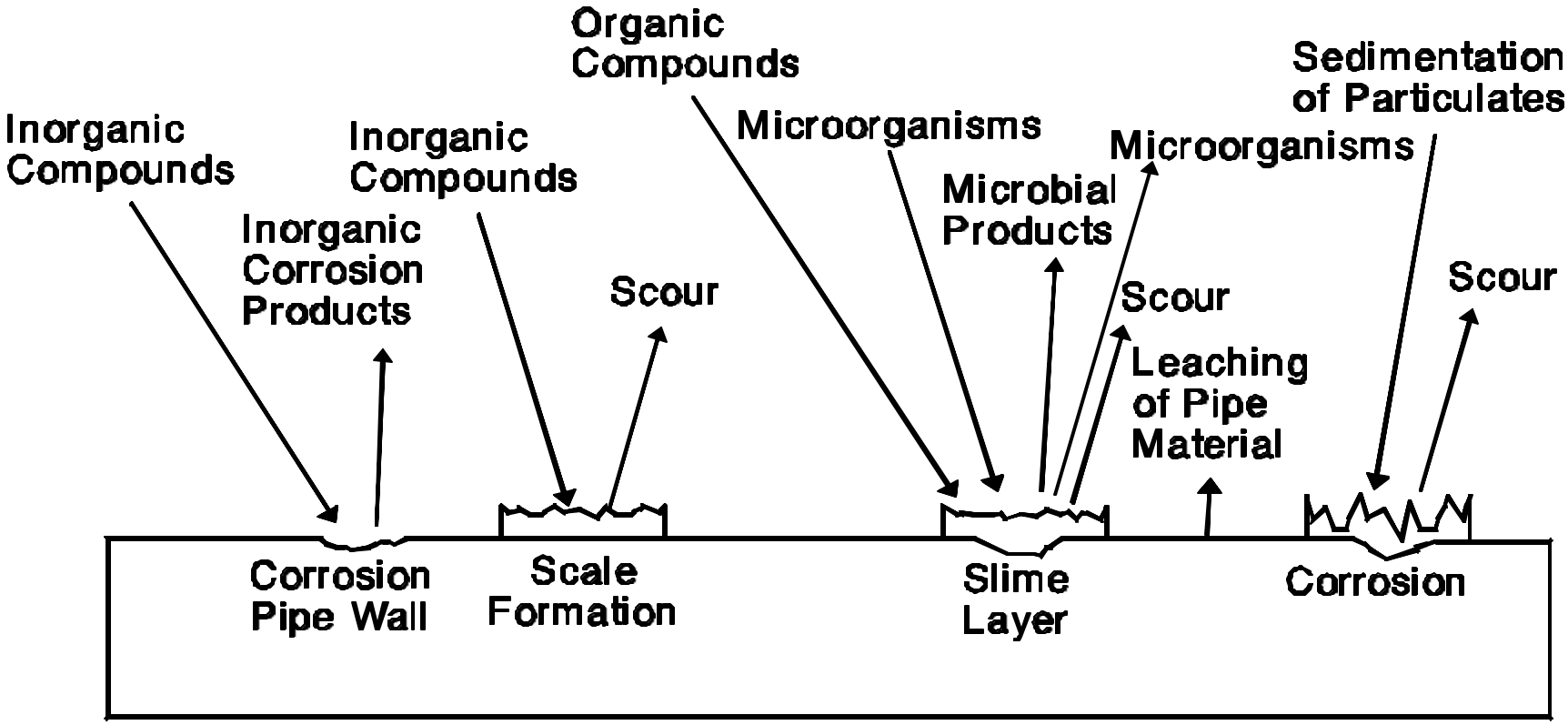

Figure 2. Schematic of Chemical and Microbiological Transformations at the Pipe Wall

Other experiments incorporated ozone into the treatment train in various configurations including ozonation before filtration but after coagulation, and pre-ozonation, and pre-ozonation coupled with ozonation before GAC filters and after sand filtration.

Finished water from the pilot plant was discharged into a network consisting of three loops. Each pipe loop was 10 cm in diameter (ID) and 31 m in length. The pipes in the loops were cement-lined cast iron containing 21 sampling devices per loop for water and biofilm. Appropriate sample tap locations facilitated removal of water samples. Biofilm formation was evaluated by placing coupons consisting of pipe material (polyvinyl chloride, polyethylene, or cement) on the end of the sampling probe which was inserted flush with the pipe wall. A shut-off valve ensured that pipe material coupons could be removed and changed while water was flowing through the pipe. Water velocity was 1 m/s with configuration and operation of the system producing a residence time of 24 h in each loop for a total of 72 h for the system. As a consequence, only a small portion of water was transferred from a given loop to each succeeding loop during a given flow cycle. An illustration of the pipe loop system is shown in Figure 3.

3.1 Research Design

In this research project, the effects studied were:

- The physico-chemical, chemical, and microbiological characteristics of the source and treated water.
- The change of these characteristics in the simulated distribution system.
- The formation of fixed biofilms on the internal surface of the pipes and its impact on water quality and network operating conditions.

During the 2-year study, the pilot plants and simulated distribution system were operated continuously. One pilot plant was used as a control (reference train) with chlorine added to the raw water and after filtration. Prechlorination was carried to breakpoint which required an average applied dose of 1.4 mg/L producing an average residual after sand filtration of approximately 0.1 mg/L. The performance of the chlorine reference pilot plant (control) was compared against alternative ozone disinfectant schemes used in the second parallel pilot plant. The three different treatment trains evaluated (T_2, T_3, T_4), as well as the control (T_1) are described in Table 1. Each treatment train configuration was evaluated using post-chlorination and post-chloramination.

Each treatment train, in parallel with the control, was evaluated at two separate times of year so that samples were collected under different temperature conditions (Table 2). Table 2 contains the various combinations examined and the dates of the experiments. For example, treatment train 1 (T1) was compared against treatment train 2 (T2) using chlorine as a post disinfectant during late December 1989 and early January 1990. The experiment was repeated in April and May of 1991.

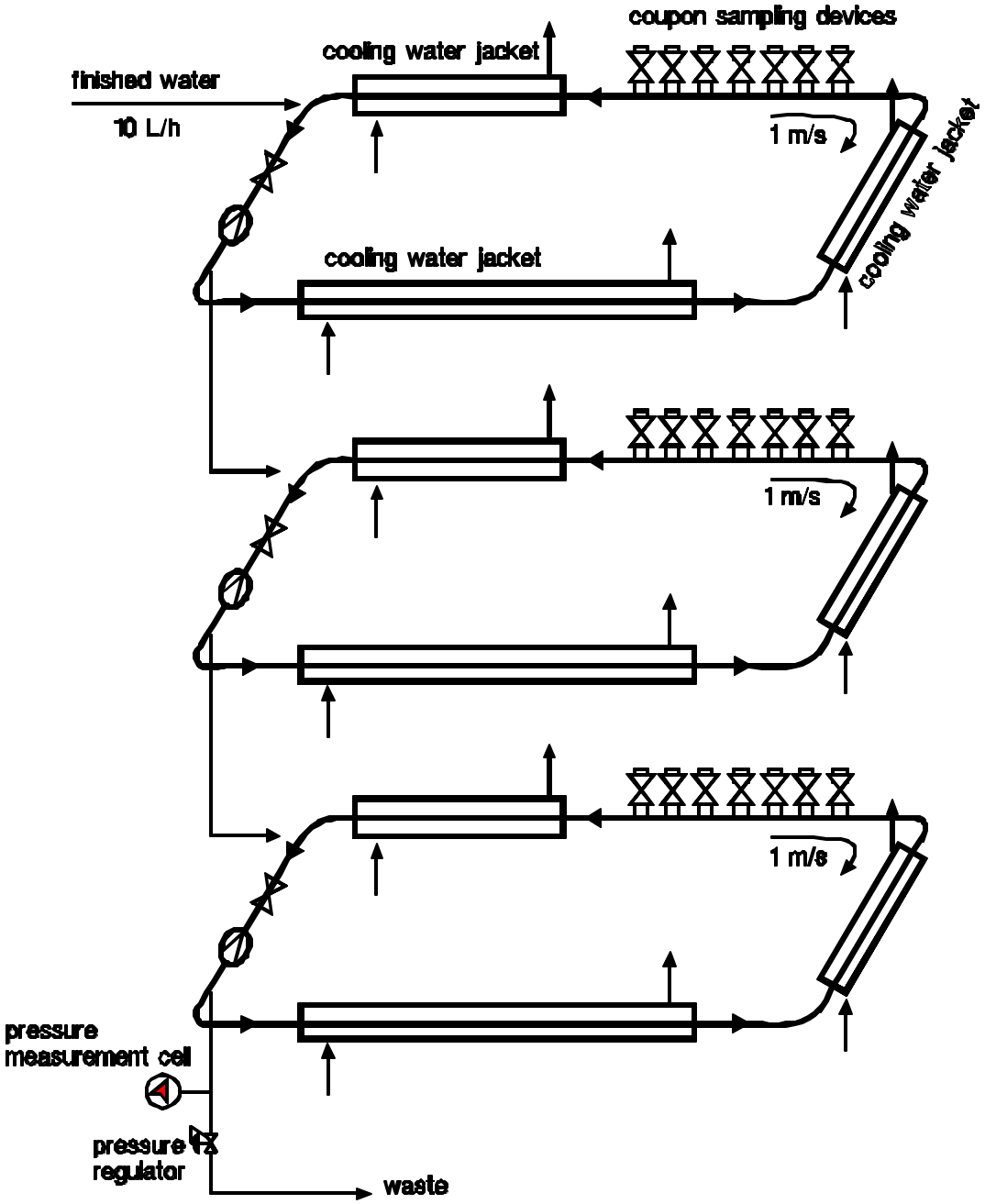

Figure 3. Experimental Distribution Network

TABLE 1. TREATMENT OPTIONS EVALUATED*	
Treatment Train Designation	Unit Processes
T_1	Prechlorination; coagulation/flocculation/settling; sand filtration
T_2	Coagulation/flocculation/settling; ozonation; sand filtration
T_3	Ozonation; coagulation/flocculation/settling; sand filtration
T_4	Ozonation; coagulation/flocculation/settling; sand filtration; intermediate ozonation; filtration (GAC)

* Each treatment train configuration was evaluated using both postchlorination and postchloramination.

TABLE 2. SEQUENCE OF SELECTED TREATMENT TRAINS			
Treatment Trains Compared	Experiment	Postdisinfection Scheme	Date of Experiment
T_1 vs T_2	1 2	Chlorination Chloramination	Dec 1989/Jan 1990 Feb/Mar 1990
T_1 vs T_3	3 4	Chlorination Chloramination	Apr/May 1990 May 1990
T_1 vs T_4	5 6	Chlorination Chloramination	June 1990 July 1990
T_1 vs T_3	7 8	Chlorination Chloramination	Sept/Oct 1990 Nov/Dec 1990
T_1 vs T_4	9 10	Chlorination Chloramination	Jan/Feb 1991 Feb/Mar 1991
T_1 vs T_2	11 12	Chlorination Chloramination	Apr/May 1991 June 1991

Each experiment consisted of three phases, a start-up phase, a transition phase, which permitted the distribution system to acclimate, and a quasi-steady-state phase during which intense sampling was conducted. The transition phase, which usually lasted 3-5 weeks depending on the disinfectant, allowed biofilm to form at the surface of the coupons inside the pipe loops. After this transition phase, the system was presumed to have attained a quasi-steady state, and sampling was conducted on three consecutive days. Data collected during the 3-day intensive portion of the study was utilized in this analysis.

3.2 Analytical Methods

Numerous analytical measurements were made on the treated and distributed water including: temperature, pH, alkalinity, ammonia, NO_2, NO_3, turbidity (NTU), Fe, Mn, particle counts (1-5, 6-10, 11-40, and $\geq$40 µm), chlorine (free, combined and total), ozone, total trihalomethanes (CHCl, $CHCl_2$ Br, $CHC1Br_2$, $CHBr_3$) and total trihalomethane formation potential, chloral hydrate, total organic carbon (TOC), assimilable organic carbon (AOC), biodegradable organic carbon (BDOC), dissolved organic carbon (DOC), colony forming units (CFU) (3 and 15 day incubation time), epifluorescence direct count and total coliforms.

Bacterial Density

Bacteria were enumerated directly from water samples and from the biofilm attached to cement, polyvinyl chloride (PVC) and polyethylene (PE) coupons. Techniques used to enumerate these bacteria in both the bulk phase and the pipe walls are described below.

Bulk Phase Samples

Water samples were collected in sterilized bottles, previously rinsed with sterile distilled water containing sodium thiosulphate at a final concentration of 17.5 mg/L. Cell densities as determined by the direct epifluorescence technique and by cultural methods (pour plate) were determined at the inlet and outlet of the first and third loops (Block et al., 1993; Standard Methods 19th Edition, 1995).

Biofilm

The colonized coupons (cement, PVC or PE) were placed in 25 mL of pH 7 bacteria-free distilled water and the attached bacteria were released from the coupons by sonication (Vibra Sonic Cells: 10 W, 20 kHz) for 2 min. Preliminary assays showed that these sonication conditions maintained the viability of bacteria and achieved removal of more than 80% of attached cells.

Bacterial Enumeration

The total number of bacterial cells were evaluated by direct epifluorescence microscopic observation. An aliquot of the sonicated biofilm or water sample was poured into a sterilized glass test tube and an aqueous solution of acridine orange was added to obtain a final concentration of 0.01% (v/v). After 30 minutes of incubation, the sample was filtered through a black polycarbonate membrane (Nuclepore SN 111156, 0.2 µm porosity). The filters were rinsed with sterile distilled water, a drop of buffered glycerine to reduce autofluorescence was added and a cover slip placed on the membrane filters. The filter was

then examined using an epifluorescene microscope (Olympus - blue light excitation) with oil immersion objective (X 1000).

The viable bacteria or colony-forming units (CFU) were enumerated by placing 1 mL of sample or diluted sample in standard nutrient agar (AFNORNF T90-402). Dilutions were made in a sterile 0.9% (v/v) NaCl solution. After 3 days and 15 days of incubation at 20-22EC, the colonies were counted. The results were expressed as viable colony-forming units per mL (CFU/mL) for water samples or CFU/cm^2 for biofilm samples (Clark et al., 1994b). Table 3 shows the average values for several water quality parameters entering the network.

As indicated in Table 3, direct count microscopic techniques (epifluoresence) were used to count cells on both the pipe wall (coupon samples) and in the bulk phase water samples. Cultural techniques were also used, however, the use of agar media for estimating bacterial numbers generally produces a result which underestimates the actual numbers present (Maul et al., 1991). Within a given population one may find variable numbers of bacteria which may be cultured in a defined media. Figure 4 illustrates the relationship between total bacteria as measured by epifluoresence and the number of colony forming units obtained by cultivation on agar media (heterotrophic organisms).

TABLE 3. AVERAGE TREATED WATER VALUES FOR THE PILOT NETWORK (for all treatment Scenarios)

Item	Mean Deviation	Standard	Minimum	Maximum
Temperature (EC)	17.3	3.8	11.3	24.1
pH	7.5	0.2	7.2	8.0
Turbidity (NTU)	0.2	0.09	0.06	0.6
DOC (mg/L)	1.50	0.5	0.7	3.6
Log_{10} CFU/mL (3 day)	-0.04	0.4	-0.3	1.3
Log_{10} CFU/mL (15 day)	1.1	0.6	0.2	3.0
Log_{10} epifluoresence (Count/mL X 10^3)	4.1	0.9	2.6	5.7

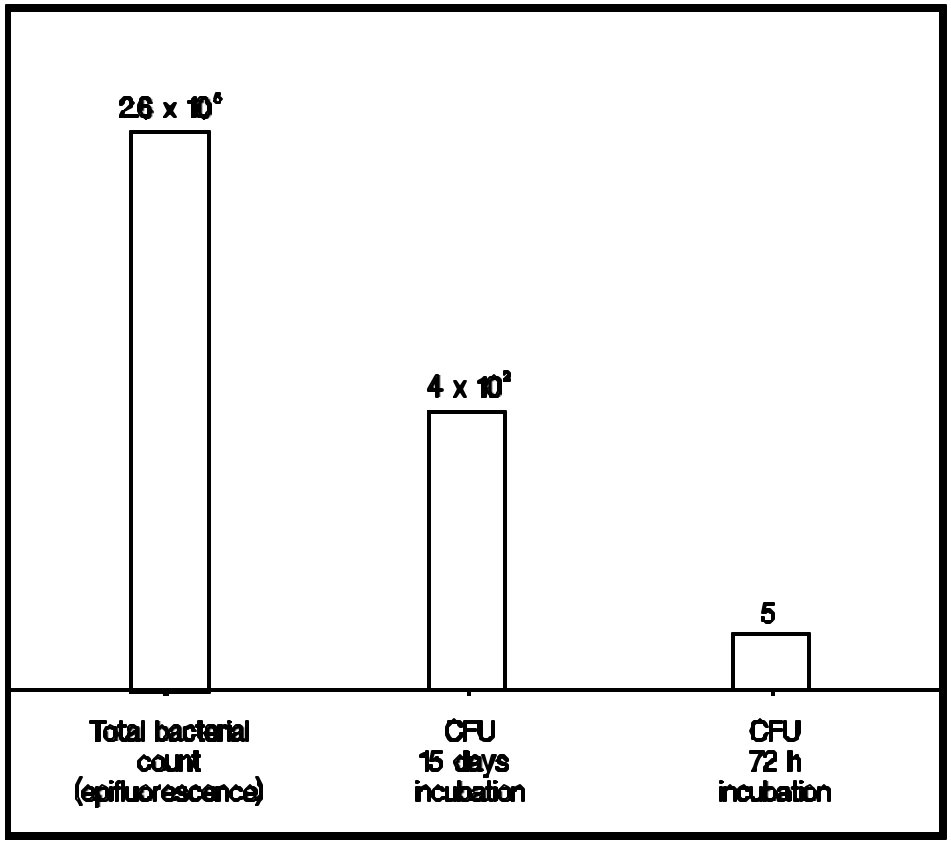

Figure 4. Numbers of Bacteria per ml. in Disinfected Drinking Water Supplies at the Outlet from the Treatment Plant (Maul et al., 1991)

4.0 THE NETWORK AS A REACTOR

The pilot network operates as a "recycle reactor," in which a certain fraction of the product stream is separated from the network and returned to the entrance of the reactor. The recycle ratio R is defined as (Levenspeil, 1972):

$$R = \frac{\text{volume of fluid returned to the reactor as feed}}{\text{volume entered the system}}$$

The value for R for each of the loops in the pilot network was:

R = 2891.85

Assumption of a first order reaction yields the following function:

$$\frac{kt}{R+1} = \ln\left[\frac{C_{A0} + RC_{Af}}{(R+1)C_{Af}}\right]$$

where C_{A0} = the concentration of the feed going into the loop in mg/L, C_{Af} = the concentration of the stream leaving the loop in mg/L, k is a reaction rate constant (day^{-1}) and ô is the mean residence time in the pipe loop in days.

Equation (1) was used as the basis for the subsequent analysis for both chlorine and chloramine decay through the loops. Reformulating equation 1 yields:

$$C_{Af} = \frac{C_{Ao}}{(R+1)e^{\frac{tk}{(R+1)}} - R} \qquad (2)$$

Figures 5 and 6 are examples of fitted curves for the chlorine and chloramine concentrations in the bulk phase. For the system R = 2891.85, ô = 24 hr and k = 6.06/day for chlorine and k = 1.64/day for chloramines.

5.0 DATA ANALYSIS

Three-day CFU/mL (CFU3), 15-day CFU/mL (CFU15) and epifluorescence counts/mL X 10^3 (EPI) measurements in the bulk phase and on the pipe wall were evaluated for their dependency on water quality variables such as Cl_2 (or NH_2Cl, depending on the final disinfection method), pH, temperature (Temp), DOC, BDOC, ozone, ammonia, NO_2, NO_3, and fluorescence.

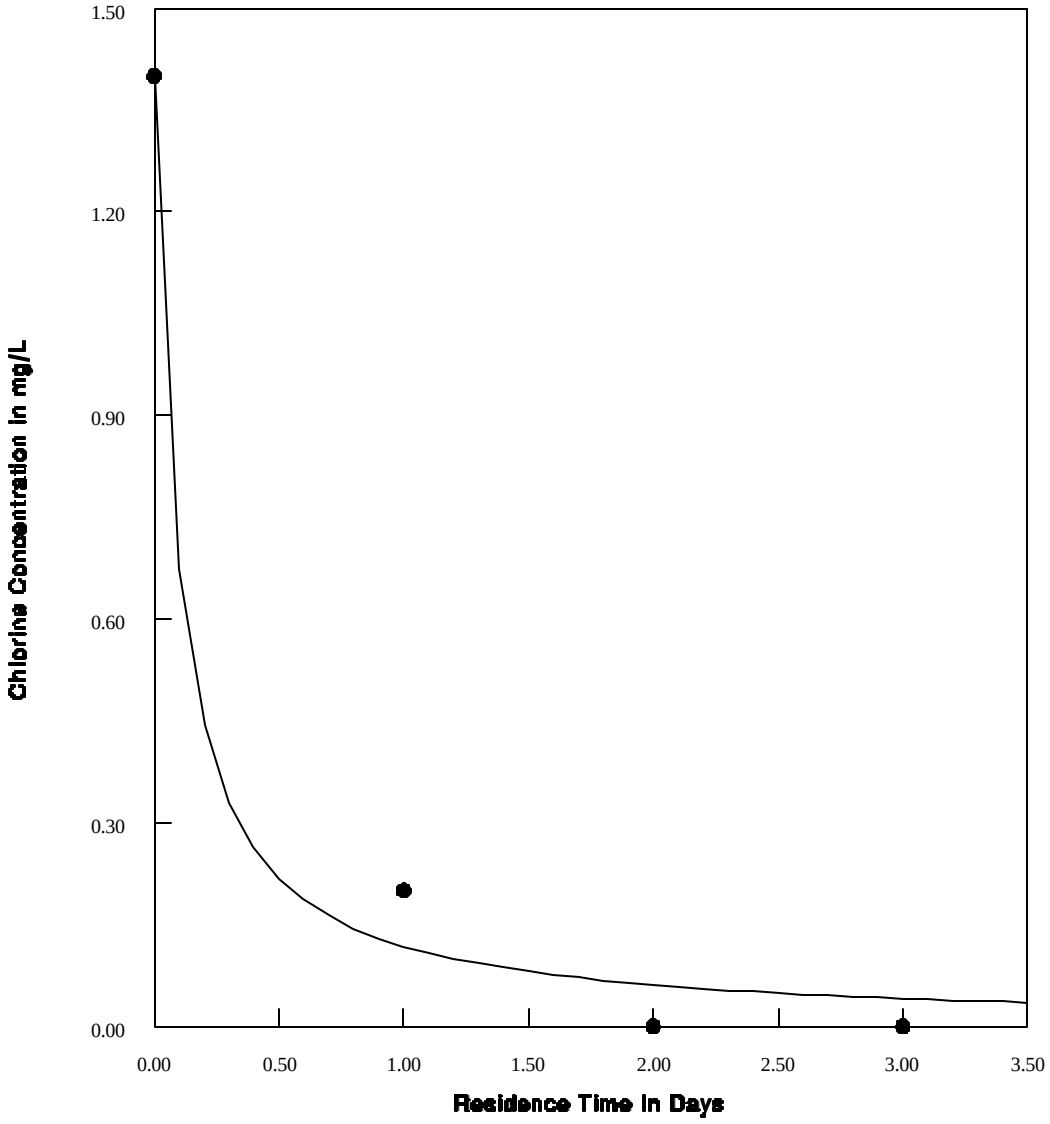

Figure 5. Chlorine Decay Curve

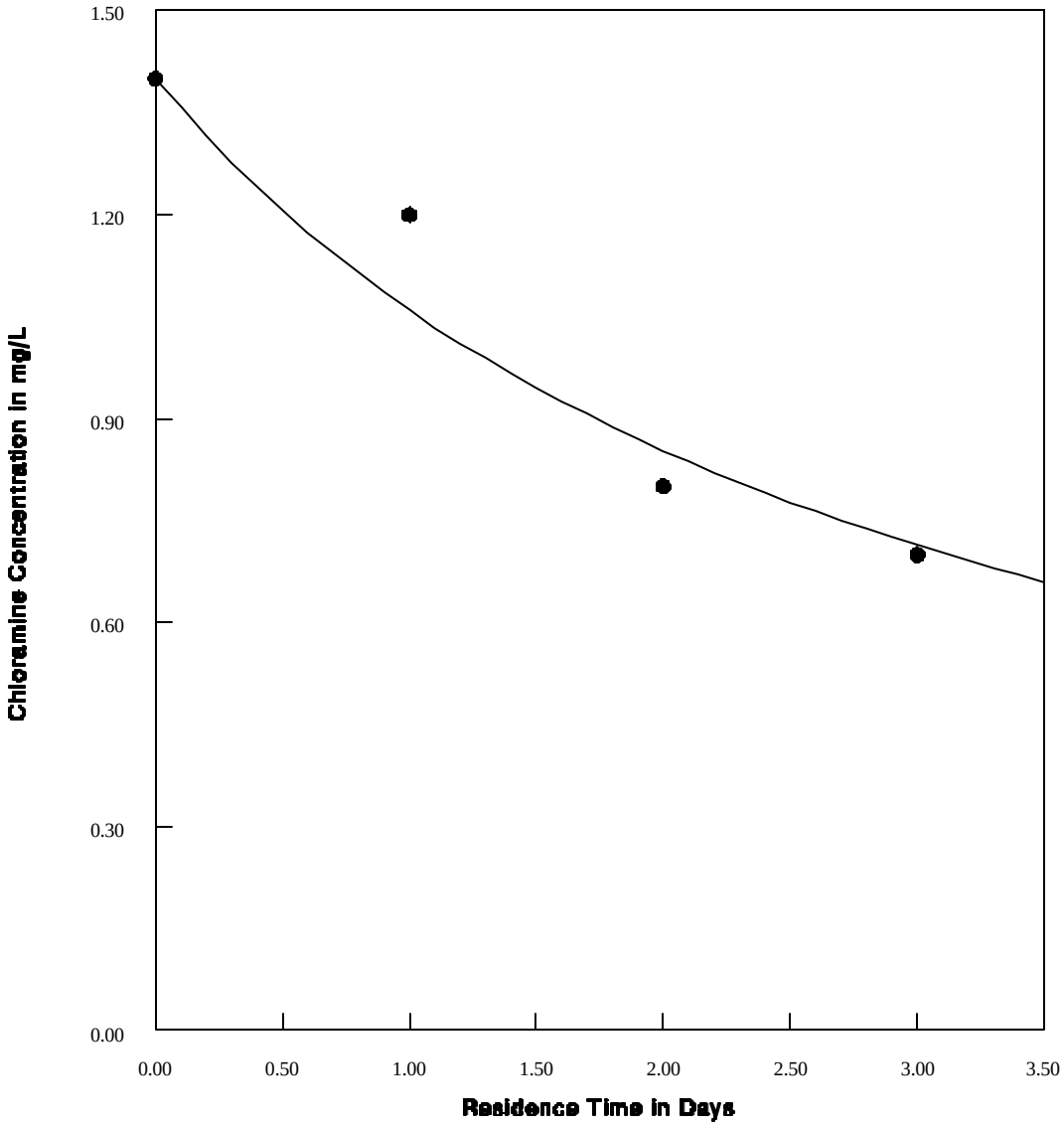

Figure 6. Chloramine Decay Curve

A correlation analysis was also performed to examine the relationship among the independent variables. It was found that many of the independent variables were correlated and were linear functions of DOC. Even though the experiment was conducted over a 12-month period, the loops were inside and the temperature of the water in the loops was relatively constant. Therefore, temperature was eliminated as an independent variable.

Scatter plots of log EPI vs. Cl_2 (or NH_2Cl) in the bulk phase and on the pipe wall exhibited a clearly decreasing trend with increasing levels of Cl_2 (NH_2Cl). The scatter plots of log CFU3 vs. Cl_2 or NH_2Cl and log CFU15 vs. Cl_2 or NH_2Cl also showed a decreasing trend with increasing disinfectant concentration but did not show as definitive a pattern as did epifluoresence. As a consequence a linear model of log EPI, log CFU3 and log CFU15 as a function of disinfectant concentration was selected for analysis. For example, the model used for EPI versus chlorine residual is given by equation (3) and for chloramine by equation (4).

$$\log EPI_i = a + b\,(Cl_2)_i + e_i, \qquad i=1,2.....n, \tag{3}$$

$$\log EPI_i = a + b\,(NH_2Cl)_i + e_i, \qquad i=1,2.....n, \tag{4}$$

In the above equations, a (intercept) and b (slope) are model parameters, n is the sample size and e_i is the error term. It is assumed that e_i's are independent and identically distributed normal variables with a mean of 0 and the same variance σ^2. A least-squares technique was used to estimate the model parameters. The same type of model was used to characterize log CFU3 and log CFU15 versus disinfectant level.

6.0 BULK PHASE ANALYSIS

Using equations 3 and 4 bacterial concentrations in the bulk phase were analyzed as a function of disinfectant concentration. Effects of both treatment and disinfectant type were evaluated. A comparison of the effects of chlorine and chloramine on epifluoresence direct count and organisms cultured for 15 (CFU15) and 3 (CFU3) days was made and will be discussed in the following sections.

6.1 Epifluorescence Direct Count

Table 4 contains parameter estimates for equations 3 and 4 for both chlorine and chloramine disinfection in the bulk water phase for all four types of treatment. The p-value for testing that the parameter b = 0 is given in parenthesis. The slopes are significantly negative for all four treatments, meaning that there is a significant linear correlation between EPI and the concentration of disinfectant in the water. The smallest R^2 occurred in the experiment involving post-chloramination for treatment T3.

TABLE 4. PARAMETER ESTIMATES FOR CHLORINE AND CHLORAMINE DISINFECTION REGRESSED AGAINST EPIFLUORESENCE IN THE BULK PHASE

Treat-ment Type	Chlorine				Chloramines			
	á	â	$ó^2$	R^2	á	â	$ó^2$	R^2
T1	5.4306	-6.556 (0.0001)*	0.1713	0.8502 (n=51)	5.5949	-1.314 (0.0001)*	0.0816	0.8298 (n=54)
T2	5.4697	-3.927 (0.0020)*	0.6821	0.6324 (n=12)	5.4445	-1.5168 (0.0001)*	0.2565	0.6066 (n=18)
T3	5.2598	-2.5876 (0.0004)*	0.4201	0.5606 (n=18)	5.3793	-0.6751 (0.0070)*	0.2906	0.3739 (n=18)
T4	5.2589	-4.4219 (0.0001)*	0.2542	0.7307 (n-18)	5.7244	-1.4422 (0.0001)*	0.2314	0.6164 (n=18)

* - At 5% level of significance

Effect of Treatment

For a given disinfection method there are differences among the estimated slopes (âs) for the different treatment trains. Assuming that the error of variance is homogeneous among treatment groups, a covariance analysis was performed on the pooled data sets to compare slopes within a disinfection method. The covariate Cl_2 (or NH_2Cl) and the effects of the treatment trains T1, T2 , T3 or T4 are included in the covariance model. The following models were used to compare the effects of chlorine (or chloramine) on EPI for the different treatment trains:

$$\log EPI_{ij} = (a + g_i) + (b + d_i)(Cl_2)_{ij} + e_{ij}, \qquad i=1,2,3,4;\ j= 1,2...,n_i \tag{5}$$

or

$$\log EPI_{ij} = (a + g_i) + (b + d_i)(NH_2Cl)_{ij} + e_{ij}, \qquad i=1,2,3,4;\ j= 1,2....,n_i, \tag{6}$$

where $\log EPI_{ij}$ is the j^{th} response for the i^{th} treatment group, a and b are average intercept and slope for the entire data set g_i and d_i are treatment effect coefficients (differences of intercept and slope from the corresponding average intercept and slope for the entire data set). The e_i's are independent and identically distributed normal random variables with mean 0 of equal variances $ó^2$ and $n = n_1 + n_2 + n_3 + n_4$ is the pooled sample size. If the four regression coefficients d_1 , d_2 , d_3 and d_4 are not significantly different from zero then there are no significant differences among the four treatments with respect to the linear effects of chlorine (or chloramine) on log EPI. If some of the d_i's are significantly different from zero then there is significant difference among the treatments. The hypothesis that all the d_i 's are zero was tested via a covariance analysis. The test revealed that the regression relationships (slopes) differed among treatment groups ($p<0.05$). Thus, within

each disinfection method, the linear effects of chlorine (or chloramine) on log EPI are not the same for each treatment.

For the chlorinated systems, the slope (linear effect) of chlorine on log EPI for treatment T1 was significantly more negative than the slopes for T2, T3 and T4 ($p<0.05$ for each comparison). Treatments T2, T3 and T4 were compared using a covariance analysis and no significant difference was seen ($p = 0.1533$). These results imply that for chlorine disinfected systems the use of ozone in the treatment train resulted in higher levels of bacterial concentration in the bulk phase than resulted from the use of pre-chlorination alone.

For the chloraminated systems, a covariance analysis showed that the slopes for T1, T2 and T4 were significantly more negative than the slope for T3 ($p<0.05$, for each comparison). No significant differences were seen among the slopes of T1, T2 and T4 ($p=0.6792$). These results imply that there were no differences in bulk phase bacterial concentrations between systems that use chloramine even if ozone is used in the treatment train.

Effect of Disinfectant

The slopes (b's) for all systems using chlorine disinfection were significantly lower than the slopes for chloramines for all four treatments. Within each treatment group, both disinfection methods were compared via covariance analysis. The model for the analysis is given by:

$$\log EPI_{ij} = (a + g_i) + (b + d_i)(x_{ij}) + e_{ij}, \qquad i=1,2;\ j=1,2,\ldots\ldots n_i, \tag{7}$$

where $\log EPI_{ij}$ is the j^{th} response for the i^{th} disinfection method, a and b are intercept and slope for the pooled data set. The parameters g_i and d_i are treatment effect coefficients corresponding to the i^{th} disinfection method (difference of intercepts and slope from the average intercept and slope for the i^{th} disinfection method), e_{ij}'s are independent and identically distributed normal random variables with a mean 0 and equal variances σ^2, $n = n_1 + n_2$ is the pooled sample size, x_{1j} is the j^{th} chlorine value, and x_{2j} is the j^{th} chloramination value.

The hypothesis that both d_is are zero was tested using a covariance analysis and shows the regression relationship (slopes) are different among disinfection methods ($p<0.01$). Within each treatment group, the linear effects of chlorine on log EPI were significantly different from the linear effect of chloramine on log EPI. Chlorine disinfection yielded more negative slopes than chloramine for each of the four treatments ($p<0.01$). Thus, it could be concluded that for all the treatments, chlorine was significantly more effective than chloramine in reducing epifluoresence in the bulk phase.

CFU15

Table 5 contains parameter estimates for log CFU15 (equations 3 and 4) for both chlorine and chloramine disinfection in the bulk phase for all four treatment conditions. The

p-value for testing that the parameter â = 0 is given in parenthesis. The slopes were significantly negative for all four treatments, meaning that there was a significant linear correlation between 15-day CFU and the concentrations of disinfectant in the water.

TABLE 5. PARAMETER ESTIMATES FOR CHLORINE AND CHLORAMINE DISINFECTION REGRESSED AGAINST CFU15 IN THE BULK PHASE

Treat-ment Type	Chlorine				Chloramines			
	á	â	$ó^2$	R^2	á	â	$ó^2$	R^2
T1	3.2224	-6.9266 (0.0001)*	0.7650	0.5866 (n=51)	3.4595	-2.5716 (0.0001)*	0.4435	0.7745 (n=54)
T2	3.2470	-4.6909 (0.0003)*	0.5860	0.7408 (n=12)	3.2243	-2.7556 (0.0036)*	0.3058	0.8102 (n=18)
T3	3.0404	-4.5898 (0.0001)*	0.7795	0.6839 (n=18)	3.2065	-1.4927 (0.0097)*	1.5730	0.3503 (n=18)
T4	3.4356	-6.8052 (0.0001)*	0.2790	0.8541 (n=18)	3.6670	-3.3338 (0.0001)*	0.2215	0.8997 (n=18)

* - At 5% level of significance

Effect of Treatment

For a given disinfection method there are differences among the estimated slopes (âs) for the different treatment trains. Equations 5 and 6 (with log CFU15 as the dependent variable) were used to compare the effects of chlorine (or chloramine) on log CFU15 for the different treatment trains. The hypothesis that all the effects were zero was tested via a covariance analysis. The test revealed that the regression relationships (slopes) differed among treatment groups for chloramine disinfection method ($p = 0.0035$) and no significant difference was seen among the slopes for chlorine disinfection method ($p = 0.0762$).

For the chloraminated systems, a covariance analysis showed that the slopes for T1, T2 and T4 were significantly more negative than the slope for T3 ($p<0.05$, for each comparison). No significant differences were seen among the slopes of T1, T2 and T4 ($p = 0.1728$).

Comparison of Disinfectants

Within each treatment group, the effects of disinfection methods were compared via covariance analysis. The model for the analysis is given by equation 7 with log CFU15 as the dependent variable. The covariance analysis shows that within each treatment group, the linear effects of chlorine on log CFU15 were significantly more negative than for chloramines ($p < 0.03$, for each comparison). Thus, it was concluded that for a given treatment, chlorine was significantly more effective than chloramine in reducing15-day CFU in the bulk phase.

CFU3

Table 6 contains parameter estimates for equations 3 and 4 (with log CFU3 as the dependent variable) for both chlorine and chloramine disinfection in the bulk water phase for all four treatment conditions. The p-value for testing that the parameter $\hat{a} = 0$ is given in parenthesis. P-values were significant and negative for all four treatments, meaning that there was a significant linear correlation between log CFU3 and the concentrations of disinfectant in chloramine disinfection ($p = 0.0716, 0.0958$, respectively). Thus, within each disinfection method, the linear effects of chlorine (or chloramine) on log CFU3 were not significantly different over the treatment groups.

TABLE 6. PARAMETER ESTIMATES FOR CFU3 VERSUS CHLORINE AND CHLORAMINE DISINFECTION REGRESSED AGAINST CFU3 IN THE BULK PHASE

Treatment Type	Chlorine				Chloramines			
	á	â	$ó^2$	R^2	á	â	$ó^2$	R^2
T1	0.9309	-3.639 (0.0001)*	0.7643	0.2816 (n=51)	0.9191	-1.2260 (0.0001)*	0.8093	0.2985 (n=54)
T2	0.5885	-1.6238 (0.0061)*	0.1672	0.5454 (n=12)	0.3917	-0.8361 (0.0036)*	0.1656	0.4206 (n=18)
T3	0.4324	-1.0852 (0.0393)*	0.2994	0.2394 (n=18)	0.4243	-0.6142 (0.0051)*	0.2191	0.3959 (n=18)
T4	0.5777	-2.1826 (0.0322)*	0.4888	0.2559 (n=18)	1.4938	-1.8824 (0.0001)*	0.3964	0.6151 (n=18)

* - At 5% level of significance

Effect of Disinfectant

The slopes (âs) for all systems using chlorine disinfection were more negative than the slopes for chloramines for all four treatments. Within each treatment group, the effects of disinfection methods were compared via covariance analysis. The model for the analysis is given by equation 7 with log CFU3 as the dependent variable. The covariance analysis showed that within treatment group T1, the linear effect of chlorine on log CFU3 was significantly more negative than the linear effect of chloramine on log CFU3 ($p = 0.0072$). For the other three treatment groups, even though the chlorine disinfection method yielded slopes that were more negative than for chloramine disinfection method, the linear effects were not significantly different ($p = 0.1478, 0.3484, 0.7587$, respectively for T2, T3 and T4).

7.0 BIOFILM ANALYSIS

As with the bulk phase analysis, models of the form shown in equations 3 and 4 were utilized to evaluate the effect of chlorine and chloramine on biofilm concentrations. The effect of these disinfectants on EPI, CFU15 and CFU3 were evaluated.

7.1 EPIFLUORESCENCE

In this section the effects of chlorine and chloramine are considered on the concentrations of epifluorescence and bacteria on cement, PVC and polyethylene.

Chlorine Disinfection

Table 7 contains the estimated model parameters, estimated error variance $ó^2$, and the R^2 for each of the four treatments, T1, T2, T3 and T4 and a given pipe material, using chlorine as a disinfectant. The p-values for the hypothesis that the parameter â is negative are given in parenthesis.

For cement the model parameter â was significantly negative for all treatments ($p<0.05$). For all four treatment types, the log EPI on the cement material decreased as chlorine level increases.

For polyethylene the model parameter â was also significantly negative for all treatments ($p<0.05$). Therefore, for all four treatments, log EPI on the wall decreased significantly as chlorine level increased. However, the slopes for Treatments T2 and T3 were smaller than the slopes for the other two treatments. The model R^2s for treatments T2 and T3 are lower than the model R^2s for treatment trains T1 and T4.

For PVC the model parameter â was significantly negative for all the treatments except for T2 ($p<0.05$). This means that for T1, T3 and T4 the amount of EPI on the PVC wall decreased significantly as the chlorine level increased. Even though the slopes for T2 and T3 were almost the same, one is significant and the other is not. The error

TABLE 7. MODEL PARAMETERS FOR WALL DENSITIES (EPIFLUORESENCE) USING CHLORINE DISINFECTION FOR CEMENT, POLYETHYLENE AND PVC

Treatment Type	Cement				Polyethylene				PVC			
	á	$\hat{a}_i$	$ó^2$	R^2	á	$\hat{a}_i$	$ó^2$	R^2	á	$\hat{a}_i$	$ó^2$	R^2
T1	6.626	-4.583 (0.0001)*	0.358	0.5736 (n=50)	6.835	-3.250 (0.0001)*	0.1158	0.6761	6.535	-1.952 (0.0001)*	0.1317	0.3986 (n=50)
T2	6.643	-3.543 (0.0134)*	1.062	0.4735 (n=12)	6.503	-1.142 (0.0470)*	0.1938	0.3389	6.565	-1.002 (0.1182)	0.2617	0.2261 (n=12)
T3	6.702	-1.996 (0.0001)*	0.1004	0.7678 (n=17)	6.730	-0.956 (0.0319)*	0.2119	0.2566	6.587	-1.052 (0.0016)*	0.0909	0.4725 (n=18)
T4	6.629	-3.9448 (0.0001)*	0.3202	0.6316 (n=18)	7.019	-2.168 (0.0017)*	0.1786	0.4923	6.540	-1.849 (0.0001)*	0.0628	0.6575 (n=18)

* - At 5% level of significance

variability ($ó^2$) for treatment T2 was higher than for T3, which may explain the insignificance of the slope for T2. Moreover, R^2 for T2 was lower than the R^2 for T3.

Chloramine Disinfection

Table 8 shows that the slopes of Treatment T3 were not significantly different from zero for cement and PE. This means that for treatment T3 and for PE and cement, chloramine had no significant (linear) effect on the reduction of EPI. Thus, treatment type T3 was dropped from further analysis. Covariance analysis was used to compare the other three treatments for each of the three wall types. For cement and PVC, no significant differences were seen among the three slopes for T1, T2 and T4. The slope of T4 was significantly more negative than the slope of T1 for the wall type PE ($p=0.0347$). Moreover, no significant difference was seen between the slope.

Comparison of Disinfectants

The slopes (b) for all three materials using chlorine disinfection method were more negative than the slopes of the corresponding materials using chloramines. Covariance analysis was performed to compare the methods for each of the four treatments (equation 7). The linear effect of chlorine on log EPI was significantly greater than the linear effect of chloramine on log EPI for the cement for all treatments ($p<0.05$ for each comparison). The effect of chlorine on log EPI was significantly greater than the effect of chloramine on log EPI for PE and PVC, for treatments T1, T3 and T4. No significant difference was seen between the disinfection methods for treatment T2.

CFU15

In this section, the effects of chlorine and chloramine are evaluated against the counts of organisms cultured for 15 days (CFU15) on cement, polyethylene and PVC.

Chlorine Disinfection

Table 9 contains the estimated model parameters, estimated error variance $ó^2$, and the R^2 for each of the four treatments, T1, T2, T3 and T4 and the pipe material, using chlorine as a disinfectant. The p-values for the hypothesis that the parameter â is zero, are given in parenthesis.

For cement the model parameter â was significantly negative for T1, T3 and T4 ($p<0.05$). For these three treatment types, the CFU15 on the cement material decreased as the chlorine level increased. The slope for treatment T2 was not significantly different from zero and the model R^2 is close to zero.

For polyethylene the model parameter â was significantly negative only for T1 ($p<0.05$). This means that for T1, CFU15 on the wall decreased significantly as chlorine levels increased.

TABLE 8. MODEL PARAMETERS FOR WALL DENSITIES (EPIFLUORESENCE) USING CHLORAMINE DISINFECTION FOR CEMENT, POLYETHYLENE AND PVC

Treatment Type	Cement				Polyethylene				PVC			
	á	$\hat{a}_i$	$ó^2$	R^2	á	$\hat{a}_i$	$ó^2$	R^2	á	$\hat{a}_i$	$ó^2$	R^2
T1	6.694	-0.644 (0.0001)*	0.0799	0.5523 (n=51)	6.756	-0.568 (0.0001)*	0.0832	0.4723	7.700	-0.766 (0.0001)*	0.0788	0.6316 (n=53)
T2	6.667	-0.807 (0.0014)*	0.1063	0.5550 (n=15)	6.717	-0.849 (0.0005)*	0.1070	0.5367	6.582	-0.801 (0.0036)*	0.1393	0.4428 (n=17)
T3	6.465	-0.016 (0.8735)	0.0653	0.0017 (n=17)	6.479	-0.012 (0.8988)	0.0504	0.0011	6.593	-0.425 (0.0059)*	0.0923	0.4059 (n=17)
T4	6.618	-0.772 (0.0040)*	0.1508	0.4137 (n=18)	7.076	-0.994 (0.0001)*	0.0983	0.6426	6.612	-0.543 (0.0175)*	0.1203	0.3050 (n=18)

TABLE 9. MODEL PARAMETERS FOR WALL DENSITIES FOR CHLORINE DISINFECTION REGRESSED AGAINST CFU15 FOR CEMENT, POLYETHYLENE AND PVC

Treatment Type	Cement				Polyethylene				PVC			
	á	â	$ó^2$	R^2	á	â	$ó^2$	R^2	á	â	$ó^2$	R^2
T1	5.082	-4.612 (0.0001)*	1.095	0.3052 (n=51)	5.447	-1.837 (0.0022)*	0.3546	0.1790 (n=50)	5.176	-0.8393 (0.2164)	0.4935	0.0317 (n=50)
T2	4.562	-0.920 (0.6925)	1.699	0.0182 (n=11)	4.615	-1.335 (0.4970)	1.190	0.00527 (n=11)	4.791	-0.2648 (0.9047)	1.155	0.0017 (n=11)
T3	5.386	-1.486 (0.0480)*	0.6173	0.2227 (n=18)	5.323	0.3686 (0.3993)	0.2321	0.0448 (n=18)	5.335	0.5442 (0.1041)	0.1273	0.1565 (n=18)
T4	5.337	-4.246 (0.0092)*	1.161	0.3539 (n=18)	5.219	0.4906 (0.8049)	2.1535	0.0039 (n=18)	5.354	-0.1861 (0.6654)	0.1007	0.0120 (n=18)

* - At 5% level of significance

For PVC the model parameter â was not significantly negative for any of the four treatments ($p>0.10$). This means the slopes for T1, T2, T3 and T4 were not significantly different from zero.

Chloramine Disinfection

Table 10 gives the estimated model parameters, estimated error variance $ó^2$, and the model R^2 for each of the four treatments, T1, T2, T3 and T4 using chloramine disinfectant. For cement the model parameter â was significantly negative for all the treatments except T3 ($p<0.05$).

For polyethylene the model parameter â was significantly negative for T1, T2 and T4. Therefore, for all these treatments, the amount of CFU15 on the polyethylene wall decreased with increased chloramine level in the water.

For PVC the model parameter â was significantly negative for T1, T2 and T3 ($p<0.05$). Therefore, for these three treatments, the amount of CFU15 on the PVC wall decreased with increased chloramine level in the water. For treatment T4 there was no significant linear effect of chloramine on 15-day CFU.

As some of the linear effects were not significantly negative for some of the treatments, comparative analysis among treatments within a disinfection method was not performed for any of the wall materials.

Comparison of Disinfectants

Most of the slopes (â) for all three materials using chlorine disinfection method were more negative than the slopes of the corresponding materials of the chloramine disinfection method.

CFU3

In this section the effects of chlorine and chloramine on biofilm concentrations for CFU3 are evaluated.

Chlorine Disinfection

Table 11 contains the estimated model parameters, estimated error variance s^2, and the R^2 for each of the four treatments, T1, T2, T3 and T4 and the pipe material, using chlorine as a disinfectant. The p-values for the hypothesis that the parameter â is negative are given in parenthesis.

For cement the model parameter â was significantly negative for T1, T2 and T4 ($p<0.05$). For these three treatment types, the log CFU3 on the cement material decreased as chlorine level increased. The slope for treatment T3 was not significantly different from zero and the model R^2 was close to zero.

TABLE 10. MODEL PARAMETERS FOR WALL DENSITIES FOR CHLORAMINE DISINFECTION REGRESSED AGAINST CFU15 FOR CEMENT, POLYETHYLENE AND PVC												
Treatment Type	Cement				Polyethylene				PVC			
	á	â	$ó^2$	R^2	á	â	$ó^2$	R^2	á	â	$ó^2$	R^2
T1	5.350	-1.059 (0.0001)*	0.5349	0.3257 (n=54)	5.591	-0.6244 (0.0004)*	0.3246	0.2167 (n=54)	5.385	-0.6937 (0.0001)*	0.3263	0.4004 (n=53)
T2	4.995	-1.303 (0.0133)*	0.6030	0.3263 (n=18)	5.518	-1.0406 (0.0095)*	0.3436	0.3514 (n=18)	5.194	-0.5268 (0.0103)*	0.0902	0.0466 (n=17)
T3	5.272	-0.5297 (0.2085)	0.9948	0.0970 (n=18)	5.544	-0.2708 (0.1782)	0.2253	0.1103 (n=18)	5.533	-0.6169 (0.0228)*	0.3097	0.3863 (n=17)
T4	5.594	-3.653 (0.0416)*	1.417	0.2348 (n=18)	5.920	-1.3187 (0.0001)*	0.1939	0.6159 (n=18)	5.469	-0.6157 (0.1272)	0.4188	0.2590 (n=18)

* - At 5% level of significance

For polyethylene the model parameter â was also significantly negative for T1, T3 and T4 ($p<0.05$). This means that for all these three treatments log CFU3 on the pipe wall decreased significantly as chlorine levels increase. However, the slope for treatment T2 was not significantly different from zero and the model R^2 was close to zero.

For PVC the model parameter â was significantly negative for all the treatments except T2 and T4 ($p<0.05$). This means that for T1 and T3, the log CFU3 on the PVC wall decreased significantly as the chlorine level increased.

Chloramine Disinfection

Table 12 gives the estimated model parameters, estimated error variance $ó^2$, and the model R^2 for each of the four treatments, T1, T2, T3 and T4 using chloramine as a disinfectant. For cement the model parameter â was significantly negative for all the treatments except T2 ($p<0.05$).

For polyethylene the model parameter â was significantly negative for all the treatments. Therefore, for all four treatments, the amount of CFU3 on the polyethylene wall decreased with increasing chloramine levels in the water.

For PVC the model parameter â was significantly negative for T1, T3 and T4 ($p<0.05$). Therefore, for these three treatments, the concentration of CFU3 on PVC decreased with increasing chloramine levels in the water.

As some of the linear effects were not significantly negative for some of the treatments, comparative analysis among treatments within a disinfection method was not performed for any of the wall materials.

Comparison Among Disinfectants

Most of the slopes (â) for all three materials using chlorine disinfection method were more negative than the slopes of the corresponding materials using chloramine disinfection. Covariance analysis was performed to compare the methods for treatments which have significant linear effects (equation 7). The linear effect of chlorine on log CFU3 was significantly more negative than the linear effect of chloramine on log CFU3 for cement and treatments T1 and T4 ($p<0.05$, for both comparison). For each of the treatments T1, T3 and T4 and for the wall type PE the disinfection methods were compared and the results showed no significant difference between methods. The linear effect of chlorine on log CFU3 was greater than the linear effect of chloramine on log CFU3 for PVC wall types and for treatment T1 ($p<0.05$ for each comparison). No significant difference was seen between the methods for treatment T3.

TABLE 11. MODEL PARAMETERS FOR WALL DENSITIES FOR CHLORINE DISINFECTION REGRESSED AGAINST CFU3 FOR CEMENT, POLYETHYLENE AND PVC

Treatment Type	Cement				Polyethylene				PVC			
	á	â	$ó^2$	R^2	á	â	$ó^2$	R^2	á	â	$ó^2$	R^2
T1	4.209	-7.044 (0.0001)*	0.1.790	0.3880 (n=50)	4.691	-3.809 (0.0005)*	1.160	0.2220 (n=50)	4.467	-3.916 (0.0001)*	0.9216	0.2760 (n=50)
T2	4.233	-2.665 (0.0055)*	0.4354	0.5539 (n=12)	4.676	0.0520 (0.9542)	0.5954	0.0003 (n=12)	4.599	-2.770 (0.6998)	0.3708	0.0155 (n=12)
T3	3.740	-1.313 (0.2171)	2.155	0.0990 (n=17)	4.573	-3.329 (0.0006)*	0.4306	0.5512 (n=17)	4.324	-3.221 (0.0128)*	1.6928	0.3291 (n=18)
T4	4.258	-9.0393 (0.0001)*	1.139	0.7168 (n=18)	4.515	-4.294 (0.0122)*	1.2545	0.3513 (n=17)	4.016	-2.887 (0.1209)	1.753	0.1436 (n=18)

* - At 5% level of significance

TABLE 12. MODEL PARAMETERS FOR WALL DENSITIES USING CHLORAMINE DISINFECTION FOR CEMENT, POLYETHYLENE AND PVC (CFU3)

Treatment Type	Cement				Polyethylene				PVC			
	á	â	$ó^2$	R^2	á	â	$ó^2$	R^2	á	â	$ó^2$	R^2
T1	4.286	-2.319 (0.0001)*	1.121	0.5350 (n=48)	4.634	-1.786 (0.0001)*	1.172	0.3843 (n=53)	4.513	-1.844 (0.0001)*	1.173	0.4004 (n=53)
T2	4.038	-0.176 (0.7864)	0.9612	0.0077 (n=12)	4.558	-1.9652 (0.0154)*	1.337	0.3325 (n=17)	4.235	-0.2614 (0.4054)	0.2413	0.0466 (n=17)
T3	3.999	-1.815 (0.0166)*	2.754	0.3264 (n=17)	4.615	-1.834 (0.0007)*	1.088	0.5456 (n=17)	4.527	-1.762 (0.0077)*	1.720	0.3863 (n=17)
T4	4.562	-3.653 (0.0008)*	2.226	0.5173 (n=18)	5.249	-2.750 (0.0012)*	1.411	0.4895 (n=18)	4.405	-2.1652 (0.0310)*	2.397	0.2590 (n=18)

* - At 5% level of significance

8.0 PREDICTING BIOFILM DENSITIES

As the sample sizes for the treatments T2, T3 and T4 are small, only treatment T1 was considered to predict EPI as a function of chlorine (or chloramine). Because of the low model R^2 for CFU3 and CFU15 no attempt was made to develop a predictive model for these bacterial concentrations. The observed chlorine (or chloramine) concentration of six randomly selected EPI values were used in the regression models (equations 3 and 4) to predict the corresponding EPI values. Tables 13 through 16 give the six observed EPI values along with the predicted values for bulk phase water and for each of the three wall materials and the two disinfection methods. In Tables 13 and 14, the first column gives the loop designation, column two is the experimental run, column three is the free residual chlorine, column four is the log of the concentration of the organism characterized by epifluoresence, column five is the predicted value for epifluoresence, and columns six and seven are the upper and lower 95% confidence intervals respectively. In Tables 15 and 16, column 1 contains the wall material, column 2 identifies the loop, and column 3 is the run. All of the predicted values fall within the upper and lower 95% confidence levels. Figures 7-10 give the plots of predicted log EPI against observed log EPI for bulk phase and for cement wall type. It should be noted that the predictive models for epifluorescence versus chlorine and chloramine can be used with confidence over the intervals over which they have been developed.

TABLE 13. OBSERVED vs. PREDICTED EPI (Bulk Phase, Cl_2)

Loop	Run	CL Free	Log EPI	Predicted	U95	L95
A1	1	0.35	3.677399	3.03060	3.83488	2.22631
A1	3	0.20	2.86362	4.07394	4.84759	3.30029
A1	5	0.15	4.38917	4.42172	5.19026	3.65317
A2	7	0.00	5.41664	5.66506	6.23431	4.69581
A1	9	0.40	2.86362	2.68282	3.50210	1.86354
A1	11	0.24	3.67765	3.79571	4.57533	3.01610

TABLE 14. OBSERVED vs. PREDICTED EPI (Bulk Phase, NH_2Cl)						
Loop	Run	CL Comb	Log EPI	Predicted	U95	L95
A1	2	1.00	4.37659	4.26388	4.84267	3.68508
A3	4	0.00	5.60638	5.62113	6.19888	5.04339
A2	6	0.10	5.44716	5.48541	6.06056	4.91026
A2	8	0.20	4.79934	5.34968	5.92283	4.77653
A3	10	0.05	5.55510	5.55327	6.12964	4.97690
A3	12	0.04	5.81023	5.56684	6.14348	4.99021

TABLE 15. OBSERVED vs. PREDICTED EPI (Wall, Cl_2)							
Material	Loop	Run	CL Free	Log EPI	Predicted	U95	L95
Cement	A1	1	0.35	4.60207	4.89311	5.99369	3.79253
Cement	A1	3	0.20	3.90312	5.66201	6.71276	4.61125
Cement	A2	3	0.00	6.78533	6.68720	7.72904	5.64536
Cement	A1	7	0.40	5.79239	4.63681	5.76143	3.51220
Cement	A1	9	0.40	6.38561	4..63681	5.76143	3.51220
Cement	A1	11	0.24	5.96848	5.45697	6.51756	4.39638
PE	A2	1	0.00	6.42813	6.83527	7.41540	6.25514
PE	A2	3	0.00	7.06070	6.83527	7.41540	6.25514
PE	A1	7	0.35	5.53148	5.77625	6.37686	5.17565
PE	A1	9	0.40	5.85733	5.62496	6.23602	5.01391
PE	A3	9	0.00	7.39041	6.83527	7.41540	6.25514
PE	A2	11	0.01	6.82866	6.80501	7.38439	6.22563
PVC	A1	1	0.35	5.53148	5.82793	6.45532	5.20053
PVC	A2	3	0.00	6.84261	6.55780	7.15414	5.96145
PVC	A1	7	0.40	6.41996	5.72366	6.36437	5.08294
PVC	A1	9	0.40	5.56820	5.72366	6.36437	5.08294
PVC	A1	11	0.24	5.86923	6.05731	6.66274	5.45188
VC	A2	11	0.00	6.03342	6.55780	7.15414	5.96145

TABLE 16. OBSERVED vs. PREDICTED EPI (Wall, NH_2Cl)							
Material	Loop	Run	CL Comb	Log EPI	Predicted	U95	L95
Cement	A2	2	0.80	5.84510	6.25685	6.75009	5.76361
Cement	A1	6	1.20	6.00000	604219	6.54868	5.53570
Cement	A1	8	1.00	5.59107	6.14952	6.64830	5.65073
Cement	A2	8	0.20	6.57171	6.57884	7.06893	6.08875
Cement	A1	12	0.98	5.43136	6.16025	6.65838	5.66212
Cement	A3	12	0.01	7.06258	6.68080	7.17417	6.18743
PE	A2	2	0.80	5.96379	6.32874	6.93867	5.71880
PE	A1	6	1.20	5.91908	6.13326	6.75883	5.50769
PE	A3	6	0.05	6.80414	6.69525	7.30475	6.08575
PE	A1	10	1.00	5.73239	6.23100	6.84746	5.61454
PE	A1	12	1.01	6.58206	6.22611	6.84297	5.60926
PE	A2	12	0.10	6.93702	6.67082	7.27916	6.06248
PVC	A1	2	0.90	6.35411	6.00615	6.60540	5.40689
PVC	A3	4	0.00	7.19201	6.68436	7.28343	6.08529
PVC	A1	6	1.20	5.90849	5.78008	6.39125	5.16890
PVC	A2	8	0.20	6.23805	6.53365	7.12808	5.93921
PVC	A1	12	0.98	5.69020	5.94586	6.54774	5.34399
PVC	A2	12	0.05	6.89265	6.64668	7.24434	6.04902

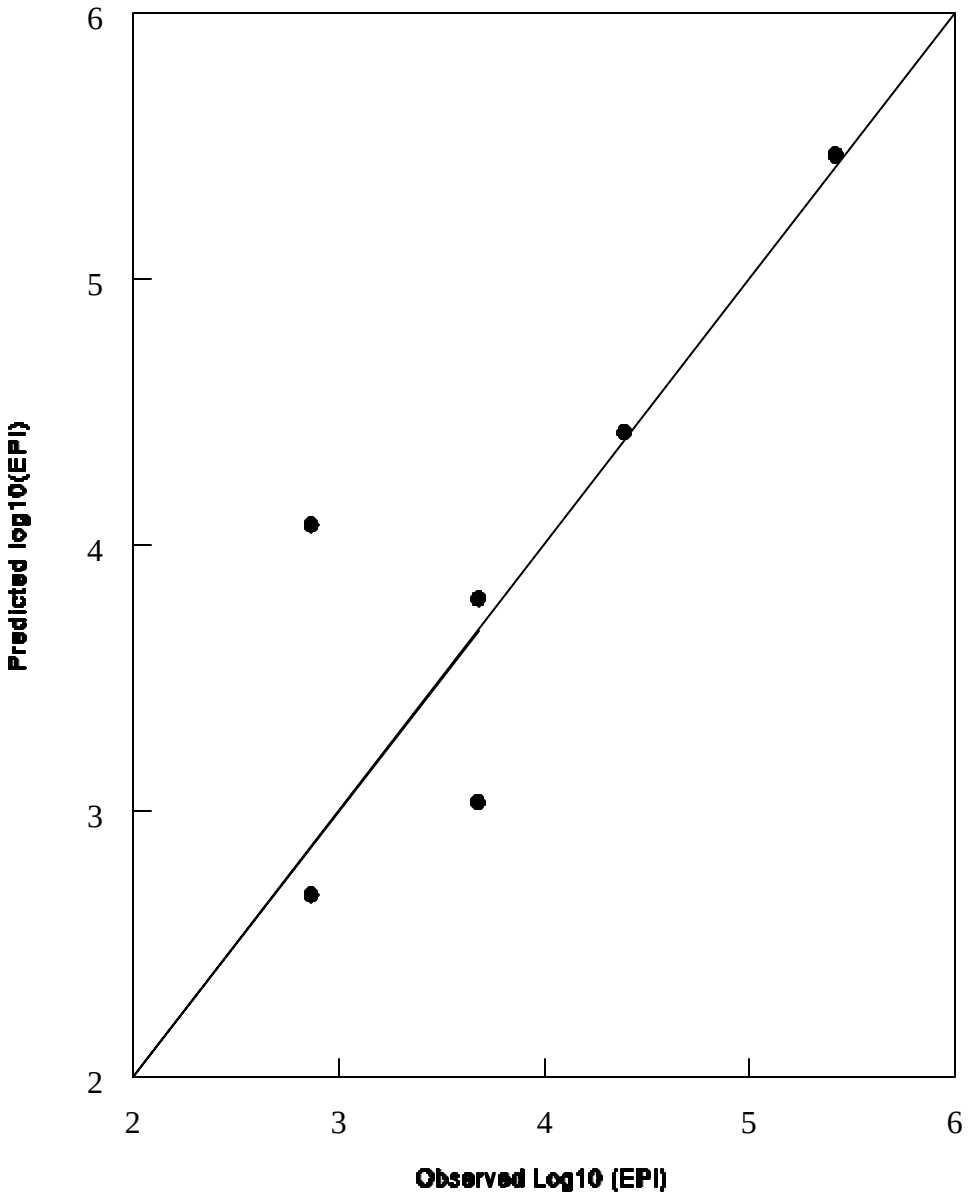

Figure 7. Observed vs. Predicted EPI (Chlorine Disinfection, Bulk Phase)

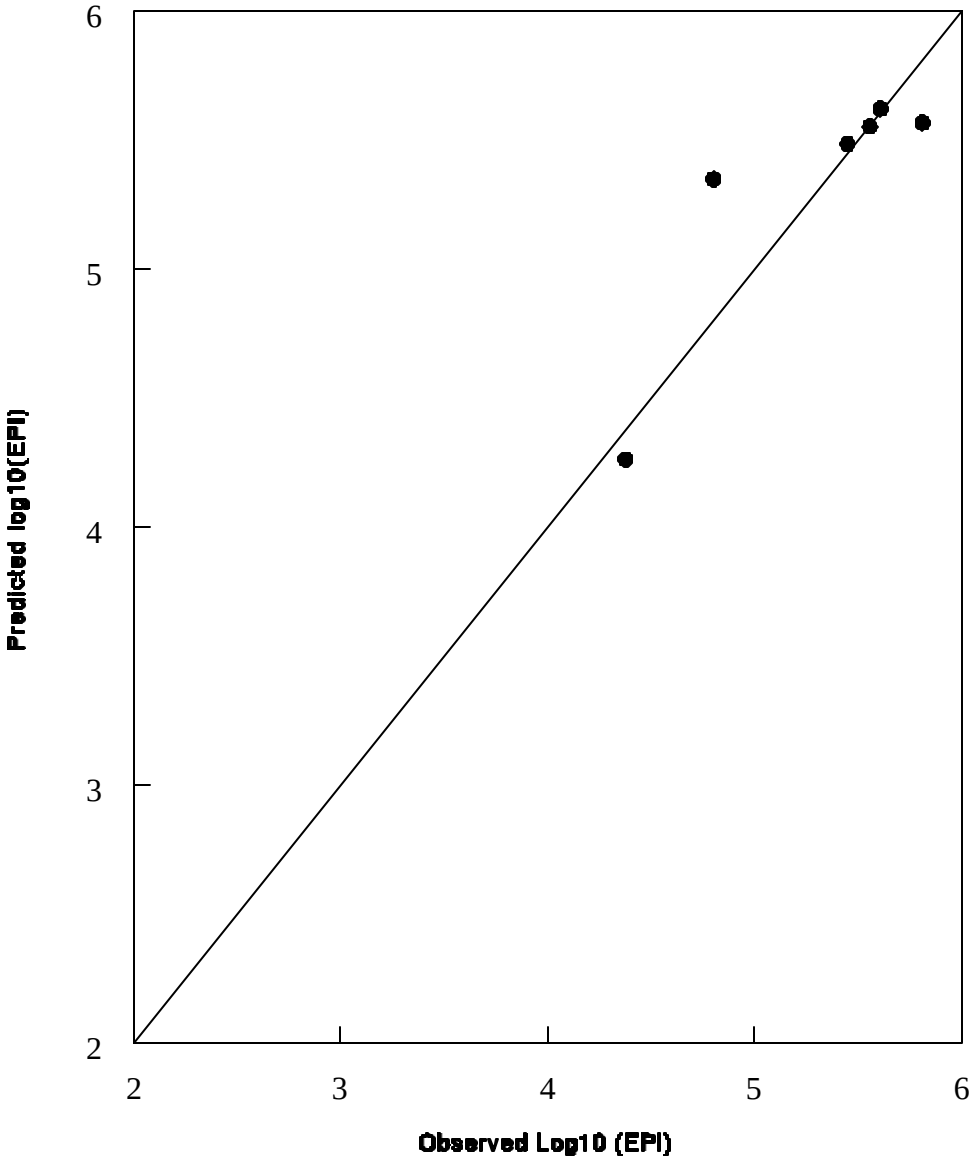

Figure 8. Observed vs. Predicted EPI (Chloramine Disinfection, Bulk Phase)

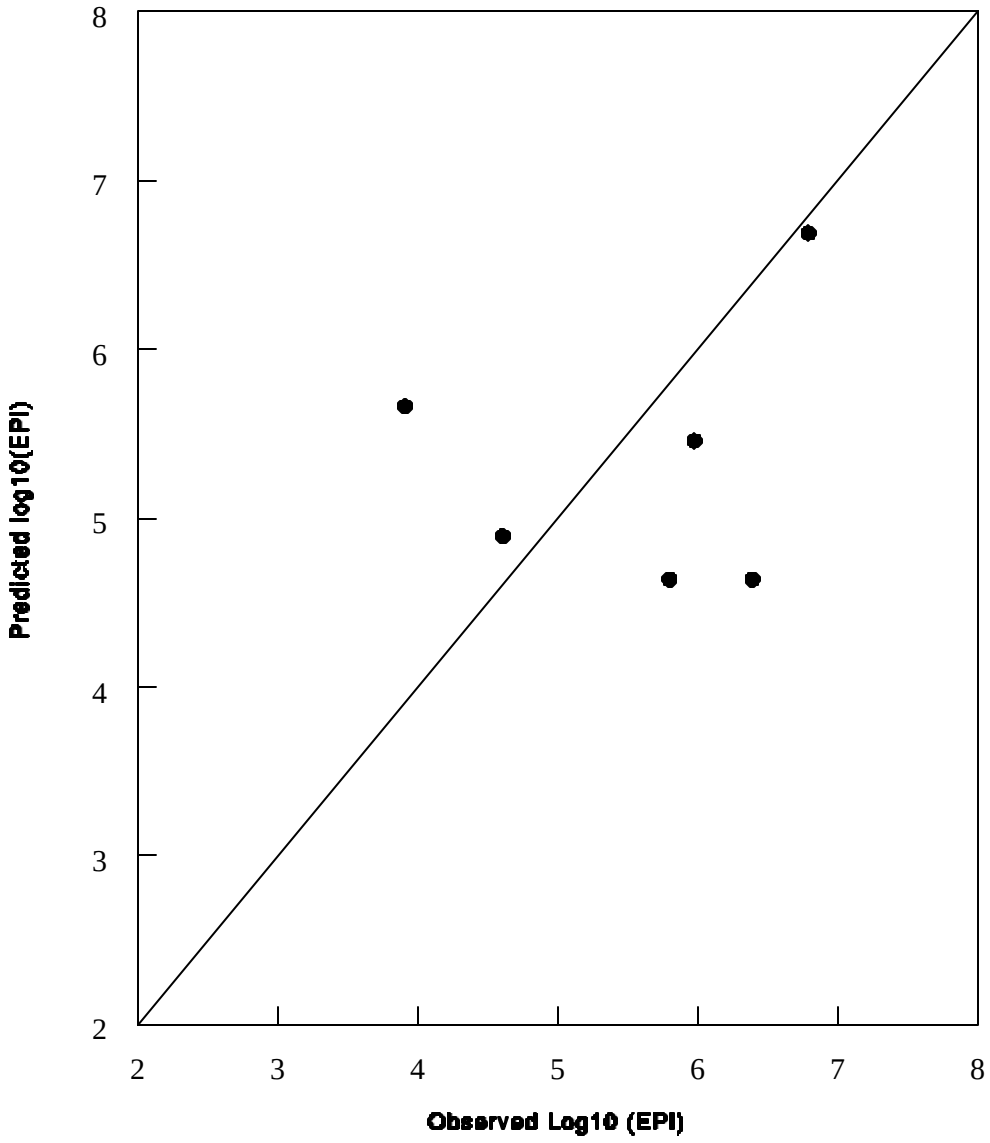

Figure 9. Predicted vs. Observed EPI (Chlorine Disinfection, Cement Wall)

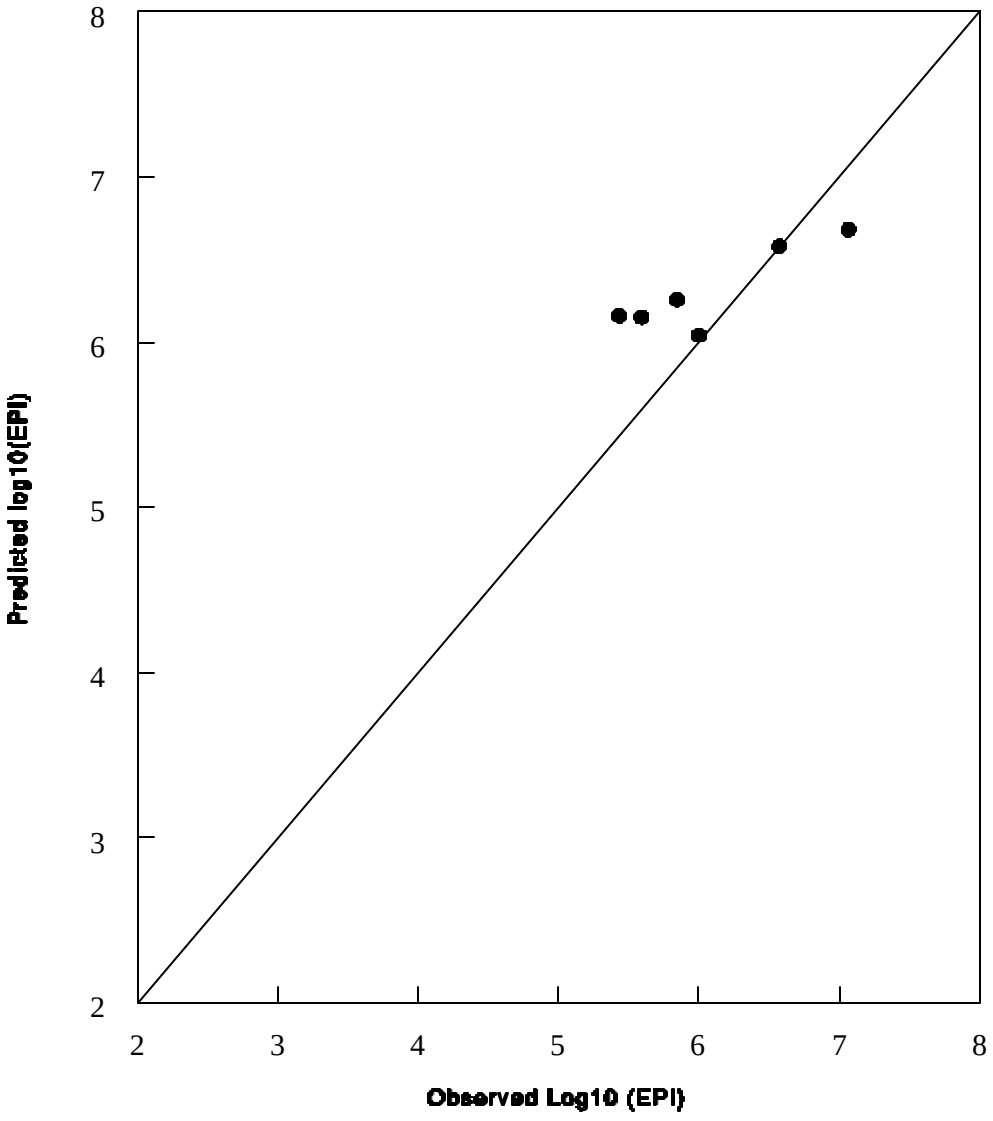

Figure 10. Predicted vs. Observed EPI (Chloramine Disinfection, Cement Wall)

9. SUMMARY AND CONCLUSIONS

Four treatment trains (T1, T2 , T3 and T4) were compared in this study. T1 represents a standard pre-chlorinated treatment process, while T2 , T3 and T4 are different pre-disinfection methods. Each treatment received a final disinfection with either chlorine (Cl_2) or chloramine (NH_2Cl).

A total of twelve experimental runs were made. Each run lasted approximately one month and consisted of parallel operation of one of the test treatment trains (T2 , T3 or T4) and the control treatment train T1. The same final disinfection method, either chlorination or chloramination, was used for each of the two treatments. Treated water from each of the treatment processes was distributed through a pipe loop system consisting of two sets of three pipe loops each, designated loops A and B, such that the output from T1 circulated through the A loops and that of the test treatment (T2, T3 or T4) through the B loops.

Removable coupons consisting of three different types of pipe wall material, cement, polyvinyl chloride and polyethylene, were inserted flush with the wall of each pipe loop. Measurements of bacterial growth on each type of material were made concurrently with measurements of the distribution water quality bulk parameters. Bacterial measurements consisted of three-day and fifteen-day CFUs and epifluorescence measurements for bacteria in the biofilm on the pipe walls and in the bulk phase.

Other water quality variables measured included pH, temperature, dissolved organic carbon (DOC), biodegradable DOC (BDOC), particle count per mL by four size ranges, total organic halides (TOX), and trihalomethanes (THM).

Each pipe loop was 10 cm in diameter and 31 m in length. Water velocity was 1 m/s with configuration and operation of the system producing a residence time of 24 hours in each loop for a total of 72 hours for the system. As a consequence, only a small portion of water was transferred from a given loop to each succeeding loop during a given flow cycle. Thus the water flow entering a pipe loop (A or B) includes both fresh feed and the recycle stream. The effect of this water flow in the water quality parameters was studied by including the recycle ratio R, where R = {volume of water returned to a pipe loop entrance /volume leaving the loop}. The measurements of water quality parameters within the loops were made after a period of equilibrium was attained in the system.

Both chlorine and chloramine reduced the bacterial growth as measured by epifluoresence direct count on the pipe wall and in the bulk phase, but chlorine disinfection was clearly more effective than chloramine disinfection. For both disinfection methods, the control group T1 was more effective than the other three treatment trains in terms of reducing the growth of epifluoresence in the bulk phase. Based on epifluoresence direct count as a measure of biofilm density the results clearly showed that chlorine was much more effective in reducing biofilm than chloramine. The slopes of the equations were consistently more negative for chlorine than for chloramine.

For CFU15 chlorine disinfection yielded consistently more negative slopes for all cases where the slopes were significant for both disinfectants. CFU3 yielded the same results.

Based on these results, it can be concluded that chlorine was consistently more effective as a disinfectant for controlling biofilm than chloramine.

Using epifluoresence direct counts, simple predictive models were developed. In all cases the predictions were within the 95% confidence intervals for the data. It is the authors' opinion that these models should be limited to the ranges of data over which the analysis was conducted.

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