THE EFFECT OF BIOFILM ON THM FORMATION IN CHLORINATED DRINKING WATER OF A PVC PIPE DISTRIBUTION SYSTEM

by

Karen C.W. Chan

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Abstract

This research investigated the interaction between chlorine residual in drinking water and the interior surface of polyvinyl chloride (PVC) distribution system pipes. The major objectives were to establish evidence of a biofilm effect on THM production, and to examine the effectiveness of chlorine on inactivating biofilm bacteria associated with PVC surfaces.

This project employed a modified SDS test, known as Material Specific Simulated Distribution System (MS-SDS) test (Brereton, 1998), to evaluate the chloroform formation in pipe-incubated water samples, collected at a treatment pilot plant in Vancouver, British Columbia.

Although the ultrafiltration membrane system installed produced an excellent finished water quality in terms of turbidity and some reduction in THM formation, the process did not selectively remove chloroform precursors. However, statistical analysis on MS-SDS test results showed that whatever impact the treatment process might have on chloroform formation in the bulk water was "drowned out" by the pipe environment. Pipe environment becomes the dominant effect on chloroform production, with residence time being the main factor of interest. Under chlorine-limited conditions, pipe wall contribution increases THM levels in the bulk water phase during the short incubation period (5 hours), but has a negative overall effect on chloroform production during the long incubation period (4 days).

Adsorption was identified as the surface mechanism responsible for the removal of THM precursors from the bulk water phase. Heterotrophic plate counts (HPCs) carried out on biofilm scrapings from the pipe surfaces revealed that exposure to chlorine stress could lead to bacterial inactivation or biofilm regrowth. Under stress, biofilm bacteria can take advantage of some form of biological resistance that resulted in additional chloroform formation in the bulk water. Humic substances previously adsorbed onto biofilm surface are released following cell damage repair; these additional

substances are now available to participate in THM formation in the bulk water phase. A pictorial model summarizes the major mechanisms interacting in a PVC pipe environment.

Experiments on re-chlorination effect showed that the practice of re-chlorination created more favorable reaction conditions of slow-reacting THM formation. Although booster disinfection has the potential of becoming an effective prevention to biofilm regrowth, it may also be out-weighed easily by other *in-situ* factors, such as the organic (and inorganic) composition of the water and the dynamic state of the biofilm.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGMENTS

Chapter 1

INTRODUCTION

Drinking water is transported from a treatment facility to the consumer's tap through a distribution system comprised of long pipes with large surface areas in contact with the drinking water. As such, while the water is transported through the system, the microbial water quality may deteriorate due to biofilm formation on the pipe walls. To protect the health of their customers, many water suppliers in the United States, the United Kingdom, and Canada distribute treated water with a disinfectant residual for the main purposes of inactivating pathogenic organisms and preserving the microbiological water quality during distribution.

The growth and persistence of bacteria in distribution systems are well documented. The bacteria typically grow on surfaces, attach themselves to pipe walls and persist as biofilm. Opportunistic pathogens such as *Legionella* species (spp.) (Wadowsky *et al.* 1982), *Mycobacterium* spp.(Engel *et al.* 1980), *Pseudomonas* spp. (Anderson *et al.* 1990) may colonize pipe surfaces, and may ultimately result in the spread of waterborne diseases. Even accumulation of non-pathogenic microorganisms is undesirable for a number of reasons: coliform and heterotrophic regrowth can place a utility in violation of regulations when biofilm organisms are sloughed into the water phase; the presence of bacterial biofilms may enhance corrosion of distribution pipe materials (Lee *et al.* 1980); iron bacteria may grow on ferrous metal surfaces and result in the presence of iron particulates in finished water (Ridgway and Olson 1981; Singley and Ahmadi 1985); and colonization by organisms such as *Actinomycetes* or fungi may result in taste and odour problems (Olson 1982).

In the United States, the application of chlorine as disinfectant residual has remained most widespread since its introduction in Jersey City in 1908, and has achieved much success in the reduction of waterborne diseases. This approach, however, has several disadvantages. Chlorine decay, as a result of reactions with pipe materials and

water components, reduces the disinfectant efficiency; and bacteria embedded in extracellular polymer substances (EPS) produced by biofilm on pipe surfaces may be shielded from the disinfectant. Consequently, even in the presence of a chlorine residual, coliforms have been observed in drinking water samples (Smith *et al.* 1991). Moreover, chlorination degrades the taste and odour of the water. Free chlorine, itself, is perceptible even at a concentration < 0.05 mg/L, and such complaints are serious signals from customers in an increasingly market-oriented society. Chlorination also leads to the formation of disinfection-by-products (DBPs), such as trihalomethanes (THMs), whose toxic properties and potential carcinogenic risk to human health have been undergoing intense scrutiny from scientists and regulators during the last 20 years.

The majority of research to date in the area of drinking water has focused on the process of treatment of water prior to distribution; however, consideration to balance beneficial and detrimental effects of a disinfectant residual on water quality in the distribution system is attracting increased attention. With this premise, the present study concentrated on the downstream aspects of DBP evolution and, in particular, the production of chloroform by-product in the presence of biofilm associated with polyvinyl chloride pipes.

Chapter 2

LITERATURE REVIEW

2.1 Health risks

Disinfection of drinking water with chlorine is known to produce a variety of chlorinated derivatives by reaction of the chlorine with the organic substances found in the source water. Recent studies suggest that the reaction of chlorine with natural aquatic humic material is a likely source of mutagen formation in drinking water (Bull and Kopfler 1991; Horth 1989; Coleman *et al.* 1984). The identification of a number of mutagenic and carcinogenic chemicals, produced during water chlorination, has raised concerns over potential genetic and carcinogenic hazards to the human population. The extremely important mutagen MX, or 3-chloro-4-(dichloromethyl)-5 hydroxy-2(5H)-furanone, has been detected as a by-product of chlorination of aquatic humic substances (Kronberg 1999; Xu *et al.* 1997; Johnson *et al.* 1989). In the mid-1970s, the U.S. Environmental Protection Agency published results of a national survey that indicated chloroform, a product resulting from chlorine application, was a ubiquitous substance in drinking water (Symons *et al.* 1975). In 1976, the National Cancer Institute (NCI) studies indicated that chloroform was carcinogenic in mice and rats (National Cancer Institute 1976). The long-term carcinogenicity study by NCI reported obvious liver tissue damage and kidney tumors. Another well conducted study, with detailed documentation, was performed by Jogensen *et al.* in 1985. This 2-year study reported increased tumors in the kidney of male rats (as was observed in the study by NCI), but there was no increase in liver tumors in female mice. Epidemiological studies on disinfected drinking water have since focused on the carcinogenic outcomes, and the large number of epidemiological data have indicated an apparent association between cancer mortality and exposure to chlorinated surface waters. However, the specific association(s) and the possible magnitude of the association are still unclear and inconclusive (Bull *et al.* 1995).

Since disinfectant residuals are often added during distribution, the potential for *in vivo* production of mutagenic products formed by the reaction of ingested residual

chlorine, with endogenous organic material, is another area of major concern related to public health risks. Halogenated compounds, with known mutagenic and carcinogenic properties, have been shown to be formed *in vivo* following oral dosing of rats with sodium hypochlorite (Mink, Coleman *et al.* 1983). Revis *et al.* (1984) have described significant increases in heart weight and myocardial fibrosis, and decreased myocardial function in rabbits and pigeons, as a result of interaction between chlorine residual and a diet marginal in calcium to produce plasma cholesterol.

Guidelines for Canadian Drinking Water Quality stipulate an interim maximum acceptable concentration (IMAC) of 0.1mg/L for total THMs (Health and Welfare Canada 1996). The USEPA standard reduces the maximum contaminant level (MCL) for total THMs to 0.08 mg/L in Stage I and to 0.04 mg/L in Stage II of the D/DBP Rule (U.S. EPA 1994). The current regulatory policy for addressing potential carcinogenicity is, however, based almost exclusively on extrapolating the results of chronic, high-dose studies in rats or mice to potential risk to humans. There are a number of physical techniques quite capable of identifying known carcinogens existing at very low concentrations in drinking water; however, such analyses are incapable of presenting information regarding the possible interactions within a mixture that could alter the carcinogenic potential of a particular contaminant. This is certainly a major limitation, since the true effective carcinogenicity potential of a substance would be expected to depend on both the concentration and its relative carcinogenicity. The traditional approach of extrapolating from effects produced at maximally tolerated doses, without consideration of the underlying biological processes, can provide a misleading picture of the effects of low-dosage exposures. Recent data on chloroform indicate that the dose-response relationship for chloroform-induced tumors in rats and mice is nonlinear, based upon events secondary to cell necrosis, and subsequent regeneration, as the likely mode of action for the carcinogenic effects of chloroform. In light of these data, Golden *et al.* (1997) suggested that it is inappropriate to extrapolate cancer risk from high doses which produce necrosis and regenerative cell proliferation, to low doses which do not, with a model which presumes genotoxicity and a linear dose-response relationship.

2.2 The roles of humic substances and organic precursors

Organics in natural water can be fractionated into humic acid, fulvic acid, hydrophilic-acid and a neutral fraction. "Humic substances" is the general term for both humic and fulvic acids, and "aquatic humic substances" can account for approximately 50% to 75% of the natural organic matters present in many surface waters. Humic substances are also responsible for the colour in natural waters. The precursors for trihalomethanes (THMs) include many classes of organics: (1) compounds containing carboxylic groups such as humic, fulvic, hydroxybenzoic, citric, β-ketoglutaric, fumaric, malic, and pyruvic acids; (2) ketones and aldehydes, such as acetone, acetylacetone, acetaldehydes, and alcohols (Rook 1974; Morris 1975; Larson and Rockwell 1979). Among these precursors, humic and fulvic acids deserve special attention, since substantial evidence has been accumulated to show that they are the principal precursors of THMs present in drinking water as a result of chlorination (Rook 1977, 1980). The terms "humic acid" and "fulvic acid" do not refer to single compounds, but rather to wide ranges of compounds of similar origin and common properties. Humic acid varies in molecular weight from 10,000 to 200,000 daltons, while fulvic acid typically is in the range of < 1,000 daltons (Manahan 1989). It has also been reported that the relative contribution to the formation of THMs by the humic fraction is greater than that of the fulvic fraction because humic acids react more readily with chlorine (Babcock and Singer 1979). Moreover, recent work by Hureiki *et al.* (1994) have suggested that some amino acids found in surface waters may represent a significant fraction of the precursors of some organohalogenated disinfection by-products. Nevertheless, the specific precursors involved in the formation of THMs are still not quite clear, even though this has been the subject of much scientific research (Pomes *et al.* 1999; Mallevialle *et al.* 1990).

Although their many structural and chemical characteristics are still not well-known, humic materials are generally considered to be poorly biodegradable because of their large molecular size. However, studies have shown that humic substances are, in fact, biodegradable (Namkung and Rittmann 1987) and that biofilm bacteria are capable of using humic substances (Volk *et al.* 1997). These organic carbon compounds tend to adsorb onto surfaces, making them more available to surface-attached heterotrophic

bacteria for production of new cellular material (assimilation) and as an energy source (dissimilation). To address this concept, Camper *et al.* (1999) completed a series of experiments where humic materials were the sole carbon and energy source for biofilm bacteria. There is evidence for biological use of humic substances in an otherwise low-nutrient condition. Camper *et al.* (1999) believe that the humic substances become utilizable when they are adsorbed to surfaces. These molecules can then undergo a conformational change and expose the utilizable attached functional groups. The immobilization on the surface is also likely to permit the cells to use exoenzymes to attack the bonds between the bound amino acids, sugars, etc. and the skeletal structure of the humic molecule. It was also found that, when there was large amount of humic materials bound in the biofilm, supplementation of additional humic material did not influence the growth rate (Camper *et al.* 1999).

2.3 Biofilm

Characklis and Marshall (1990) defined a biofilm as an organic or inorganic surface deposit consisting of microorganisms, microbial products, and detritus. A more recent description of biofilm in an oligotrophic environment, offered by Costerton *et al.* (1994), consisted of microcolonies, or stacks, of bacteria embedded in an organic polymer matrix and interlinked by water channels.

Biofilms are formed in distribution system pipelines when microbial cells attach to pipe surfaces and multiply to form a slime layer on the pipe. Fletcher and Marshall (1982) observed that macromolecules tend to accumulate at solid-liquid interfaces to form a film, called the substratum, creating favourable growth conditions in an otherwise oligotrophic environment, where the bulk water phase is low in nutrients. The substratum tends to accumulate enough nutrients, from the water flowing through the distribution system, to support the growth of microorganisms as biofilms. Extracellular polysaccharides (EPS), produced by attached bacteria, help to anchor the bacteria to the pipe surface, and may also be a factor in nutrient capture (Fletcher and Marshall 1982). Biofilms are dynamic microenvironments, encompassing processes such as metabolism, growth, product formation, and finally detachment, erosion, or "sloughing" of the biofilm

from the surface. The biofilm may grow until the surface layers begin to slough off into the water. Attachment has been shown to be a major factor in resistance to disinfection (LeChevallier *et al.* 1988). It was postulated that the attachment of cellular masses to the interior surfaces of pipe wall most likely protected embedded cells from the action of some of the disinfectants, and these embedded cells could serve as a reservoir for subsequent spread through the system, following detachment or biofilm sloughing caused by changes in nutrient, disinfection, or hydrodynamic status (Anderson *et al.* 1990). The pieces of biofilm released into the water may continue to provide protection for the organisms, until they can colonize a new section of the distribution system (Characklis and Marshall 1990).

A survey of organisms found in biofilms published by the United States Environmental Protection Agency (1992) includes the following types of microorganisms: bacteria, opportunistic pathogens, *Actinomycetes*, fungi, protozoa, and other invertebrates. Bacteria comprise the largest portion of the biofilm population. Heterotrophic bacteria are the most common, and their source normally is not known. Among the heterotrophic bacteria are a group of closely related microorganisms, the coliforms, which are used as the primary microbial indicator of drinking water quality. Opportunistic pathogens include some species of *Mycobacteria*, *Pseudomonas* spp., *Legionella* spp., *Aeromonas* spp., *Favobacterium* spp., and some species of *Klebsiella* spp. and *Serratia*. Meanwhile, *Actinomycetes* and fungi can cause taste and odour complaints (Olson 1982).

The structure of biofilms from drinking water distribution systems is often very difficult to study because of the large amounts of detritus, corrosion products, and other inorganic matter. In spite of such difficulties, Camper *et al.* (1999) have noticed that disinfection has a profound influence on the general structure of the natural mixed-population biofilms. Chlorinated biofilms differ in structure from non-chlorinated biofilms, but often the number of cells is similar. Non-chlorinated biofilm cells are distributed as clumps and single cells across the entire surface. Intact chlorinated biofilm cells tend to remain only in clumps. The biofilm is more patchy and the cells are

approximately 50% smaller than those in non-chlorinated biofilm. There is also a shift in morphology from longer rod-shaped cells to more rounded organisms.

2.4   Chlorine disinfection: microbiological changes

An inability to maintain a disinfectant residual may allow bacterial growth in drinking water supplies; however, many researchers have observed coliforms in drinking water samples even in the presence of a chlorine residual (Wierenga 1985; LeChevallier *et al.* 1991; Smith *et al.* 1991). During 1983, the occurrence of coliforms was reported at one particular water system in the United States and preliminary experiments were conducted to evaluate the possible sources of the coliforms and the resistance of coliform isolates recovered (Olivieri *et al.* 1984). Coliforms were recovered in the presence of free residual chlorine as high as 5 to 8 mg/L and it was found that the coliforms were a result of regrowth in the distribution system. Coliform isolates were recovered from the water system and were exposed as suspension to chlorine levels at about 1 mg/L. They were found to be not particularly resistant to free chlorine. However, when the same microorganisms were prepared and exposed as biofilm to free chlorine, they were noticeably resistant and persisted in the presence of free chlorine concentrations commonly found in water distribution systems. The chlorine apparently did not reach the microorganisms in the biofilm. However, the study did not determine whether the chlorine was consumed or did not penetrate the biofilm at all.

It appears that chlorination acts selectively on bacteria which, in turn, promote a wide range of survival strategies. Many different bacteria appear to be capable of taking advantage of some form of resistance mechanism (Ding *et al.* 1995; Payment 1999; Momba *et al.* 1999). Leriche and Carpentier (1995) documented the survival of *Salmonella typhimurium* and *Pseudomonas Fluorescens* in biofilm after being exposed to chlorine stress. Viable but nonculturable (VNC) state resulted, in which survival was enhanced by a dormant or injured condition that prevented culturing on selective media. In addition, the presence of *P. fluorescens* resulted in an increased resistance of *S. typhimurium* to chlorine. Other studies (Vess *et al.* 1993) were conducted to examine six common water bacteria for their ability to colonize polyvinyl chloride (PVC) surfaces, to

survive various germicidal treatment, and to re-establish themselves in sterile distilled water. These studies showed that some species of *Pseudomonas* spp. and *Mycobacteria* could attach and colonize the interior surface of PVC pipes and develop significant resistance to the action of 10 to 15 ppm free chlorine. Moreover, scanning electron microscopy revealed bacterial attachment and possible formation of extracellular material embedded with *Pseudomonas* and *Mycobacteria* on remnant PVC surfaces; these may be the primary factors that allowed organisms to survive and re-establish in PVC pipes after chlorination.

A better understanding of free chlorine consumption in distribution systems is also necessary to account for the occurrence of bacteria in highly chlorinated waters. Free chlorine consumption in distribution systems is due to chemical reactions occurring both in the bulk water phase and at the pipe walls. Knowledge of the relative importance of these various reactions is needed in order to improve the chlorine decay modeling. Four main parameters that influence the free chlorine consumption in distribution system are: (1) reaction with organic and inorganic chemicals in the bulk aqueous phase; (2) reaction with biofilm at the pipe wall; (3) consumption by the corrosion of ferrous material; and (4) mass transport of chlorine and other reactants between the bulk flow and the pipe wall (Clark 1998). From numerous laboratory and pilot-scale experiments, Kiéné *et al.* (1998) published several important findings related to chlorine decay modeling, which include: chlorine consumption by synthetic materials (high density polyethylene, PVC, polypropylene, glass reinforced polyester) is negligible and does not appear to be an important parameter for the modeling of chlorine decay. By contrast, the non-disinfecting chlorine consumption of unlined, old iron-cast pipes is a major parameter for the chlorine decay modeling — for cast iron or steel pipes, the rate of chlorine consumption depends directly on the zero-order, corrosion-induced reactions at the pipe wall (Vasconcelos *et al.* 1997). Chlorine decay due to bulk reactions varies greatly according to water temperature and concentration of organic matter — variation of the kinetics can be predicted by a simple model taking into account the total organic carbon (TOC) and the temperature. Chlorine decay, due to attached biofilm, varies

according to colonization time, but the reaction rate eventually reaches a steady state depending on the water quality (Kiéné *et al.* 1998).

2.5 Pipe materials: iron pipe vs. PVC pipe

Disinfectant decay, as a result of reactions with pipe material and water components, poses a realistic threat to regrowth control and disinfectant efficiency, especially in the presence of unlined iron-cast pipe materials in the distribution system. Many older distribution systems contain unlined cast or ductile iron pipes, frequently characterized by accumulations of corrosion products or tubercles that can nearly occlude the pipe diameter. Iron surfaces are particularly reactive and contribute to the deterioration of water quality through a variety of processes. Brereton (1998) has noted that iron tubercles, recovered from existing water mains (in Greater Vancouver, circa 1930), exhibit a potential for chloroform formation when exposed to chlorinated water, presumably due to the accumulation of organic precursors into tubercles by interaction with corrosion by-products. Depending on the chloroform concentration in the bulk water, these surface-related chloroform products may subsequently be released into the bulk water at lower aqueous chloroform concentrations (Brereton 1998). Unlined iron-cast pipe materials consume chlorine and are found to be a major interference in disinfection efficiency (LeChevallier *et al.* 1987, 1990). Consequently, iron surfaces are prone to substantial microbial colonization and have been implicated as a key component in microbial regrowth in distribution systems (Camper *et al.* 1996, LeChevallier *et al.* 1993).

Another reactive characteristic of iron corrosion products (iron oxides) is that they have a large potential for the adsorption of natural organic matter (LeChevallier *et al.* 1990). The biological use of adsorbed humic substances has been discussed in section 2.2. Camper *et al.* (1999) carried out experiments to examine the potential for corrosion products from ductile iron to support microbial growth in humic water. There was a 2-log increase in bacterial numbers over a 3-day time period, again providing circumstantial evidence that the adsorbed humic material was available for microbial metabolism. Utilities, with a large proportion of unlined ferrous metal pipes that have

had coliform regrowth problems, include Vancouver, British Columbia; Boston, Massachusetts; and Washington, DC. New plastic materials — for example, unplasticised polyvinyl chloride (PVC) and medium density polyethylene (MDPE) — are replacing the much older cast iron pipes in the industry; their biofilm-forming potential is currently under investigation (Kerr *et al.* 1999, Vess *et al.* 1993, Anderson *et al.* 1990).

In a 20-month, pilot-plant study of microbial regrowth on pipe wall after the action of chlorine disinfection on humic water, Lund and Ormerod (1995) found that a residual of 0.04 to 0.05 mg/L free chlorine was sufficient to prevent biofilm formation in PVC pipes. Momba *et al.* (1998) further evaluated the impact of chlorination in a laboratory scale unit and showed formation of biofilm on both cement and stainless steel coupons, with free chlorine residual concentration as high as 0.2 mg/L. LeChevallier *et al.* (1990) showed that bacteria grown on galvanized, copper, or PVC pipe surfaces were readily inactivated by a 1 mg/L residual of free chlorine. However, biofilms grown on iron pipes, treated with free chlorine doses as high as 4 mg/L (3 mg/L residual) for two weeks, did not show significant changes in viability. Kerr *et al.* (1999) completed a study to compare biofilm accumulation and heterotrophic bacterial diversity on three pipe materials -- cast iron, medium density polyethylene (MDPE), and unplasticised polyvinyl chloride (PVC). The greatest number of biofilm heterotrophs was always found on cast iron, followed in decreasing order by MDPE and PVC. Organisms growing on ferrous metal surfaces were less susceptible to free chlorine than when present on other materials, presumably because metal exerts a non-disinfecting chlorine demand of its own.

The importance of surface material on organism numbers, including coliforms, was substantiated by Camper *et al.* (1999). Even in the absence of a disinfectant, mild steel surfaces were consistently colonized by nearly 10-fold more heterotrophs and 2- to 10-fold more coliforms than polycarbonate surfaces, when the reactors were operated under the same conditions. The impact extended to effluent bacterial concentrations as well. Furthermore, the presence of mild steel affected population densities on polycarbonate surfaces in the same reactor. Therefore, it appeared that the mild steel surface was capable of enhancing biofilm growth, rather than only protecting it from the

action of a disinfectant. Norton and LeChevallier (2000) recently confirmed that iron pipe stimulated the rate of biofilm development and iron pipe surfaces dramatically influenced composition, activity, and disinfectant resistance of biofilm bacteria. In that study, bacterial levels on disinfected iron pipes again exceeded those for chlorinated PVC pipes.

Kerr *et al.* (1999), using the number of distinct colony types as a simple indication of heterotrophic diversity, found that the diversity of heterotrophs was greatest on cast iron, followed by MDPE, then PVC. However, this preliminary study showed a decrease in diversity over time in cast iron and an increase in the other materials tested. In Germany, Kalmbach *et al.* (2000) documented a new, ubiquitous genus *Aquabacterium* spp. (rod-shaped bacteria which are able to metabolize a broad range of organic acids, but no carbohydrates at all) in drinking water systems. In this study, material-induced population shifts within the microbial community were demonstrated when the population composition of *Aquabacterium* was compared on different substrata, including glass, polyethylene and soft-PVC. The community composition on soft-PVC differed significantly from the other materials. These early studies provide evidence to support the hypothesis that the pipe materials select unique microbial communities and can also influence their diversity. In addition, Kerr *et al.* (1999) found that the dominant colony types recovered from the biofilms altered over time, so that there was no stable biofilm population on any of the surfaces over longer periods of time. Biofilm populations were continually changing, probably as a result of competition with new biofilm members.

2.6 Chlorine reaction dynamics: formation of THMs

The aqueous chlorination of humic matter is a complex reaction system in water chlorination, because the composition and structure of the organic reactant is only known in a limited way and is, furthermore, not constant from sample to sample. Most organic matter in water supplies is natural in origin and is derived from living and decaying vegetation. These compounds may include humic and fulvic acids, polymeric carbohydrates, proteins and carboxylic acids. There are probably numerous organic

compounds having diverse functional groups of natural origin. They may either be present in the water after treatment or originate from materials in contact with the drinking water.

The diverse structure and chemical nature of these humic materials give rise to different THM formation potentials by way of several mechanisms. However, the reaction chain of THM formation is generally initiated by the ionization of an organic compound, and then substitutes either chlorine or bromine into an activated carbon site in the organic compound to form a derivative. More successive ionization and substitution steps occur until the methyl group is fully halogenated; then, base-catalyzed hydrolysis produces the THM end-product (Morris 1984). Ionization rates vary greatly with different organic structures; in fact, under the typical water distribution conditions (pH ~ 5 to 9; 10^{-4} M concentrations), observed rates of THM formation with natural organic matters suggest organic precursors with highly activated structures (Rook 1976). Plot of THM concentration against humic substance levels reveals a "convex-shape," suggesting that a multi-step reaction occurs (Adin *et al.* 1991). In the presence of small amounts of chlorine, the first chlorination reaction is considered as the slow, rate-determining step, and the initial ionization reaction a rapid reversible, pre-equilibrium step. Another step in the reaction chain for chloroform formation may also be slow. When a later step is a slow step, the reaction tends to come to a halt, just prior to the slow step, and the intermediate that has been formed at this point accumulates in identifiable quantities. Some of the chlorinated intermediates found in water chlorination may be accounted for in this way (Morris 1984).

Although chloroform is most often the predominant THM species, brominated THMs can occur in waters containing bromide ion. During chlorination, a portion of the inorganic bromide ion concentration is converted into organically bound bromine in the brominated THM species. Oxidized bromide tends to react more rapidly than free chlorine, and it is noted that total THM (TTHM) increases as a function of increasing bromide ion (Minear and Bird 1978; Amy *et al.* 1984). TTHM is the sum of the four main compounds in this group: trichloromethane ($CHCL_3$) — or chloroform — dichlorobromomethane ($CHCl_2Br$), dibromochloromethane ($CHBr_2Cl$), and

tribromomethane ($CHBr_3$) — or bromoform. The extent of brominated THM formation depends on the bromide (Br^-) to chlorine (Cl) concentration ratio (Nokes, Fenton and Randall 1999) and the efficiency of bromination process compared with chlorination (Heller-Grossman et al. 1993). Common cases covered in the literature generally show chloroform to be the dominant species and bromoform the least commonly found, because bromide content in most waters is usually low.

Table 2.1 summarizes the ranges of concentration and speciation of THMs found by other researchers in various distribution systems.

Location	Elizabethtown, New Jersey	Abu-Dhabi, United Arab Emirates	S. Bohemia, Czech Republic	Montreal, Quebec
TTHM	25 - 30 $\mu g/L$	32 - 41 $\mu g/L$	4.25 - 7.1 $\mu g/L$	14 $\mu g/L$; 43 $\mu g/L$[b]
$CHCL_3$	18 -22 $\mu g/L$	1.6 - 2.7 $\mu g/L$	3.9 - 6.1 $\mu g/L$	N/A
$CHCl_2Br$	5.9 - 6.8 $\mu g/L$	2.4 - 4.1 $\mu g/L$	N/A	N/A
$CHBr_2Cl$	1.2 - 1.4 $\mu g/L$	3.9 - 11.1 $\mu g/L$	N/A	N/A
$CHBr_3$	0.12 - 0.18 $\mu g/L$	17 - 28 $\mu g/L$	N/A	N/A
Main Species	chloroform (73%)	bromoform (62%)	chloroform (82%)	[b]$CHCl_2Br$ (42%) [b]chloroform(39%)
Source Water Characteristics	minimal bromide	high bromide (0.8 mg/L)	low DOC (1.9 mg/L)	low BDOC (0.2 - 0.45 mg/L)
Max. Limit[a]	0.08 mg/L	0.08 mg/L	0.1 mg/L	0.1 mg/L
Specification	D/DBP Stage II	D/DBP Stage II	Czech	CDWQ
Reference	Chen and Weisel (1998)	Elshorbagy et al. (2000)	El-Shafy and Grünwald (2000)	Desjardins, et al. (1997)

Table 2.1 Concentrations and speciations of THMs found in various distribution systems. [a]maximum limit for TTHM.   [b]measured in a dead end of the distribution system.

2.7 Control of biofilm regrowth and THMs

Controlling the levels of THM precursors, prior to chlorination, and of nutrients available for bacterial growth, is the most direct means of resolving both biofilm and THMs problems. Unfortunately, they are also the most difficult. From the perspective of downstream control in the distribution system, the treatment process of drinking water

should ideally play the important role of reducing THM precursors in the finished water prior to chlorination, and the choice of treatment should also play a direct role in limiting the concentration of compounds serving as growth substrates for microorganisms in the distribution system. The German Association on Gas and Water Supply (DVGW) is preparing the technical guideline W296 on measures to reduce or to avoid THM formation (Hoyer 1998). In this document, measures used to overcome THM formation are the same as those used to prevent from the need of residual disinfectants during distribution. Moreover, these measures are not only limited to water treatment and distribution network; the quality of raw water is decisive to quite the same extent.

Conventionally, biologically available organic carbon is thought to be the only limiting nutrient in drinking water, and, based on this presumption, regrowth in a system is monitored by controlling the biologically available organic carbon. Carbon in drinking water is measured in three ways: as total organic carbon (TOC), which is the total amount of soluble and insoluble organic carbon compounds present in the water; as dissolved organic carbon (DOC), which is the soluble fraction of TOC; and as assimilable organic carbon (AOC), which is the fraction of DOC that can be readily digested and used for growth by aquatic organisms. Often, AOC constitutes just a fraction (0.1% to 9.0%) of the total dissolved organic carbon (van der Kooij and Hijnen 1985). Van der Kooij et al. and Hijnen (1985) also indicated that HPC bacterial growth may be limited in distribution water containing AOC levels of less than $50\mu g/L$. This level is consistent with the findings of LeChevallier et al. (1991), who showed that AOC levels greater than $50\mu g/L$ were associated with an increased incidence of coliform bacteria. In his study, AOC was the only nutrient observed to decline as the water moved through the distribution system.

However, phosphate has recently been shown as the growth-limiting nutrient in drinking water systems with high contents of organic carbon (Miettinen et al. 1997), and a study developed in the University of Tokyo (Sathasivan and Ohgaki 1999) showed that controlling phosphorus might be a more effective nutrient control. It has also been postulated (Kalmbach et al. 2000) that the combination of phosphates and carbon sources might be the essential factor leading to a population shift in the microbial community of

Aquabacterium biofilm on soft-PVC surfaces, since phosphates are contained in various concentrations in soft-PVC as plasticizers and as anti-flammable substances. The control of THM precursors and bacterial regrowth remains very interesting candidates for future research; however, the challenge for engineers and applied scientists is to achieve a level of control to provide 'safe' water to the public in the face of all uncertainties.

2.8   Simulated distribution system

The simulated distribution system (SDS) testing method (Koch *et al.* 1991; APHA *et al.* 1995) uses bench-scale techniques to evaluate the development of THM (or other DBPs) after disinfection at any point in a distribution system. Bench-top procedures used in the SDS test are intended to mimic conditions in a distribution system. However, the test assumes zero contribution from the distribution system by incubating water samples in standard glass bottles and, therefore, cannot account for possible interaction between the bulk water and the internal pipe environment (see Sections 2.4 and 2.5).

One very important modification of the standard SDS test was developed by Brereton (1998) to incorporate the potential pipe/biofilm impact in the assessment of DBP formation: chlorinated water samples are incubated in containers constructed from the same type of pipe material recovered from the actual distribution system in the same condition. The material specific simulated distribution system (MS-SDS) test is founded on the same premise as the standard SDS test; by running the two tests simultaneously, it is possible to distinguish DBP yield attributable to the bulk water alone and total yield including pipe/biofilm influences. The MS-SDS test can further be used to estimate concentrations of THMs or other DBPs under different distribution system conditions, by varying the type and condition of the pipe containers.

In that study (Brereton 1998), MS-SDS tests showed that cast iron pipes generated chloroform, depending on the initial chlorine dose, and that subsequent release of chloroform formation to the bulk water was dependent on the *in-situ* aqueous chloroform concentration. A portion of the chlorine demand associated with the pipe wall is nonTHM-yielding; thus, at lower chlorine applications where the residual was below the threshold (~5 mg/L free chlorine), this nonTHM-yielding component of

overall chlorine demand dominated, resulting in bulk water chloroform levels lower than those attributable to the water phase alone. These findings suggest that standard SDS test may overestimate predictions of THM occurrence, and prove MS-SDS test a useful tool in the assessment of pipe effects on DBP evolution.

2.9   Haloacetic acids

Aside from THMs, many other organic halides have been found in chlorinated drinking water. Studies of chlorination by-products have suggested that chlorinated carboxylic acids, such as dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA), could account for a high percentage of the nonvolatile TOX (total organic halide) formed in chlorinated drinking waters (Christman *et al.* 1980; Johnson *et al.* 1982; Miller and Uden 1983). Like THMs, haloacetic acids are common chlorination products of a wide range of different organic compounds (Quimby *et al.* 1980; Christman *et al.* 1983). Highly activated compounds, with functional groups that can be readily oxidized by chlorine, proceed according to the general chlorination pathway for haloform reaction described in Section 2.6, until fully halogenated. Depending on the nature of its oxygenated functional groups, such precursor can form either DCAA or TCAA. Increasing chlorine application generally leads to the preferential formation of DCAA and TCAA over monochloroacetic acid (MCAA). Biological degradation of HAAs has been reported in aquatic environment (Pitter and Chudoba 1990); MCAA and DCAA are considered slowly degradable, and TCAA is considered more refractory. Moreover, Reckhow and Singer (1984) have shown evidence to suggest that common intermediates exist for both chloroform and TCAA formation, where the base-hydrolyzable trichloromethyl species may be oxidized to produce TCAA or hydrolyzed to produce chloroform. However, chloral hydrates are apparently transformed into THMs once the chlorine residual disappears (Clark *et al.* 1994).

High bromide content in water leads to the formation of the brominated species of HAAs, including monobromo- (MBAA), dibromo- (DBAA), tribromo- (TBAA), bromochloro-, dibromochloro-, and bromodichloroacetic acids. However, distribution of the HAA species among the mono-, di-, and trihalogenated forms appears to be

independent of bromide concentration (Cowman and Singer 1996). Heller-Grossman *et al.* (1993) studied the chlorination of a bromide-rich lake water (Lake Kinneret, Israel) and identified six HAAs, four of which contained bromine. The identified THMs and HAAs accounted for 75% of TOX, and brominated species constituted over 85% of the THMs and HAAs. Tribromoacetic acid was also found to undergo decomposition to form bromoform.

Table 2.2 summarizes the results from studies on HAAs concentration and speciation by various researchers.

Scott and Alaee (1998)	drinking water, surface water, groundwater, rain, snow, lake water (14 locations, Canada)
	MCAA, DCAA: 0.02 - 8 $\mu g/L$ (all samples); **MBAA**: 4 $\mu g/L$ (drinking water); **DBAA** (snow, drinking water); **TCAA** (few samples); TBAA: Not Detected
Heller-Grossman *et al.* (1993)	lake water (high content of bromide) (Lake Kinneret, Israel)
	high yield: TBAA, dibromochloro-AA, bromochloro-AA **common reported ranges**: DCAA, THMs **low yield**: TCAA
Cowman and Singer (1996)	surface water, groundwater
	major components: bromochloro-AA, bromodichloro-AA, dibromochloro-AA (readily formed and constituted at least 10% of HAA$_9$ in waters containing as little as 0.1 mg/L bromide)
Chen and Weisel (1998)	drinking water (Elizabethtown, New Jersey)
	HAA$_5$: 14 - 24$\mu g/L$; **TCAA**: 5.5 - 7.5$\mu g/L$; **DCAA**: 6.2 - 13$\mu g/L$ **MCAA**: Not Detected; **DBAA**: 0.5 $\mu g/L$; **MBAA**: 1.4 $\mu g/L$

Table 2.2 Studies on concentrations and speciations of HAAs by various researchers.

Some reports have demonstrated that the mutagenic activity of chlorinated drinking water is more closely associated with the nonvolatile fraction of TOX (Bull 1982; Coleman *et al.* 1984). Bull and McCabe (1984) reviewed the health effects identified with common DBPs and briefly described decreased serum glucose and lactate, neurotoxic, aspermatogenesis and ocular lesions associated with DCAA, and induction of peroxisomes with TCAA. Accordingly, the USEPA standard introduces a maximum

contaminant level (MCL) for the total concentration of the 5 major HAAs (mono-, di-, and trichloroacetic acids, mono- and dibromoacetic acids) of 0.06 mg/L in Stage I and 0.03 mg/L in Stage II of the D/DBP Rule (US EPA, 1994).

2.10   Summary

The reaction of chlorine from disinfection with natural aquatic humic material, to form THMs and other DBPs, is a likely source of potential mutagen and carcinogen formation in drinking water. Unfortunately, aquatic humic substances are ubiquitous in many surface waters and the most effective DBP control strategy would appear to be controlling the organic level prior to chlorination. Yet, the specific precursors involved in the formation of different DBPs are still not clear.

At the same time, many studies have indicated that disinfection does not always correlate with reduced bacterial growth in water. It is likely that chlorination acts selectively on bacteria, which in turn promote some form of resistance mechanism. Moreover, it is believed that humic substances may become biologically utilizable to bacteria in an otherwise low-nutrient condition, when they are adsorbed to surfaces.

Biofilms are formed in most distribution system pipelines when microbial cells attach to pipe surfaces and multiply to form a slime layer on the pipe. The water industry is replacing pipes with less reactive synthetic materials, such as PVC, in the hope of minimizing biofilm growth, but their biofilm forming potential are still under investigation. A modified SDS test method has been proposed to account for any possible influence imposed by the pipe environment, since chlorine consumption in a distribution system is due to both chemical and biological reactions occurring in bulk water and at pipe walls. Thus, an understanding of THM formation in the presence of biological growth, under chlorine-limited condition, is necessary to provide 'safe' water to the public.

Chapter 3

RESEARCH OBJECTIVES

3.1   General

Many research efforts have been made to investigate the occurrence of trihalomethanes; however, while most of these efforts are concerned primarily with the quality of the raw water and its treatment, comparatively little attention has been paid to examine the possible impact of the drinking water distribution system. Yet, the application of chlorine residual and re-chlorination throughout the distribution network is a very common practice. Various opportunities exist to further enhance THM development beyond finished water capacity during distribution. When considering the conditions in a distribution system, the ubiquitous presence of microbial communities should not be neglected. Despite the oligotrophic environment in a distribution system, the growth and persistence of bacteria in such an environment is well documented. It can then be hypothesized that surface biofilm may give rise to certain impacts on the formation of THM during distribution, given the many common factors in THM formation mechanisms and microorganism survival strategies in biofilm: Both exert a chlorine demand on applied disinfectant residual and both rely heavily on natural organic matter for their existence (as an organic precursor for THM formation; as a carbon and energy source for biofilm).

3.2   Objectives

The major objectives of this study were to establish evidence of a biofilm effect on THM production, and to examine the effectiveness of chlorination on inactivating biofilm bacteria associated with PVC pipes. More specific research objectives were identified as follows:

- test the chloroform formation potential of bulk waters from different treatment processes under exposure to residual chlorine;

- set up a modified SDS test to accommodate possible impact on chloroform production from the internal pipe environment (MS-SDS test);

- compare directly the chloroform formation potential of waters incubated in standard SDS glass bottles and MS-SDS pipes;

- test the presence of viable microorganisms within the surface biofilm on PVC pipe walls under exposure to residual chlorine;

- investigate a possible association between biofilm inactivation by chlorine and chloroform production in bulk water.

Chapter 4

GENERAL METHODS AND ANALYTICAL PROCEDURES

This chapter describes the experimental methods and procedures applied in the present study. For the most part, standard procedures were followed in accordance with *Standard Methods* (APHA *et al.* 1995). Where appropriate, modifications to these procedures are described in detail.

4.1   Vancouver water system

The Province of B.C. owns all of the fresh water inside its borders. British Columbia is one of only two provinces in Canada where the Health Act is the main legislative base of drinking water quality (the other being Saskatchewan). The Safe Drinking Water Regulations give local authorities responsibility for ensuring that fresh water is safe for the Province's 4.1 million residents. Greater Vancouver Regional District (GVRD) is the local authority responsible for its 18 municipalities and the 1.8 million residents it serves in the Greater Vancouver area.

Drinking water is drawn from underground wells and taken from lakes, rivers and other surface sources. The latter method is most prevalent in British Columbia, with surface water supplying about 80% of the overall drinking need, on average. Greater Vancouver's water system is fed by three reservoirs — Capilano, Seymour and Coquitlam — all protected wilderness watersheds north of Burrard Inlet and the Fraser River. These watersheds cover 586 km^2 and remain one of the few highly secure watershed areas in North America, off-limits to public access instead of being forced to share water resources with other users such as industry and agriculture. Elsewhere, risks are presented by various activities which may include: farming, logging, transportation corridors, air pollutions, septic fields, golf course fertilizations, and gravel pits. The GVRD's 550 km of water mains make up the basic framework for a water distribution system linking the 18 municipalities — with each reservoir designed to supply up to 1.2 billion litres/day during peak summer flows (GVRD 2000).

Although there has been no disease outbreak associated with this regional drinking water supply, the region's water system repeatedly fails Canadian drinking water standards. Heavy rainfall in the watersheds (up to 4.5 m annually) has been the source of turbidity exceeding water quality guidelines in Canada — 54 days on average per year in Capilano, 18 days in Seymour and 4 days in Coquitlam (GVRD 2000). A series of factors — naturally acidic rainwater, turbidity during heavy rains, and the potential for dangerous bacteria to grow in the distribution pipes, especially in summer — have necessitated the upgrade of the region's water treatment systems. An ozonation plant is being built at Coquitlam and Capilano, and plans to build a filtration plant at Seymour is under active consideration. At present, all three Greater Vancouver reservoirs rely only on coarse screening and chlorination to disinfect drinking water. However, eight automated rechlorination stations, designed to boost chlorine levels to safe levels during distribution, have been added and are already in operation since mid-1998. The goal of the system is to pump out drinking water spiked with chlorine at 1.0 ppm, reduced to 0.2 ppm by the time it reaches residential homes. Municipalities have been assisting by flushing out their own water pipelines connecting to the GVRD main network, thereby reducing the buildup of sediments that work against the effectiveness of the residual chlorine.

4.2   Seymour water treatment pilot plant

The GVRD's pilot plant facility is located at Seymour Falls Dam in the Seymour Watershed (North Vancouver) and was first commissioned to investigate an optimal process design for the Seymour filtration plant. The present study utilized the same pilot plant facility, with a few modifications, to provide treated water from the Seymour source for secondary chlorination in order to evaluate the formation of DBPs.

The pilot plant facility consists of the following unit components (Figure 4.1):

- Rapid mix accomplished by chemical injection upstream of in-line static mixers;
- Flocculation / Dissolved air flotation (DAF) unit, which consists of a single rectangle tank baffled to form an integral flocculator/DAF cell, a mechanical skimmer, an air saturator and associated controls;

- Duel media (sand / anthracite) filter columns with automatic control of outflow and backwashing;

- Ultrafiltration membrane system with hollow fiber configuration.

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┌──────────┐   ┌──────────┐   ┌──────────────┐   ┌──────────────────┐
│   Raw    │───│  Rapid   │───│ Flocculation/│───│ Sand / Anthracite│
│  Water   │   │   Mix    │   │     DAF      │   │    Filtration    │
└──────────┘   └──────────┘   └──────────────┘   └──────────────────┘
     │                                            ┌──────────────────┐
     └────────────────────────────────────────────│  Ultrafiltration │
                                                   │     Membrane     │
                                                   └──────────────────┘
```
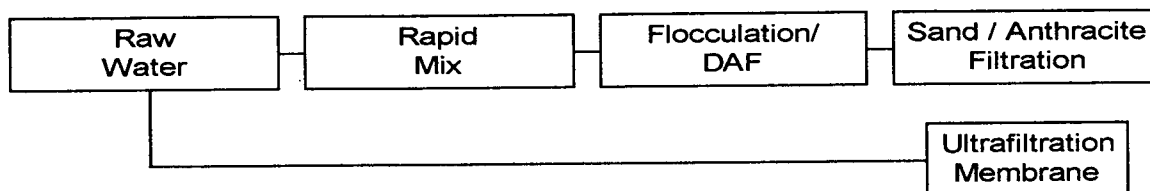
Figure 4.1 Schematic diagram of the modified pilot plant

At the raw water inlet, a gallery of connected PVC pipe segments were installed to allow continuous flow of untreated water through the pipes, thus establishing *in-situ* surface biofilm on the internal pipe walls. These PVC pipe segments were disconnected and used as pipe incubators in the MS-SDS test, for the analysis of chloroform production.

Seymour raw water is naturally soft and low in pH, alkalinity and turbidity. Chloroform is the only THM formed upon chlorination due to naturally low bromide content. Table 4.1 summarizes the average monthly Seymour raw water quality during 1997-1998 (Crowther *et al.* 1998).

Table 4.1 Summary of average monthly Seymour raw water quality during filter design optimization pilot testing
August 20, 1997 through February 13, 1998

Parameter	Aug. 97	Sept. 97	Oct. 97	Nov. 97	Dec. 97	Jan. 98	Feb. 98
Turbidity (NTU)	0.47	0.94	0.84	0.60	0.90	0.52	0.40
pH	6.3	6.2	6.2	6.4	6.4	6.3	6.3
Temperature (°C)	16.0	14.5	10.1	8.7	5.0	3.0	3.6
Total number of Particles (3-5 μm) (No./mL)*	9.5×10^2	2.3×10^3	2.2×10^3	NR	NR	NR	9.9×10^2
Total number of Particles (5-15 μm) (No./mL)*	5.0×10^2	9.8×10^2	8.3×10^2	NR	NR	NR	3.7×10^2
Total number of Particles (15-750 μm) (No./mL)*	1.5×10^3	3.3×10^3	3.0×10^3	NR	NR	NR	1.4×10^3
Alkalinity (mg/L as $CaCO_3$)	4.0	4.1	2.8	2.9	2.8	2.9	2.7
Hardness (mg/L as $CaCO_3$)	5.0	5.6	4.2	4.6	4.2	4.7	NR
UV-254 Absorbance (per cm)	0.065	0.096	0.116	0.097	0.095	0.074	0.072
Specific UV Absorbance (SUVA) (L/mg•m)	3.8	4.1	4.0	4.1	3.6	3.8	3.5
Total Organic Carbon (TOC) (mg/L)	1.9	2.6	2.9	2.5	2.8	2.2	2.1
Dissolved Organic Carbon (DOC) (mg/L)	1.7	2.3	2.8	2.4	2.6	1.9	2.0
Apparent Colour (ACU)	19	27	24	20	19	16	15
True Colour (TCU)	16	18	20	18	17	13	13
Total Aluminum (mg/L)	0.09	0.09	0.20	0.10	0.18	0.09	0.12
Total Iron (mg/L)	0.21	0.43	0.10	0.10	0.12	0.09	0.07

(*) indicates the average value shown is a geometric mean. All other average values are arithmetic means.
(NR) indicates the parameter was not recorded.
Source: Crowther R., Watson, M., and Sandwell. 1998.

4.3   Experimental Design

The simulated distribution system (SDS) test (APHA *et al.* 1995) allows test variables to be modified to mimic local distribution system conditions, with the exception of internal pipe environment exposure, with bench-top procedures. These conditions include temperature, pH, disinfectant dose and residual, bromide ion concentration and reaction time (corresponding to the residence time of water within the distribution system). The material specific simulated distribution system (MS-SDS) test (Brereton, 1998) is founded on the same premise as the SDS test, but with the modification of incubating water sample in a pipe container, rather than in a glass bottle (to account for internal pipe environment effect). The procedure used to simulate a distribution system in both tests can be standardized according to specific needs.

4.3.1   SDS Test

In the present study, water samples were collected from the Seymour pilot plant at different stages of the treatment process, and were then transported to the Environmental Laboratory at University of British Columbia for chlorination and analyses. Waters were spiked with chlorine at 2-3 ppm, reduced to approximately 0.2 ppm by the end of the incubation period, and were stored headspace-free in clean, 125 mL standard SDS glass bottles, sealed with PTFE-lined, open-top screw type caps, for a preset incubation period at 25 °C. The standard SDS test was performed in accordance with *Standard Method* 5710C (APHA *et al.* 1995) under both short term and long term incubation periods of 3-5 hours and 4 days. At the conclusion of the incubation period, 40 mL samples were quenched with sodium sulfite and subjected to GC analysis for chloroform.

4.3.2   MS-SDS Test

At the heart of this test was the container used to store water samples during the incubation period. The storage apparatus used in the present study was constructed from new PVC material into pipe sections 14 inches (36 cm) long with a diameter of 2 inches (50 mm). Each pipe section was threaded at both ends, to allow connection to the raw

water inlet at the Seymour water treatment pilot plant between MS-SDS test runs; this allowed for *in-situ* growth of surface biofilm on the internal pipe walls. Immediately prior to an MS-SDS test run, pipes were disconnected and filled headspace-free with chlorine-spike waters. The MS-SDS test was performed in conjunction with the standard SDS test, under the same conditions for the same incubation period at 25 °C. The pipe incubators were completely wrapped in Teflon tape at both ends and capped off with PVC screw caps. At the conclusion of the incubation period, the pipe incubators were uncapped and drained, and the waters were collected and quenched for chloroform analyses. The interior surface of the pipe incubator was then scraped with a sterile cotton-tipped swab for bioactivity analysis (Section 4.4.5), prior to reconnecting to the raw water inlet.

4.4 Laboratory Analysis

4.4.1 Chlorine residual

Free chlorine residual was measured by the DPD (N,N-diethyl-*p*-phenylenediamine) colorimetric method, using a field kit (Hach Model CN-70). In this procedure, free chlorine reacts with commercially packaged DPD Free Cl_2 Reagent Powder for 25 mls sample in the absence of iodide ion to produce a red color, which can then be matched to a calibrated colored disk. The use of the field kit allowed rapid determination of Cl_2 residual over the range of values, from 0 to 3.5 mg Cl_2/L. The method detection limit and precision are 0.02 mg/L for chlorine concentrations from 0 to 0.7 mg/L, and 0.1 mg/L for concentrations from 0.7 to 3.5 mg/L.

4.4.2 Trihalomethanes

Total trihalomethanes were measured by liquid-liquid extraction gas chromatography, according to *Standard Method* 6232B, using pentane (Fisher Chemical, HPLC grade) exclusively as the extraction solvent in the ratio of 2 mL pentane : 10 mL sample (as recommended). Background interference associated with the extraction solvent was eliminated by column chromatography, using basic alumina (Brockman

Activity I; 60-325 mesh; Fisher Chemical); this was maintained at 230°C for a minimum of 2 hours, prior to use.

A Hewlett Packard 6890 series gas chromatograph (GC), equipped with a Hewlett Packard 7673 GC/SFC injector and an autosampler, was used to analyze samples. Instrument control, data acquisition and data evaluation were controlled by HP ChemStation for GC systems. Table 4.2 shows the programmed GC operating conditions. 3.0 μL aliquots of extraction were injected into the GC. As a minimum, duplicates (duplicating the entire procedure, including sample collection) were analyzed every 10th sample, and typically at least one duplicate for every sample set. A sample set included the raw and effluent water samples, collected before and after treatment, and subjected to parallel SDS and MS-SDS tests.

Calculations of chloroform concentration were accomplished by comparing the response peaks of samples to those of external standards analyzed in each GC run. A minimum of three (typically five) standards, enveloping the anticipated range of sample concentrations, were used to construct a calibration curve. Solvent blanks were run at the beginning and end of each run. The method detection limit ranges from 0.1μg/L to 200 μg/L.

4.4.3   Haloacetic Acids

Currently, the widely used method for analyzing HAAs in drinking water is *Standard Method* 6251B. In this method, diazomethane is employed as a methylating agent to convert HAAs to their methyl esters. Diazomethane is generally prepared as needed from a commercially available precursor compound, the potential carcinogen 1-methyl-3-nitro-1-nitrosoguanidine (MNNG). Because of the toxicity of MNNG and the hazardous nature of diazomethane, there is concern about using these chemicals in HAA analysis. Thus, it is desirable to develop a procedure to avoid the use of carcinogenic reagents.

In the present study, haloacetic acids were measured by liquid-liquid microextraction, acidic methanol derivatization gas chromatography, in accordance with a recent acidic methanol methylation procedure proposed by Xie, Reckhow and

Springborg (1998). Figure 4.2 shows the analytical procedure for the Xie *et al.* method using acidic methanol. A 30 mL sample was dechlorinated with ammonium chloride (NH_4Cl) and supplemented with sulfuric acid (H_2SO_4) and sodium sulfate (Na_2SO_4) to facilitate extraction of the acids from the aqueous phase to the organic solvent. Each sample was then extracted with 4 mL of methyl tert-butyl ether (MTBE, Fisher Chemical, HPLC grade) spiked with 300 μg/L of dibromopropane (the internal standard) and agitated for 15 min with a mechanical shaker. For the acidic methanol derivatization, 1 mL of this extract was transferred to a 20 mL vial containing 2 mL of methanol and 0.1 mL of concentrated sulfuric acid. The vial was capped with PTFE-faced septa and screw caps, then kept in a 50°C water bath for 1 hour. After addition of 5 mL of 10 percent Na_2SO_4 solution and 1 mL of fresh MTBE, the vial was recapped and shaken for 2 min by hand. After settling, 1 mL of MTBE extract was transferred to an autosampler vial and analyzed by gas chromatography (Xie *et al.*, 1998). 3.0 μL aliquots of extraction were injected into the GC. The GC and the control software employed were the same as for THM analysis (Section 4.4.2). The GC operating program is shown in Table 4.2.

Parameter	THMs (Section 4.2)	HAAs (Section 4.3)
Capillary column		
Type	DB624 (J&W Scientific 125-1334)	DB624 (J&W Scientific 125-1334)
Length and diameter	28m x 530 μm i.d.	28m x 530 μm i.d.
Film thickness	3.0 μm	3.0 μm
Carrier gas	Helium @ 5.5 mL/min	Helium @ 5 mL/min
Make-up gas	Nitrogen	Nitrogen
Combined flow rate	(He + N_2) @ 60 mL/min	(He + N_2) @ 60 mL/min
Oven temperature		
Initial temperature	30 °C, holding for 2 min	35 °C, holding for 2 min
Ramp 1	5 °C/min	5 °C/min to 136 °C, holding for 3 min
Ramp 2	N/A	20 °C/min to 236 °C, holding for 3 min
Final temperature	90 °C	250 °C
Post-run	N/A	holding for 5 min
Injector		
Type	splitless	splitless
Temperature	90 °C	157 °C
Detector		
Type	electron capture detector	electron capture detector
Temperature	260 °C	297 °C

Table 4.2   Gas chromatograph operating conditions for trihalomethane and haloacetic acid analyses (source: *Standard Method* 6250B, APHA *et al.*, 1995; Xie *et al.*, 1998)
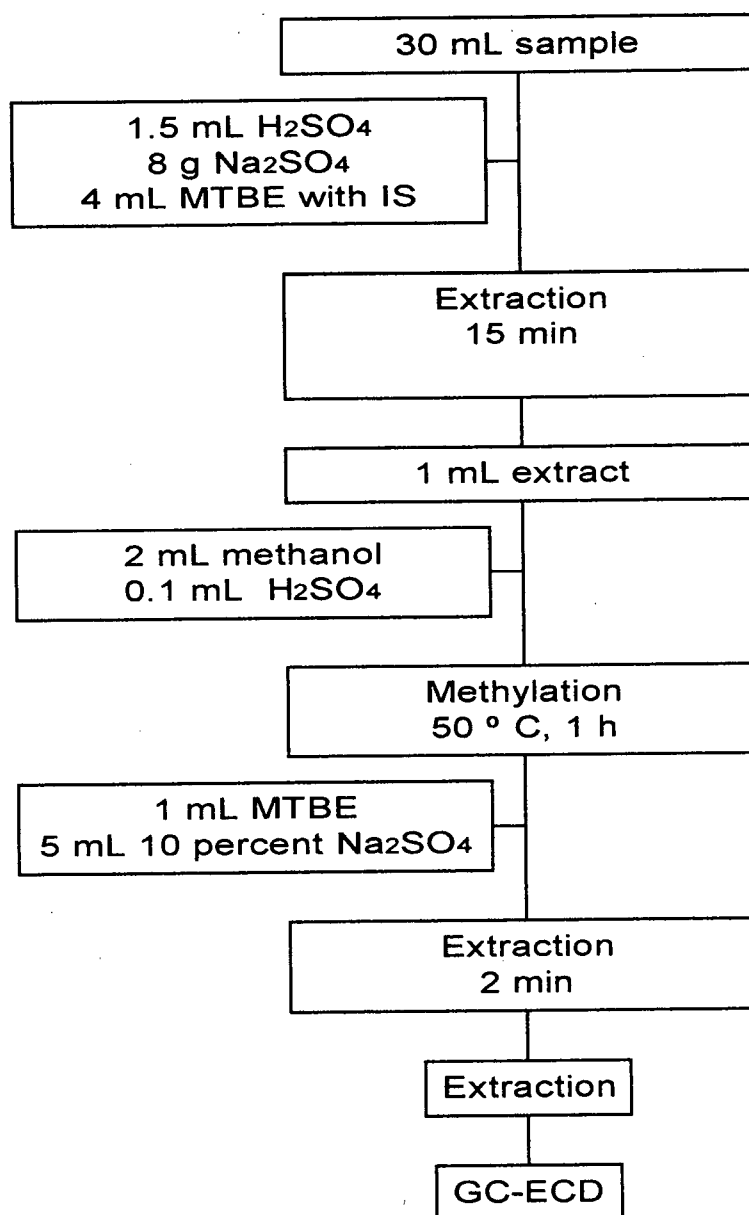
Figure 4.2   HAA analytical procedure for liquid-liquid microextraction gas chromatography using , acidic methanol derivatization (source: Xie, Reckhow and Springborg, 1998)

4.4.4   Glassware and reagent preparation

Sample collection vials (40 mL, amber glass) and bottles (125 mL, 250 mL, and 2.3 L; amber glass) were washed with detergent, rinsed with tap water and distilled water, and heated at 350°C for one hour. All volumetric glassware were washed with detergent, rinsed with tap water and distilled water, and dried at 105°C, prior to use. Autosampler vials were rinsed with the appropriate clean solvent (pentane for THM analysis and MTBE for HAA analysis) and heated at 350°C for one hour. Open-topped plastic caps (for sample vials and bottles) and PTFE lined rubber septa were washed with detergent, rinsed with tap water and distilled water, and dried at 105°C for one hour.

Organic-free water was prepared from Vancouver tap water by Millipore Alpha-Q Ultra-pure water system, with a purification pack containing media — including activated carbon, ion exchange resin, and organic scavenger. The system produces 18.2 Megohm-cm reagent water (Type I water, ASTM) directly from potable tap water. Residual chlorine quenching agents (sodium sulfite solution for THMs and ammonium chloride solution for HAAs), DBP aqueous calibration standards, travel blanks, reagent blanks, method blanks, chlorine dosing solution, and solution dilutions were all prepared with this water.

Chlorine dosing solution was prepared from organic-free water and stock hypochlorite solution (5.25% sodium hypochlorite; Javex brand, Colgate-Palmolive Inc., Toronto, Canada). The stock solution was checked for hypochlorite concentration prior to use each time by the DPD colorimetric method.

4.4.5   Heterotrophic plate counts

Following the conclusion of the MS-SDS test run, the pipe incubators were uncapped and drained. A small area (0.5in x 2πin) of the incubator's interior surface was then scraped with a sterile cotton-tipped swab to estimate active cell counts. The swab was immediately inserted into a sterile test tube containing 5 mL dilution water (0.1692g KH_2PO_4 : 0.0020g $MgCl_2 \cdot 6H_2O$) and sonicated for 12 minutes (AQUASONIC ultrasonic cleaner 50HT, VWR Scientific Products, West Chester, PA.). The suspension was then enumerated for heterotrophs according to *Standard Method* 9215. For most

water samples, plates suitable for counting were obtained by plating 0.1 mL undiluted sample and 0.1 mL of 10^{-1} dilution. Dilutions were prepared by transferring 1 mL of sample into a sterile test tube containing 9 mL of sterile dilution water, using a sterile pipette. From each tube, 0.1 mL was spread on plates prepared with tryptone glucose extract agar (TGE agar, BDH) using a turntable and a bent-glass rod. Under U.S. EPA's Surface Water Treatment Rule (40 CFR 141.74), provision on heterotrophic bacteria, plates were incubated at 35°C for 48 hours and enumerated for colony forming units (CFUs) on a Quebec type colony counter.

Plate counts have been described and used as simple, reliable methods in past studies to measure biofilm cell densities. However, the cells in the biofilm often became impossible to culture on selective media over time (Camper 1999), and samples were either homogenized (Kerr *et al.* 1999; Brereton, 1998; LeChevallier *et al.* 1990) or sonicated (Momba *et al.* 1998) to disrupt cell aggregates prior to enumeration. Thus, plate count results of biofilm heterotrophs have to be interpreted with caution and treated as estimates.

4.5   Statistical analysis

Statistical analysis and graphical illustration were completed using Microsoft Excel 97 (Microsoft Corporation ©, Redmond, WA).

4.5.1   Hypothesis testing

Statistical hypothesis tests tell us how likely it is that a particular parameter of two sample distributions differs from each other. The term "null hypothesis", H_0, is used for any hypothesis set up primarily to see whether it can be rejected. The null hypothesis, which assumes that the difference in the parameter between two distributions is zero, is tested against the alternative hypothesis, which usually suggests that a change in the parameter has taken place. The test of a statistical hypothesis is a procedure by which we accept or reject H_0.

The idea of setting up a null hypothesis is not an uncommon one, even in non-statistical thinking. In fact, this is exactly what is done in North American court of law, when an accused is assumed to be innocent unless he or she is proven guilty "beyond a reasonable doubt" (Miller and Freund 1985). The phrase "beyond a reasonable doubt" reflects the probability α of rejecting a true null hypothesis in error. Thus, a level of significance is always specified for a hypothesis test, to denote the maximum probability of rejecting a true null hypothesis. For most statistical tests, α is chosen to be 0.05. A test is conservative if the observed level of significance of the hypothesis test is smaller than the stated level of significance.

4.5.2   Hypothesis concerning two means

Paired-sample Student's t-test is used to compare the means of two distributions with small sizes, based on a Student's t distribution, in a "before and after" kind of situation, where the samples are no longer independent. Pairing up sample values helps us to control or account for variability that arises from differences among the experimental units (Hogg and Ledolter 1992). A one-tailed t-test returns the probability that two sample means are equal. If the observed significance level, or probability (P value), is less than 5% ($P < 0.05$), we reject H_0 and accept the alternative hypothesis that one sample mean is either greater or small than the other. This kind of alternative is one-sided, since the inequality may go one way or the other. If the observed P value is less than 1% ($P < 0.01$), the result is considered to be highly, statistically significant. P values are given within the text when statistical significance is identified.

4.5.3   Analysis of variance

The analysis of variance (ANOVA) technique is used to compare the effect of different treatment factors on data. For example, in the analysis of disinfection by-products, we want to investigate chloroform formation in pipe-incubated water samples under different incubation periods. Data is classified into groups based on the

differences in treatment; water samples incubated under short incubation period are grouped together, and samples under long incubation period grouped separately. If the analysis reveals significant difference in the chloroform formation between the various data groups, the factor of incubation time can be said to significantly influence the chloroform formation. This illustration is also an example of an one-way ANOVA because there is only one factor. In a two-way ANOVA, the data is grouped based on two factors; for example, water samples incubated under short incubation period and low temperature are grouped together, and samples under long incubation period and high temperature grouped separately (Montgomery 1991).

Microsoft Excel 97 has a built-in program to calculate and summarize the ANOVA results in a convenient table format. The most important part about this ANOVA table is the Fisher value (F_0). If the computed Fisher value exceeds the critical Fisher value (F_0), or if the Fisher test probability (P value) is smaller than the stated significance level α, then the treatment factor is considered to be significant.

4.5.4   Model checking

The single-factor ANOVA model assumes that the observations are independent and normally distributed with the same variance in each treatment. Thus, the checking procedure focuses on the *residuals*, that is, the differences between the observations and their respective group averages. A normal probability plot of the residuals can be made to check whether the plotted residuals lie more or less on a straight line. A test of $\sigma_1^2 = \sigma_2^2$ can also be used to check the equality of the variances; however, most statisticians find this test not to be very useful (Hogg and Ledolter 1992).

4.5.5   Correlation

The sample correlation coefficient provides a simple measure of association and quantifies the degree of linear association between two variables. It is important to emphasize that it only applies to linear association. The sample correlation coefficient is

always between -1 and +1. The sign of the coefficient tells us about the nature of the association; positive values imply a positive association: smaller (larger) x-values occur with smaller (larger) y-values, negative values imply a negative association: smaller (larger) x-values occur with larger (smaller) y-values. The absolute value of this coefficient tells us about the strength of the linear association, with -1 or +1 being a perfect linear association. A coefficient of zero, on the other hand, implies that there is no linear association (Hogg and Ledolter 1992).

4.5.6 Regression

Regression models are used to inspect more closely the relationship between x and y in paired data sets. One important objective of regression models is to predict a value of y where only the value of x is known. This is done based on finding the best-fitting line through the existing data points, also known as the method of least squares. The R-squared value helps determine the line of best-fit; a value near 0 indicates a poor fit, and a value near 1 indicates a good fit. Microsoft Excel 97 can determine the R-squared values in nonlinear least-squares procedures as well as in linear least-squares procedures.

Chapter 5

RESULTS AND DISCUSSION

5.1   Preliminary results

In a preliminary effort to measure chloroform production under chlorination at low concentrations, a series of 5 sets of raw and filtered water samples were collected from the Seymour Pilot Plant and subjected to SDS tests. All five effluent samples had been treated by sand/anthracite direct filtration with ferric chloride ($FeCl_3$) added and rapidly mixed as coagulant.

The following parameters are defined based on the SDS data, to describe and compare different treatment performances from a downstream THM controlling perspective:

- Cl_2 consumption = applied chlorine - free chlorine residual measured at completion of SDS run
- chloroform conc. = the amount of $CHCl_3$ formed during incubation
- chloroform yield = chloroform conc. / Cl_2 consumption
- normalized production = chloroform conc. of effluent / chloroform conc. of raw water
- normalized yield = effluent yield per unit Cl_2 / raw water yield per unit Cl_2

Table 5.1 shows the actual measured chloroform level in water samples, before and after treatment, and the normalized production for each SDS run. The normalized production provides a direct comparison of chloroform formation potential between the effluent and the raw water within the same incubation period. Values less than 1.00 indicate reduction of THM formation achieved by means of treatment process.

Table 5.2 presents the calculated chloroform yield of water samples, before and after treatment, and the normalized yield for each SDS run. The normalized yield compares the effluent and the raw water on the basis of equivalent chlorine consumption and provides a general indication of the presence of reaction intermediates and/or other chlorine demand, given the organic make-up of the two waters. Values > 1.00 indicate

reduction of other chlorine demand, inorganic or organic, by the treatment process; values < 1.00 reveal the presence of secondary reaction intermediates as a result of chlorination of organic precursors.

run	after treatment chloroform (μg/L)	before treatment chloroform (μg/L)	normalized production	incubation time
1	6.2	6.77	0.92	3h
2	18.91	22.41	0.84	15h
3	6.41	10.45	0.61	4d
4	3.77	17.04	0.22	4d
5	18.13	17.91	1.01	5d

Table 5.1   Chloroform reduction by direct filtration (normalized production < 1.00 indicates reduction)

run	after treatment			before treatment			normalized yield	incubation time
	Cl_2	chloroform	yield	Cl_2	chloroform	yield		
1	1	6.2	6.2	0.9	6.77	7.5	0.82	3h
2	0.3	18.91	63.0	1	22.41	22.4	2.81	15h
3	2	6.41	3.2	2.65	10.45	3.9	0.81	4d
4	1.3	3.77	2.9	2.1	17.04	8.1	0.36	4d
5	2.64	18.13	6.9	2.5	17.91	7.2	0.96	5d

Table 5.2   Normalized chloroform yield after direct filtration (Cl_2 measured in mg/L; chloroform measured in μg/L; yield measured in μg/mg)

SDS test results indicated a wide range of chloroform yield, as high as 18.9μg/L within 15 hours and as low as 3.8μg/L within 5 days. Moreover, free chlorine residual measurements at the end of each SDS run translated into variable chlorine consumption rates: 2.6 mg/L within 5 days for one sample, 1.3 mg/L within 4 days for another, and 1 mg/L within only 3 hours for yet a third sample. These early data suggested that criteria used to operate a treatment process may not achieve the same level of performance consistency when used as criteria to evaluate downstream distribution system conditions.

Except in one case (run 5), normalized production < 1.00 (Table 5.1) confirms the common findings of other studies covered in the literature (Childress *et al.* 1999;

Albidress *et al.* 1995; Hureiki *et al.* 1996): the effectiveness of using ferric chloride as coagulant and filtration/adsorption process in removing THM precursors. Childress *et al.* (1999) noted that, in their study, the reduction in THM formation potential is consistent with the trends observed for the THM precursor removal data (i.e. UV_{254} and TOC data). Hureiki *et al.* (1996) conducted a research on amino acids at two Canadian water treatment plants from Laval City (Quebec, Canada) and showed a strong decrease (34 to 72%) of total amino acids by coagulation-flocculation-settling. Although free and combined amino acids represent a small percentage of DOC (1 to 3% of DOC), total amino acids have been found earlier to account for an important part of the chlorine demand of treated water, and some amino acids may represent a significant fraction of the DBP precursors (Hureiki *et al.* 1994).

However, Childress *et al.* (1999), in the same study, also found coagulation to be less effective for a source water with lower SUVA. Specific UV absorbance, the ratio of UV absorbance to DOC, provides a relative index of humic content of the DOC (Owen *et al.* 1993). However, since the SUVA of Seymour source water is in the range of 3.5 to 4.1 L/mg•m (Table 4.1), ferric chloride is still believed to be an effective precursor-remover in this case.

Filtration/adsorption has also been shown effective in removing BDOC (Albidress *et al.* 1995). However, Hureiki *et al.* (1996) found that duel-media (sand and anthracite) filtration might increase or decrease the concentrations of amino acids; this may explain the increase in normalized production of Run 5 (Table 5.1).

Normalized yield < 1.00 (Table 5.2) reveals the presence of secondary reaction intermediates as a result of chlorination of organic precursors in all but one case (run 2). Adin *et al.* (1991) have described a mechanistic model for THM formation, in which organochlorine intermediates are produced in the first step and are converted to THM in the second step. Clark *et al.* (1994) found that these chloral hydrates were apparently transformed into THMs once the chlorine residual disappeared; thus, they are considered to be long-lived intermediates. U.S. EPA has proposed to include chloral hydrate in the National Primary Drinking Water Regulations (U.S. EPA 1994).

5.2   Ultrafiltration membrane

SDS tests were conducted on raw and permeate water samples which had been treated by the ultrafiltration membrane system; Table 5.3 and Table 5.4 summarize the average percent reduction of chloroform formation and the average normalized yield, respectively, over 15 runs, excluding blanks. The actual measured chloroform levels in water samples, before and after treatment, and the actual free chlorine residual measured at completion of each SDS run are presented in Appendix A. Figure 5.1 and Figure 5.2 present these measured chloroform concentrations graphically.

Table 5.3 indicates that the average chloroform production levels in both the 5-hour and the 4-day incubation periods were reduced by 11 and 9 percent, respectively, relative to their pretreatment levels. Chloroform levels in the permeate water samples were compared, statistically, to their counterpart in the raw water samples, using a paired-sample t-test, to test the null hypothesis. Since the t-test for small samples requires that the underlying population variances in the two groups are the same, normal distributions were first tested and confirmed (Hogg and Ledolter 1992). For long term incubation, chloroform formation is not significantly reduced by ultrafiltration membrane treatment ($P = 0.13$); however, for short term incubation, there is a highly significant reduction ($P = 0.001$).

These reductions were not high, and only under the 5-hour incubation period the reduction was significant. However, chloroform production levels in the raw water samples were quite low, averaging $18.57 \mu g/L$ in 5-hour incubation periods and $29.62 \mu g/L$ in 4-day incubation periods, which are well below the EPA maximum contaminant level (MCL) of 0.04 mg/L in Stage II of the D/DBP Rule (U.S. EPA 1994). Therefore, large reduction with the treatment is not expected. The mean values of normalized yield shown in Table 5.4 also confirm reduction of other nonTHM-yielding chlorine demand, inorganic or organic, by the treatment process: chloroform yielding per unit free chlorine consumed was more prominent in permeate waters than in raw waters since free chlorine was more available for chloroform formation in permeate waters than in raw waters.

| SDS | percent reduction | | number of |
incubation time	average	range	samples
5 hours	10.88 %	30.80 % to -1.91 %	9
4 days	8.68 %	39.57 % to - 43.79 %	6

Table 5.3   Chloroform reduction by ultrafiltration membrane system (negative reduction means an increase in chloroform production)

| SDS | normalized yield | | standard | number of |
incubation time	average	range	deviation	samples
5 hours	1.06	0.81 to 1.17	0.12	9
4 days	1.06	0.72 to 1.69	0.35	6

Table 5.4   Normalized chloroform yield after ultrafiltration membrane treatment (actual chloroform concentrations measured in μg/L)

Although the ultrafiltration membrane system consistently produced excellent finished water quality with turbidity < 0.25 NTU, SDS tests demonstrated a range of chloroform production in permeate waters, from 5.6μg/L to 29.3μg/L in 5-hour incubation periods (Figure 5.1) and from 7.9μg/L to 63.1μg/L in 4-day incubation periods (Figure 5.2). These results are in agreement with the findings of another independent study conducted by Lin *et al.* (1999). Lin *et al.* found that ultrafiltration membrane, having a relatively large molecular weight cutoff, was effective in reducing turbidity, particulates, organics and bacteria. However, although it was able to remove a significant portion of THM formation potential in large AMW fractions, the permeate THM yield in terms of μg THMs / mg DOC was still relatively high. Therefore, despite excellent, low turbidity being accomplished through a very effective inorganic and NOM removal process, the treatment does not selectively remove those organic precursors that are responsible for the occurrence of chloroform. The earlier result of a highly significant reduction of chloroform formation during the short incubation period, however, suggests that the ultrafiltration membrane system installed is more sensitive in removing fast-reacting precursors. It may be postulated that organics with large AMW constitute the fast-reacting precursors.
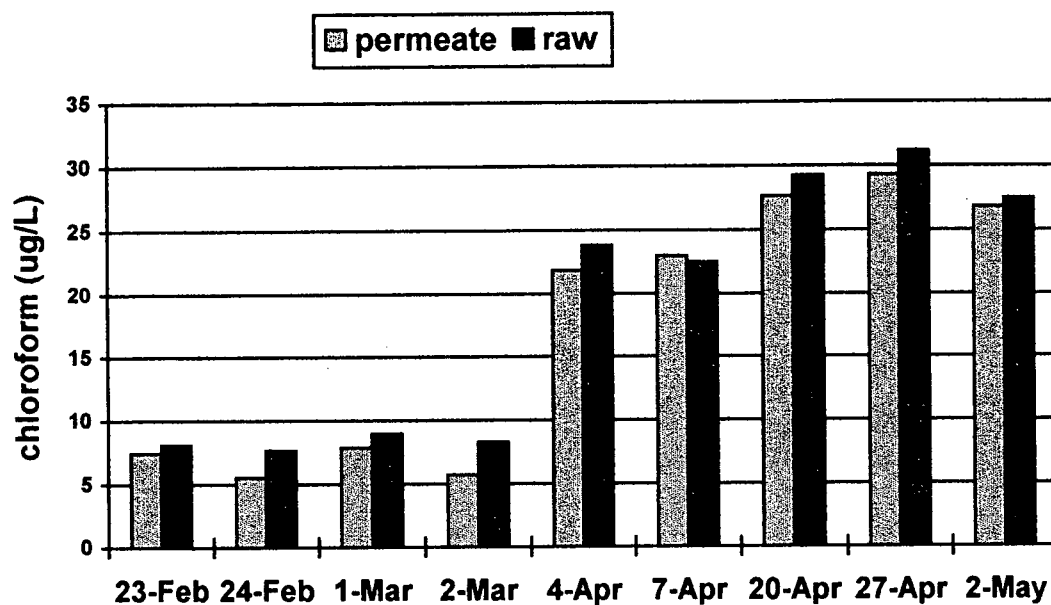
Figure 5.1   Chloroform production in raw and permeate waters (5-hour incubation)
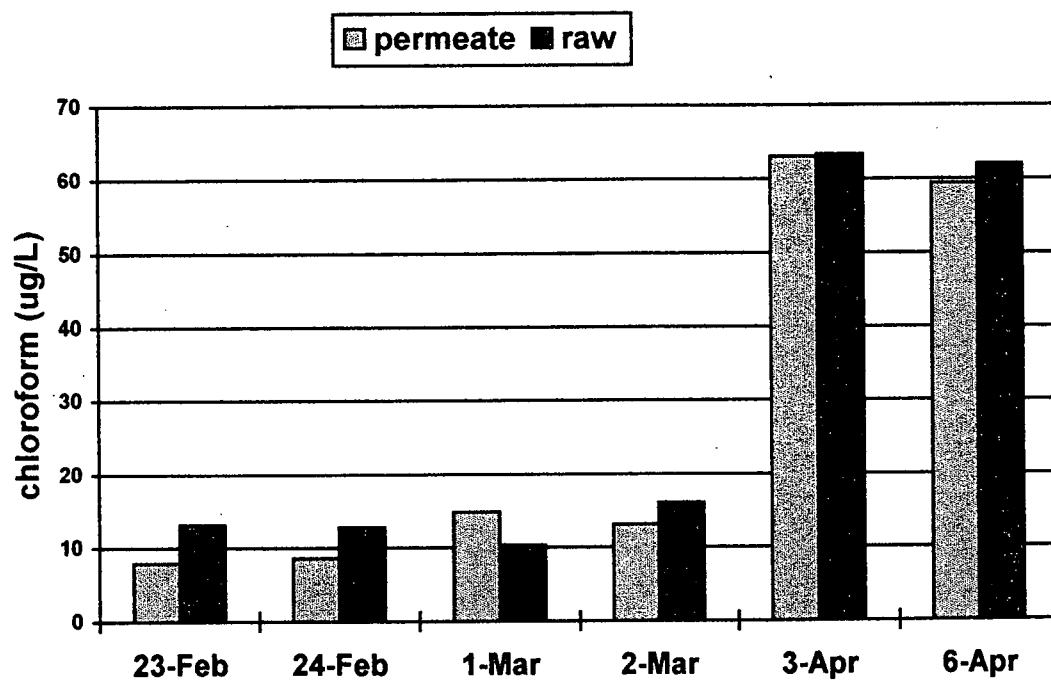

Figure 5.2   Chloroform production in raw and permeate waters (4-day incubation)

The mean concentration of chloroform production in the warm season (April and May) was more than three times that in the cold season (February and March) for 5-hour incubation, and for 4-day incubation, the mean concentration of chloroform production in the warm season was five times that in the cold season. Water temperatures in the cold season were recorded to be at 4 °C, and water temperatures in the warm season ranged from 6 °C to 11 °C. Seasonal differences in total THMs concentrations has been reported previously (Chen *et al.* 1998; Brett and Calverley 1979); one explanation for the seasonal differences was that chemical reaction rate constants increased with increasing temperature and higher temperatures in the warm season accelerated the rate of THM production in the distribution system as compared with the cold season. Although Figures 5.1 and 5.2 show essentially the same results, this rationale for the observed pattern cannot be applied in this case because the SDS tests employed in this study were carried out under constant temperature (25 °C). However, basic chemistry tells us that the solubility of solids, including organic matters, increases as the temperature increases. Thus, the observed pattern for chloroform production may be explained by an increase in dissolved organic content in the source water due to higher temperature in the warm season than in the cold season. Unfortunately, an evaluation of how changes in the source water organic matter affected the THM concentration could not be done, because organic carbon was not measured nor characterized. However, it may be inferred that DOC content was still relatively high in the permeate waters, since the chloroform reduction by ultrafiltration process was relatively low and the seasonal differences were much greater under the long incubation period.

5.3   MS-SDS test

Subsequent to the SDS tests, a series of MS-SDS runs were conducted to identify the relative role that the pipe environment may have on the final THM production. The total chloroform concentration measured in a water sample drained from an MS-SDS pipe container reflects the combined result of bulk water reactions and pipe wall influences; in comparison, the chloroform yield from bulk water reactions alone can be estimated from the parallel SDS test result. The following parameters are defined, based

on parallel SDS and MS-SDS data, to describe the overall impact from the pipe environment under certain laboratory conditions:

- total chloroform conc. = the amount of $CHCl_3$ formed during pipe incubation (MS-SDS)

- chloroform conc. = the amount of $CHCl_3$ formed during incubation (SDS)

- pipe effect (PE) = total chloroform conc. / chloroform conc.

PE values >1.00 indicate an overall increase in THM formation as a result of pipe wall surface interaction with residual chlorine. PE values <1.00 reveal a net loss of chloroform yield as a result of nonTHM forming chlorine demand imposed by the pipe environment (Brereton 1998).

In the following series of MS-SDS runs, the main factor of interest was the incubation time (5 hours, 4 days). Table 5.5 summarizes measured PE statistics collected over 26 MS-SDS runs, which employ both raw and permeate waters collected from the ultrafiltration membrane system, excluding blanks. The total chloroform concentrations measured in pipe-incubated water samples, and the free chlorine residual measured at completion of each MS-SDS run are presented in Appendix B.

incubation time	water sample	average PE	PE range	standard deviation	number of samples
5 hours	raw	1.13	0.51 to 1.79	0.50	7
5 hours	permeate	1.18	0.75 to 1.85	0.37	7
4 days	raw	0.75	0.61 to 1.10	0.19	6
4 days	permeate	0.83	0.59 to 1.24	0.23	6

Table 5.5 Effect of incubation period on PE values (actual chloroform concentration measured in $\mu g/L$)

In the 5-hour runs, PE values predominately exceed 1.00, while the 4-day runs result in PE <1.00 in almost every instance. Further analysis of the PE data can be done with ANOVA (the analysis of variance) to determine whether the treatment factor (incubation time) has any statistical significance on the PE data. The main factor of interest was the incubation time; therefore, one-way ANOVA is chosen as the most appropriate method, grouped by incubation period. The experimental design is

considered unbalanced because the two groups do not have the same number of data points. Moreover, a random-effects model of ANOVA is employed. Although all the data from each group are characterized by the same factor, the samples are taken from a larger population and are subject to variation. The random-effects model allows conclusions made from the data to be extended to the whole population (Hogg and Ledolter 1992). The Fisher test probability is $P = 0.012$; at $\alpha = 0.05$, the impact of incubation time on PE values is highly significant.

The residuals for all the PE values should follow a normal distribution, for the ANOVA method to be valid. Figure 5.3 shows a normal probability plot of the residuals against the normal scores; a linear pattern provides evidence that the underlying distribution is normal (Hogg and Ledolter 1992). The result fully confirmed that the data sets did not violate the essential assumption of the ANOVA method.
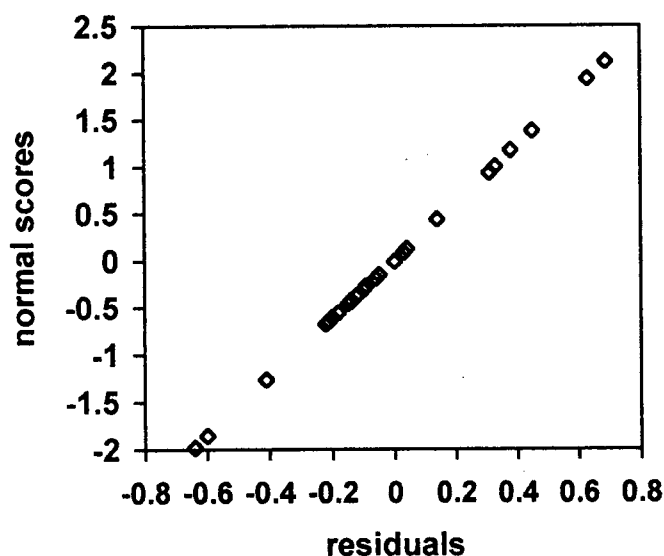
Figure 5.3   Normal probability plot for ANOVA of PE data

So far, it is evident that pipe wall contribution increased THM levels in the bulk water phase only during the short incubation period, and had a negative overall effect on chloroform production in the long incubation period. Based on the assumption that all haloform reactions occur simultaneously in the bulk water, it would then appear that slow-reacting precursors in a pipe environment are being removed from the bulk water

phase, most likely by means of adsorption onto pipe surfaces, thus leaving the fast-reacting precursors to react with the available chlorine. The long incubation, however, allows slow-reacting precursors in the SDS to overtake the overall chloroform production level in bulk water, due to the persistence of free chlorine in the glass bottles. This hypothesis is supported by the fact that chlorine was completely exhausted in most pipe containers by the end of the 4-day incubation period, while measurable residuals remained in all SDS bottles (Appendix B). The larger data spread evident at the 5-hour contact time is indicative of the variation in concentrations and reaction rates of a heterogeneous mix of precursors across the individual runs and the difficulty in achieving steady state, in this short incubation period.

The average PE values of permeate waters (Table 5.5) are slightly greater than that of the raw waters under both short and long incubation periods; however, the differences are not statistically significant at $\alpha = 0.05$ ($P = 0.358$ for the 5-h incubation period and $P = 0.256$ for the 4-d incubation period), when analyzed by the paired-sample t-tests. Pairing up certain sample data helps to control or account for variability that arises from differences among the experimental units. The advantage of pairing or blocking is to increase the precision, by eliminating the differences among the experimental units from the measurements, in sacrifice of the degrees of freedom. More degrees of freedom would have made the analyses more sensitive to detecting differences (Hogg and Ledolter 1992). Although paired PE values of raw and permeate waters help to account for the variability among different process treatment runs, the small difference between the two mean PE values may still be "drowned out" by the variability among different MS-SDS pipe containers. Thus, the primary purpose of this test is not so much to investigate the effectiveness of the treatment process, but to investigate the significance of the treatment process on chloroform reduction in a pipe environment.

In view of the highly significant chloroform reduction during short term incubation, as observed previously in Section 5.2, the pipe environment seems to have "drowned out" whatever impact the treatment may have on chloroform formation in the bulk water. Whether the pipe effect is positive or negative, it depends on the pipe surface mechanisms, with incubation time being the main factor of interest. Adsorption is most

likely the mechanism in the pipe environment that is responsible for precursor removal from the bulk water. The next challenge is to study all surface mechanisms involved in a pipe environment. Further MS-SDS experiments were conducted and their results are discussed in Section 5.4.

5.4 Pipe surface mechanisms

5.4.1 Organic free water incubation

A series of MS-SDS and parallel SDS runs were performed on organic-free waters in the same manner as the preceding runs. Chlorinated organic-free waters, prepared according to Section 4.4.4, were incubated in either pipe containers or SDS bottles for short (5 hours) or long (4 days) incubation period. Chloroform concentrations and free chlorine residual concentrations measured at completion of the MS-SDS and parallel SDS runs are presented in Appendix C. Figure 5.4 and Figure 5.5 present these results graphically.

The following assumption can be made because the water is organic free in nature, thus precursor free. Any THM production from this organic-free water has to be considered as either background concentration or end product resulted from the reaction of chlorine with precursors not originally present in the water column.

Figure 5.4 shows results from the MS-SDS and parallel SDS tests under the 4-day incubation period. Chloroform levels measured in these water samples were consistently about or below $2\mu g/L$ in the SDS test, but ranged from $1.33\mu g/L$ to $32.87\mu g/L$ in the MS-SDS test. Chlorine residual measurements at the completion of each run revealed almost zero chlorine consumption for SDS runs, while the applied chlorine was almost completely exhausted in the corresponding MS-SDS pipe containers. Chloroform concentrations at or below $2\mu g/L$ are generally accepted as background; however, CH_3Cl concentrations were observed to be 4, 5 or even 15 times background level in some MS-SDS runs. This was evidence of transfer of precursor materials from the pipe wall to the bulk water.

Figure 5.4   MS-SDS and parallel SDS tests on organic-free water

(incubation = 4d; applied chlorine = 2 mg/L; measurement above each bar
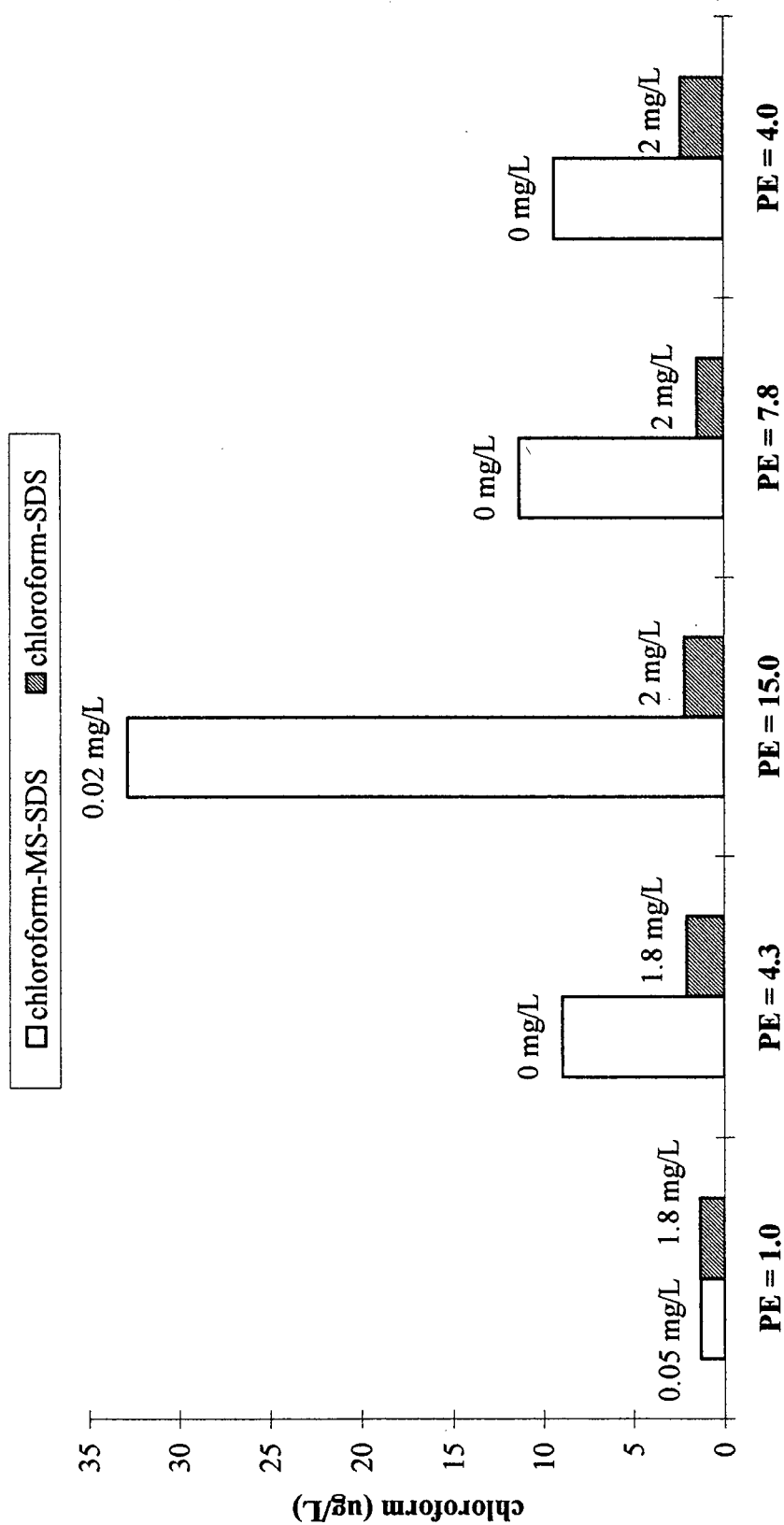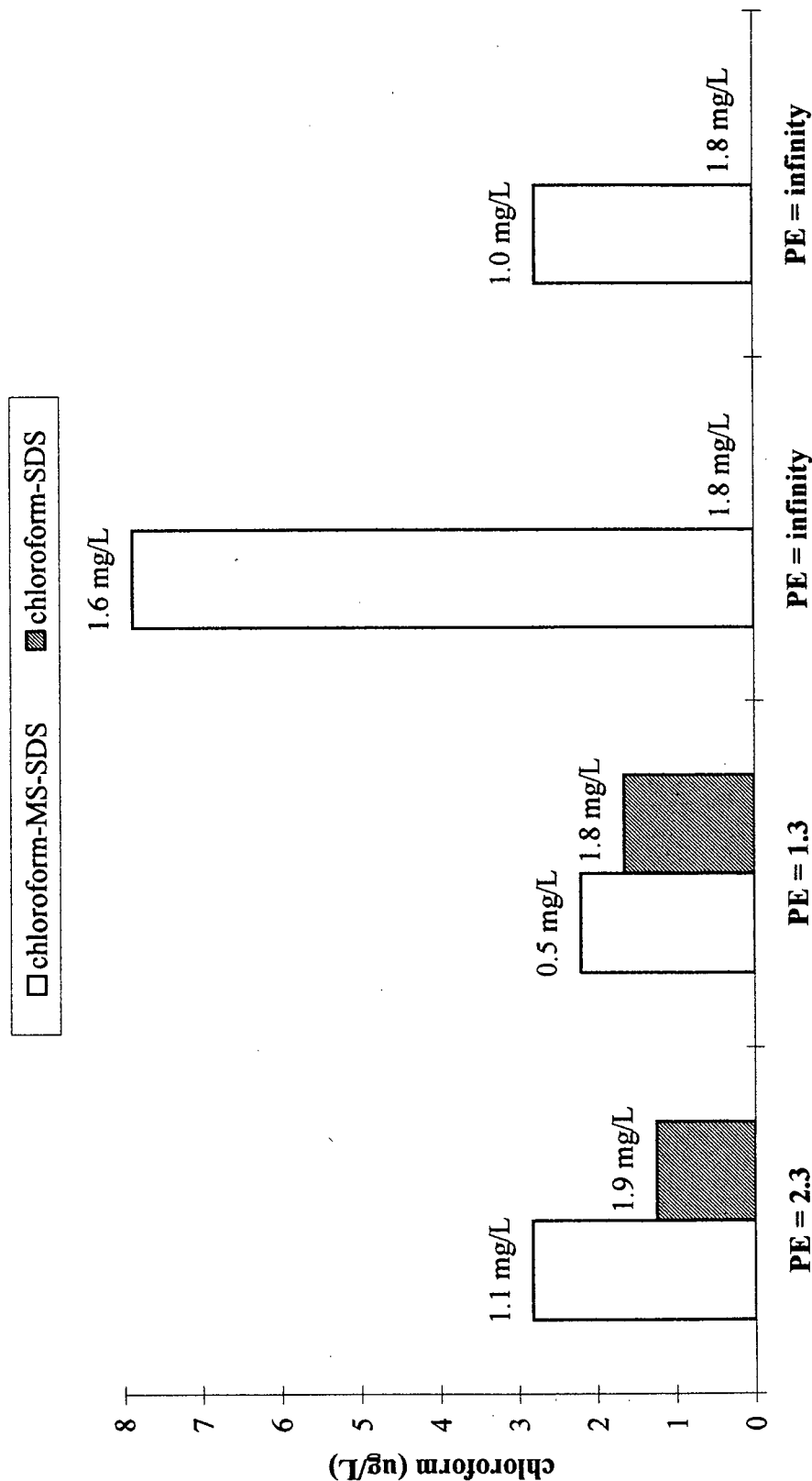indicates chlorine residual measured at completion of each run)

Figure 5.5 MS-SDS and parallel SDS tests on organic-free water

(<u>incubation = 5h</u>; applied chlorine = 2 mg/L; measurement above each bar
indicates chlorine residual measured at completion of each run)

Similar results from the SDS test were observed under the 5-hour incubation period (Figure 5.5), namely, almost zero chlorine consumption and chloroform concentrations at background level. However, MS-SDS test under the short incubation period indicated a lesser range of CH_3Cl concentrations, from $2.20\mu g/L$ to $7.88\mu g/L$, and the presence of free chlorine residual at the completion of each run. Nevertheless, more chlorine was consistently consumed in pipe containers than in SDS bottles by the end of the incubation period.

Regardless of incubation time, chloroform production maintained PE $\geq$ 1.00 in every instance (Figure 5.4 and Figure 5.5), indicating that the pipe environment was definitely an active contributor to aqueous chloroform formation. In one case (Figure 5.5), CH_3Cl concentration exceeding the background level was observed in the MS-SDS run, when the parallel SDS run produced no detectable chloroform formation. This suggests that the source of these additional precursor compounds may be organic substances previously adsorbed onto pipe surfaces, when the pipe containers were exposed to flow-through, raw water for *in-situ* biofilm establishment. These observations support the speculative discussion in Section 5.3, in which adsorption is perceived as the surface mechanism responsible for the removal of slow-reacting precursors.

5.4.2   Heterotrophic plate counts (HPC)

Heterotrophic plate counts were carried out with each pipe incubation of organic free water, to assess the relative involvement of PVC biofilm organisms in surface interaction. Prior to each run, pipe containers were connected to the raw water inlet at Seymour Pilot Plant, to allow at least 72 hours of continuous flow of untreated water, thus maintaining a film of healthy heterotrophic organisms. Free chlorine residual measurements, immediately upon the completion of the runs, maintained the trend that chlorine was completely exhausted by the end of most long incubation runs, while measurable chlorine remained after 5 hours of contact time (Appendix C). HPC's, following these runs, revealed that bioactivity was reduced after chlorination and that the applied chlorine residual of 2 mg/L was sufficient for biofilm inactivation in most runs (Table 5.6). This is consistent with previous observations by LeChevallier *et al.* (1990),

where bacteria grown on PVC pipe surfaces were readily inactivated by a 1 mg/L residual of free chlorine.

Cl_2 remaining (mg/L)	chloroform measured (μg/L)	HPC following MS-SDS / HPC prior to MS-SDS (cfu/ml)	contact time
0.05	1.33	none detected	4 days
0	8.93	none detected	4 days
0	11.32	200/1000	4 days
0.02	32.87	60/1000	4 days
0.04	21.32	4900/5500	4 days
1.1	2.82	none detected	5 hours
0.5	2.2	none detected	5 hours
1.6	7.88	10/340	5 hours
1.0	2.76	none detected	5 hours

Table 5.6 Heterotrophic plate counts following MS-SDS test on organic-free waters (Figure 5.4 and Figure 5.5)

However, runs with higher chloroform production in the bulk water were found to be accompanied by active bacterial growth in biofilm. It appears that chlorination acts selectively on bacteria which, in turn, promote some form of resistance mechanism that results in chloroform formation. Humic substances were shown to be utilizable as carbon and energy sources for biofilm bacteria, when they were adsorbed to surfaces (Camper *et al.* 1999); thus, chlorinated cells probably incorporate the humic substances among those organic compounds previously adsorbed to pipe surface (Section 5.4.1) to repair damage due to disinfection. There was also visual evidence that humic substances were adsorbed, as scrapings of biofilm exhibit a characteristic brown colour. Such "survival strategy" apparently occurs at chlorine concentrations sufficient to penetrate the biofilm, yet insufficient to effect cell death. HPC's associated with high aqueous chloroform measurements present strong evidence to suggest that these humic substances are subsequently released, allowing them to become available as precursors for THM formation.

Colony forming units grown on agar were smooth, sometimes with mineral-like deposits and ranged in color from light yellow to orange. Extracellular polysaccharides (EPS), produced by attached bacteria, are believed to provide a barrier to chlorine

penetration; they help to anchor the bacteria to the pipe surface, and may also be a factor in nutrient capture (Fletcher and Marshall 1982). This suggests that humic substances are most likely incorporated with inorganic materials and bounded within the EPS.

5.4.3 Sorption

Brereton (1998) observed that partitioning of pre-formed chloroform in an iron pipe environment was a one-way, irreversible desorption to the aqueous phase, driven by a concentration gradient of the chloroform level in the bulk water. Therefore, several cursory experiments were conducted to study the movement of pre-formed chloroform in a PVC pipe environment. Chloroform-spiked ($30\mu g/L$) non-chlorinated water was incubated in a pipe container for 4 days. No significant change in chloroform was measured in the spiked water. This indicates that adsorption of pre-formed chloroform does not occur. Following a series of random MS-SDS runs in which measurable chloroform formation occurred, the pipe containers were drained, re-filled with non-chlorinated organic free water and incubated for 7 to 10 days. No significant chloroform was observed in the organic free water. This indicates that carry-over interference does not occur.

Thus, any increase / decrease in chloroform production in the aqueous phase is the net result of (1) an increase / decrease in background concentration, (2) a decrease in CH_3Cl concentration due to the removal of precursors by means of surface adsorption, and (3) an increase in CH_3Cl concentration due to the release of previously adsorbed precursors, subsequent to cell damage repair.

5.5 Bioactivity and THM production

Figure 5.6 illustrates the relationship between bioactivity measured by HPC and chloroform production for several sets of raw and permeate waters, in the 4-day pipe incubation runs. Heterotrophic plate counts performed on-line, prior to the runs, enumerated between 250 cfu/cm^2 and 450 cfu/cm^2. After 5 hours of incubation, HPCs were repeated on randomly selected pipe containers: enumeration was largely reduced to between zero and 340 cfu/cm^2. However, by the end of the 4-day runs, HPC results

clearly showed that bioactivity had not only recovered but proliferated (Figure 5.6). This trend does not contradict the biofilm inactivation observed in Section 5.4.2, in view of the difference in nature of the incubated waters — organic free water versus humic waters. On the contrary, the proliferation is in agreement with the discussion on biofilm survival strategy presented in Section 5.4.2.
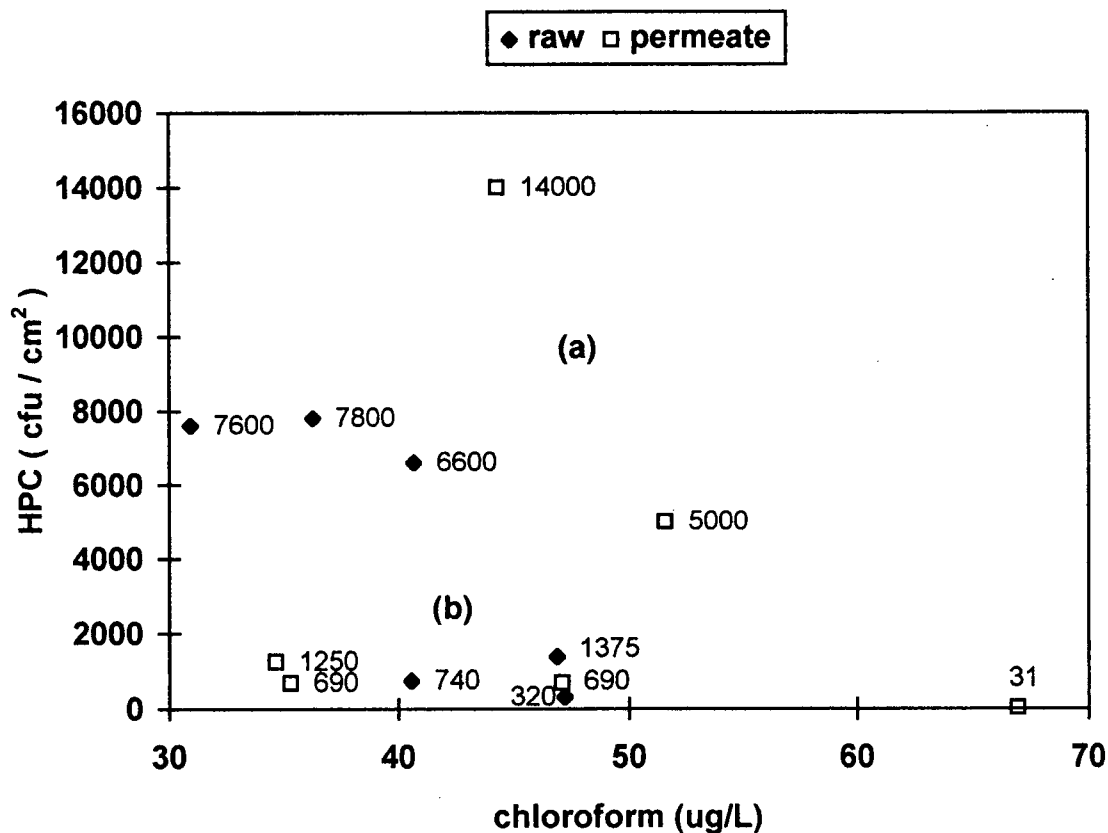
Figure 5.6   Heterotrophic plate counts and chloroform production (incubation time = 4 days)

The ubiquitous presence of humic substances in raw and permeate waters, when adsorbed to pipe surfaces, provides an abundant carbon and energy source for excellent biofilm damage repair and cell proliferation. Figure 5.6 fails to prove a linear relationship between bacteria regrowth and increased chloroform formation ($r^2 = 0.66$ for raw water; $r^2 = 0.083$ for permeate water) because this formidable task is confounded by so many interactions involved in chloroform formation within a pipe environment. The

relative mix of fast- and slow-reacting precursors and other chlorine demanding compounds in the bulk water promote inconsistent reaction conditions. This is further complicated by reaction dynamic of haloform intermediates which may be partially or fully chlorinated. Thus, the additional chloroform formation associated with bioactivity is carried out in concert with the slow haloform reaction proceeding under increasingly chlorine-limiting condition, and the effect may be difficult to isolate or correlate.

Chloroform production data from the permeate water samples was compared to their counterpart from the raw water samples, and the paired-sample t-test at $\alpha = 0.05$ shows the two groups of data to be statistically indifferent. This is expected because the pipe environment have "drowned out" whatever impact the treatment may have on chloroform formation in the bulk water. Thus, Figure 5.6 may now be simplified into one homogeneous block with completely randomized data. Two clusters, (a) and (b), become apparent under such light, and they are re-plotted as Figure 5.7 and Figure 5.8.

Figure 5.7 shows a poor fit for linear relationship between HPC and chloroform production ($r^2 = 0.004$) for the upper data set (a); however, by visual inspection, one point stands out as outlier. After the outlier is taken away, the regression line improves greatly, with the adjusted r^2 being 0.917. On the other hand, the best-fit regression line shown in Figure 5.8 for the lower data set (b) is an exponential curve ($r^2 = 0.747$). Furthermore, the regression lines for both data sets (Figures 5.7 and 5.8) are "negatively" sloped; this indicates that small (large) values of HPC are associated with large (small) values of chloroform production. In order to quantify the strength of association between these two variables, the correlation coefficient is employed as a measure. It is important to emphasize that the correlation coefficient only measures the degree of *linear* association between two variables; therefore, a linear transformation has to be performed on Figure 5.8 before the correlation coefficient can be calculated. The transformation is presented graphically in Figure 5.9. The correlation coefficients for Figure 5.7 (without the outlier) and Figure 5.9 are, respectively, -0.96 and -0.86.
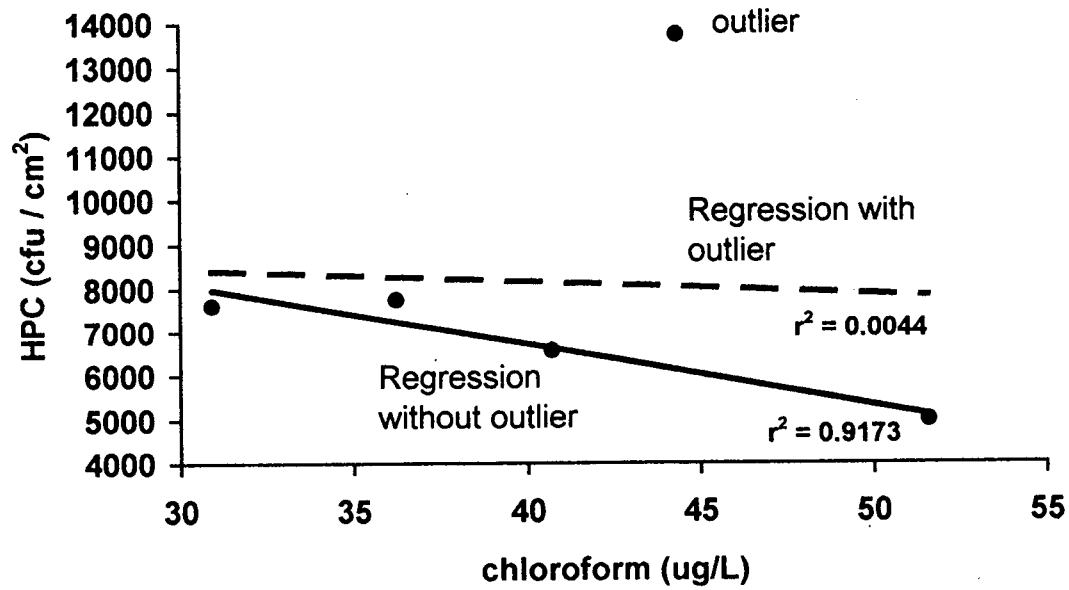
Figure 5.7   Regression plot of heterotrophic plate counts and chloroform production (cluster (a), Figure 5.6)
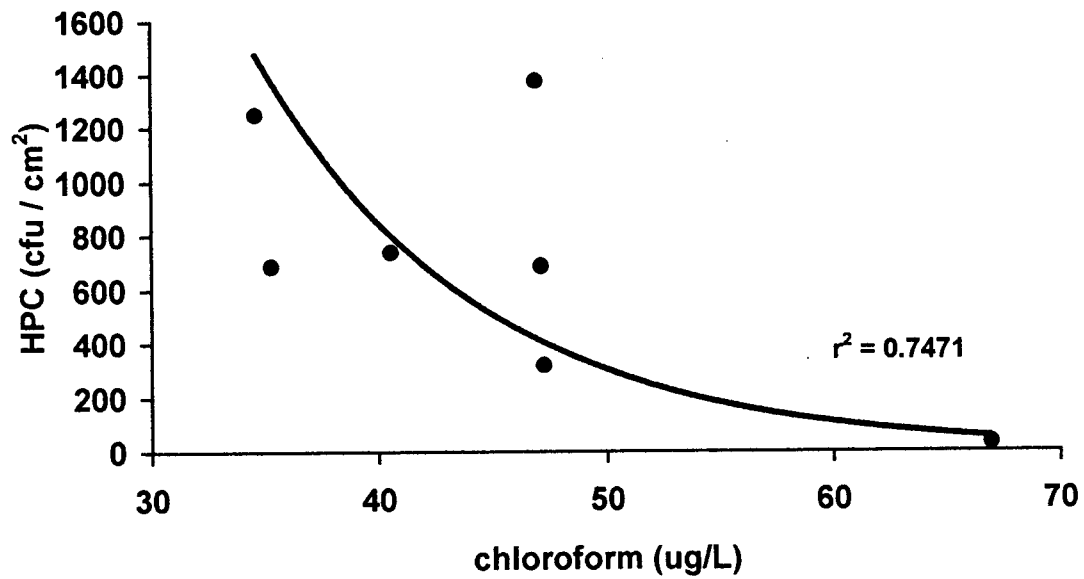
Figure 5.8   Regression plot of heterotrophic plate counts and chloroform production (cluster (b), Figure 5.6)
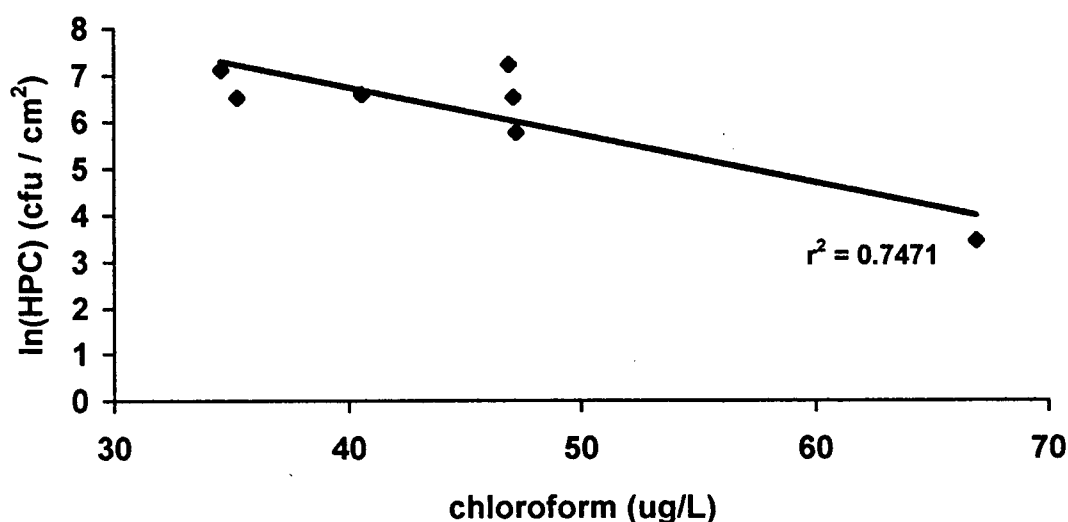
Figure 5.9   Linear transformation of Figure 5.8

The sign of the coefficient gives information about the direction of association between the two variables: negative values imply an inverse association, which confirms the "negatively" sloped regression lines in Figures 5.7 and 5.8. This trend, if accurate, agrees with the two surface mechanisms described in Section 5.3 and Section 5.4. Surface adsorption, which is responsible for the removal of precursors from the bulk water phase, requires a healthy film of microorganisms attached to the pipe walls. The higher the bioactivity (high HPC), the more effectively precursors are removed (low chloroform production). However, at chlorine residual concentrations where biofilm is susceptible to disinfection (low HPC), bacteria are more inclined to jump-start a repair mechanism that results in the release of precursor compounds for additional chloroform formation. It is also noteworthy that the relationship between bioactivity and chloroform production follows a linear one at higher HPC levels, but an exponential one at lower HPC levels. The cell damage repair mechanism may be speculated to be in full gear only when inactivation is at risk; however, as bacteria continue to grow, chloroform formation occurs only by the reaction of available chlorine with aqueous precursors not adsorbed onto biofilm but does not necessarily benefit from additional release of adsorbed substances during biofilm damage repair.

5.6   Re-chlorination effect

The Greater Vancouver area uses automated rechlorination stations to boost chlorine levels during distribution. Thus, an earlier effort was made to perform a couple of MS-SDS and parallel SDS runs on raw and filtered waters in a slightly different manner, to consider the effect of booster disinfection. The effluent samples had been treated by dual-media direct filtration, with $FeCl_3$ as a coagulant at the Seymour Pilot Plant prior to chlorination. At time zero, a free chlorine residual, at 2 mg/L, was added to all water samples incubated in both SDS glass bottles and MS-SDS pipe containers. After 24 hours, the same water samples were re-chlorinated with 1 mg/L of free chlorine (booster effect). By the end of the incubation period (4 days from time zero), chlorine residual measurements, chloroform formation analyses and heterotrophic plate counts were carried out accordingly. Table 5.7 and Table 5.8 summarizes all measured and calculated data on two separate sampling events.

sampling event	MS-SDS		SDS		PE	water type
	Cl_2 residual	chloroform	Cl_2 residual	chloroform		
#1	0	8.61	0.65	10.45	0.82	raw
#1	0	7.41	1.3	6.41	1.16	filtered
#2	0	10.65	1.2	17.04	0.63	raw
#2	0	5.57	2.0	3.77	1.48	filtered

Table 5.7   MS-SDS and SDS test results (Cl_2 measured in mg/L; chloroform measured in μg/L; sampling events correspond to those in Table 5.8)

sampling event	HPC following MS-SDS	HPC prior to MS-SDS	water type
#1	4000	2500	raw
#1	900	2500	filtered
#2	none detected	2500	raw
#2	none detected	2500	filtered

Table 5.8   Heterotrophic plate count results (HPC measured in cfu/mL; sampling events correspond to those in Table 5.7)

As discussed earlier in Section 5.3, most long incubation runs resulted in PE values < 1.00, regardless of water type. This trend, however, is observed only in raw waters in this experiment (Table 5.7). The action of re-chlorination offers additional free chlorine and more favorable reaction conditions for slow-reacting precursors; the increased chloroform production indicates that a substantially higher concentration of precursors is masked by the chlorine-limited state of the bulk water. Nevertheless, only the filtered waters show signs of taking advantage of such favorable conditions for chloroform formation. This suggests that raw waters contribute a higher proportion of nonprecursor chlorine demand than filtered waters. Such observation do not contradict the calculated mean values of normalized yield presented in Table 5.2; this also suggests a reduction of nonTHM-yielding chlorine demand (inorganic or organic) by the treatment process. Moreover, PE values > 1.00 implies the adsorption of organic matters to pipe surfaces prior to the runs, which may translate into downstream THM control problem in a real distribution system.

In spite of the same booster treatment, Table 5.8 shows different HPC outcomes following the MS-SDS test for the two sampling events. Although booster disinfection has the potential of becoming an effective prevention to biofilm regrowth (sampling event #2), it may also be out-weighed easily by other *in-situ* factors, such as the organic (and inorganic) composition of the water and the dynamic state of the biofilm.

5.7   Haloacetic Acids

A cursory assessment of haloacetic acid occurrence in the PVC pipe environment was conducted using the acidic methanol derivatization procedure, described in Section 4.4.3 (specifically Figure 4.2 and Table 4.2). As bromide levels in Vancouver water are naturally low, brominated species of the haloacetic acids are not of concern. Moreover, despite being one of the five major HAAs, MCAA (monochloroacetic acid) was not analyzed because there were concerns about the poor extraction efficiency or the poor GC-ECD response for MCAA using this procedure (Xie *et al.* 1998). Attempts were made to analyze DCAA and TCAA (di-, and trichloroacetic acids) only. Nonetheless, no

valid HAA result is presented in the present study, due to the unsuccessful attempt by this researcher to execute the acidic methanol derivatization procedure.

Great difficulty was experienced with this procedure in achieving the quality control desired. Firstly, plotted points for DCAA often produced a calibration curve with a negative slope which could not pass through zero (Figure 5.10). Peak area data for each HAA compound and an internal standard peak area were used to calculate the relative response, and plotting such responses versus standard concentrations should generate a calibration curve passing through zero for each HAA compound. Secondly, injected samples were often unacceptable because results did not meet the quality control criteria (*7d. Internal standard assessment*) described in *Standard Method* 6251B (APHA *et al.* 1995).
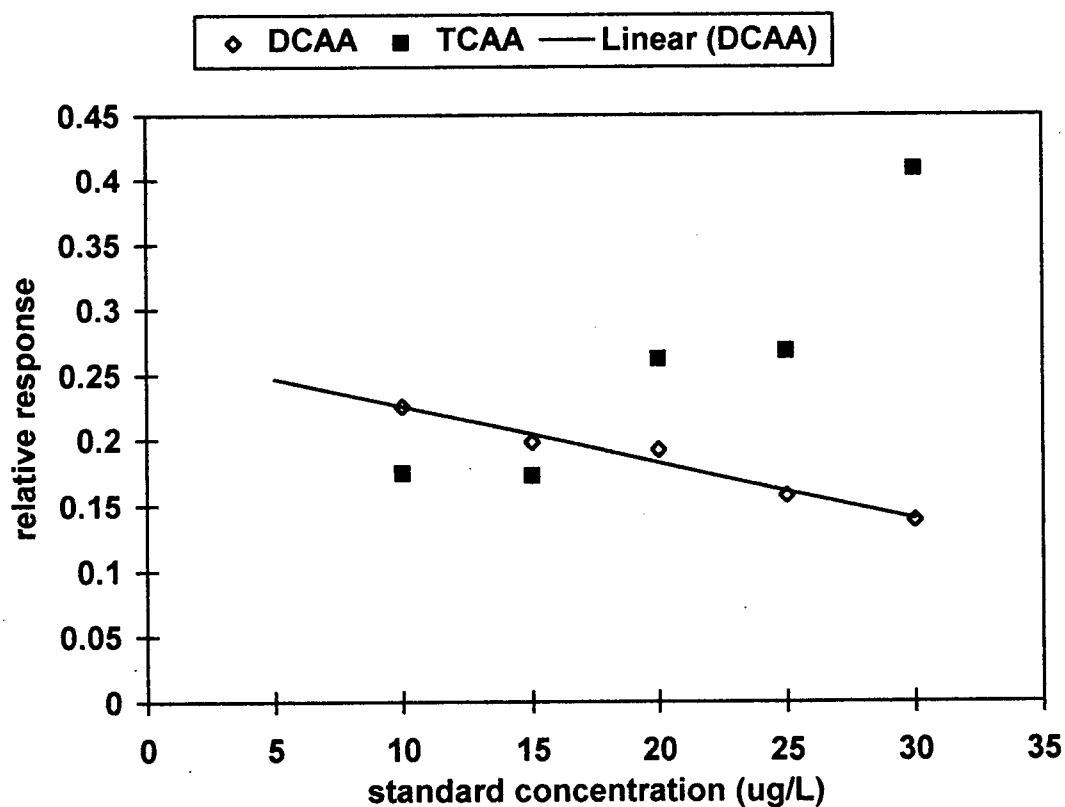
Figure 5.10 Calibration curve for HAA analysis using acidic methanol methylation technique proposed by Xie *et al.*, 1998.

Although no further attempt was made in the present study to validate this procedure, the same method has been incorporated as EPA Method 552.2 in determination of haloacetic acids in drinking water (U.S. EPA 1995), and a recent study has been conducted to compare HAAs results using both the diazomethane derivatization and the acidic methanol derivatization techniques (Urbansky 2000). All the nine species of HAAs gathered under the Information Collection Rule were analyzed by both techniques. For HAA$_5$, diazomethane was considered satisfactory as a derivatization agent. However, for HAA$_9$, acidified methanol outperformed diazomethane, which suffered from photochemical-promoted side reactions, especially for the brominated species.

One explanation for this researcher's unsuccessful attempt to execute the acidic methanol technique may lie in the reversible mechanism of esterification. Esters are formed by the reaction of acids (analytes) and alcohols (methanol); the reaction between low-molecular-weight organic acids and alcohols is never complete. In dilute acid, hydrolysis occurs and a reversible reaction results (Sawyer et al. 1994; Schaum's Outline Series). Xie et al. (1998) proposed 1 hour at 50 °C for methylation (or esterification) in their method. In the present case, acid-catalyzed hydrolysis may have occurred before extraction is completed.

Chapter 6

SUMMARY AND CONCLUSIONS

6.1   Summary

6.1.1   General review

At the focus of this study was chloroform production in drinking water, under the influence of two distinct environments: the bulk water phase and the pipe environment. In preliminary experiments, various treatment processes with an excellent level of performance produced effluents with a wide range of chloroform yield under chlorination; this indicated that water treatment did not selectively remove those organic precursors that were responsible for the occurrence of chloroform in bulk water phase. The next challenge was to investigate any change in behaviour of aqueous chloroform production in pipeline conditions.

The MS-SDS procedure proved extremely useful in the direct comparison of THM formation in the two environments mentioned above.   Generally, extra chlorine demand exerted by the pipe environment may contribute to the decreased chloroform production in bulk water at chlorine concentrations typical of most utility practice. Declining yield, due to the loss of chlorine residual through disinfection, is then merely a problem of maintaining disinfectant. However, several other aspects of this study reveal more complex reaction dynamics.

Firstly, chlorinated water incubated in pipe containers produced more chloroform than that in SDS glass bottles during the first 5 hours of contact. This phenomenon may be attributed to the adsorption of a proportionate majority of slow-reacting precursors to the pipe surface, thereby allowing the applied chlorine free to combine rapidly with the fast-reacting precursors in the bulk water. Secondly, in the chlorine booster effect experiments, re-chlorination significantly increased THM production beyond SDS capacity in treated water, indicating a higher concentration of 'borrowed' precursors masked by a chlorine-limited condition.   Thus, at least some of the time, the susceptibility of these adsorbed precursors to residual chlorine is likely to pose a threat

downstream of the THM control efforts in a distribution system. Finally, and perhaps most telling, is the substantial chloroform occurrence in pipe-incubated, organic-free water, as revealed by the MS-SDS test.

The accumulation of microorganisms in the form of a slimy surface layer (biofilm) on pipe surfaces may promote opportunities for precursor-chlorine reactions; bioactivity, indicated by HPC measurements, thus becomes the fundamental factor of interest. A pattern of increasing chloroform production with bioactivity emerges in response to the bacterial inactivation pressure exerted by chlorination, indicative of a biofilm resistance mechanism that results in the release of adsorbed humic substances for aqueous chloroform formation. Previously, Camper *et al.* (1999) showed the potential of humic substances as carbon and energy source for bacterial growth in an otherwise low nutrient environment, when they are adsorbed to surfaces; however, the present study expands this knowledge to the drinking water chlorination practice and, for the first time, observes and reports an associated chloroform formation.

6.1.2 Reaction Dynamics

This section offers a condensed narration of the reaction dynamics for THM production in water chlorination (typical of practice in Vancouver) within PVC pipes, based on the completed research. A pictorial model is provided in Figure 6.1.

In the standard SDS test, a finished water is chlorinated with the desired dosage to allow haloform reactions to proceed under incubation for a time period, resulting in measurable THM production and chlorine residual. These reactions take place inclusively in the bulk water phase; they include direct formation of THMs and partially or fully chlorinated intermediates, based on the relative concentrations of a heterogeneous mix of fast- and slow-reacting precursors in the finished water (as well as the interactions of chlorine with other nonTHM yielding compounds). The residual chlorine, upon completion of the incubation period, is indicative of the bulk water chlorine demand. Since the potential influence of a pipe environment is absent, the PE parameter is equal to unity by definition.

Figure 6.1   Pictorial model for THM evolution within a PVC pipe environment

Abbreviations

P   organic precursors
I   intermediates
THM   trihalomethanes
OD   nonTHM-yielding chlorine demand

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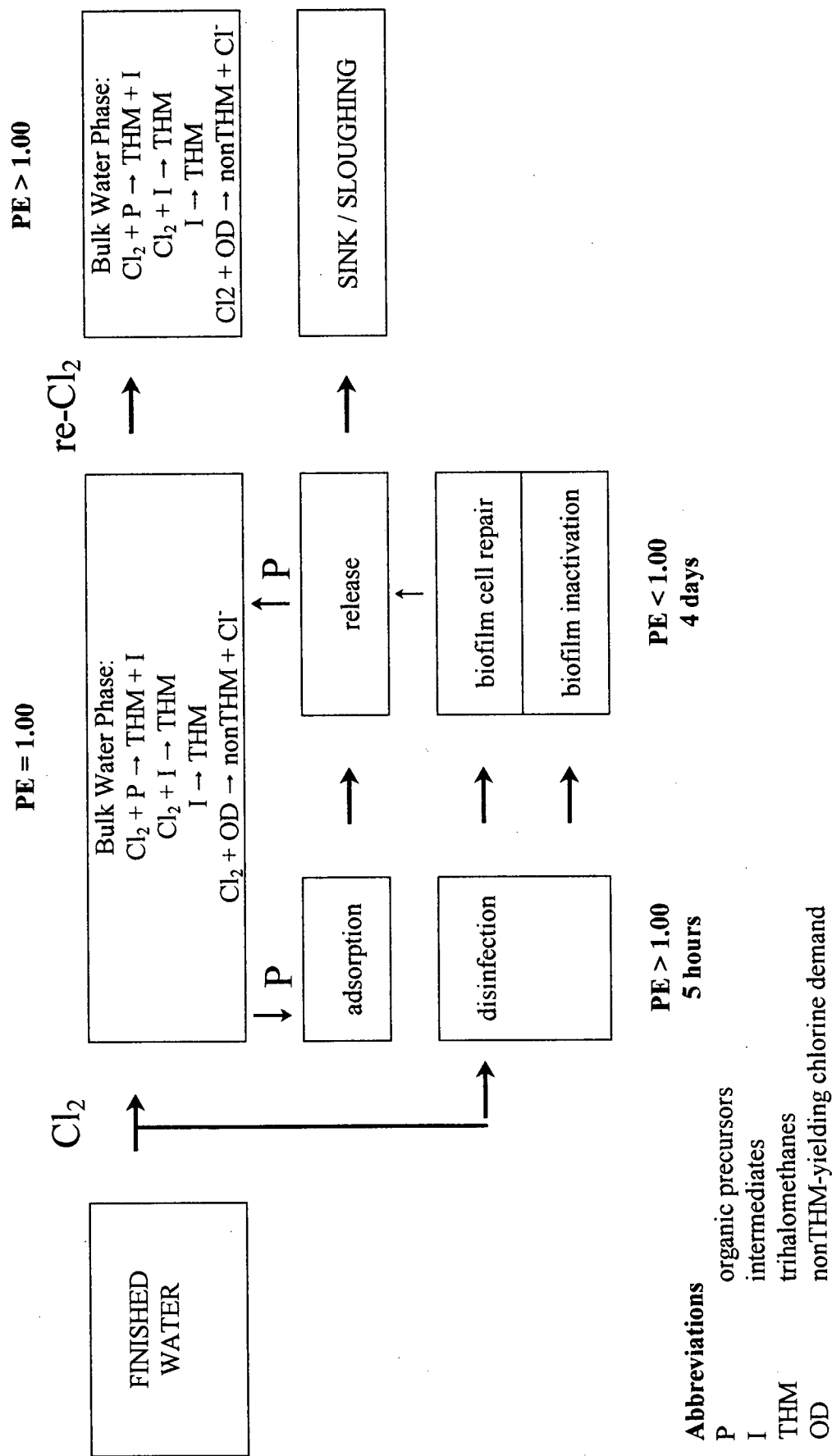
In the presence of a PVC pipe environment, however, two general outcomes are possible, dependent on reaction time. During short incubation period (5 hours), the removal of slow-reacting precursors from the bulk water phase provides excess available chlorine to react with the fast-reacting precursors. For long incubation (4 days), the participation of the pipe wall produces lower, overall THM production in the bulk water under limited-chlorine situation, due to proportionately higher chlorine disinfectant decay.

In both MS-SDS cases, bulk water reactions proceed as in the SDS test, the primary difference being the adsorption of a portion of the precursor materials from the bulk water to biofilm surfaces. During the first several hours of contact, humic substances are incorporated into the EPS matrix produced by the attached bacteria, resulting in more available chlorine for "rapid" THM production. At the same time, there is sufficient chlorine to satisfy both the bulk water demand and the biofilm disinfection demand. This typically yielded higher THM production in relation to SDS test, and hence PE values exceeding 1.00, although the relative organic make-up of the finished water would tend to dictate the outcome in each individual instance.

For the increasingly limited chlorine condition in long-period incubation, chlorine consumption is proportionately higher in disinfection (nonTHM producing reactions), initiating a bacterial cell damage repair mechanism and release of bounded humic substances to the water column. Bulk water haloform formation reactions still occur, albeit less efficiently in terms of overall consumption. These reactions include direct formation of THMs by virtue of a chlorine reaction with previously adsorbed precursors, as well as intermediate (partially and fully chlorinated) production. Depending on the residual concentration gradient in relation to bacterial counts and other chlorine demand, biofilm growth may be inactivated or reduced sufficiently to promote the resistance mechanism. In some instances, however, the residual concentration may be insufficient to penetrate the biofilm and cell proliferation was subsequently observed (likely due to chlorine consumption of the diverse mix of nonprecursor compounds present in the finished water). Nonetheless, the net result is lower THM production and lower chlorine

residual in relation to the SDS test. Thus, PE values are almost exclusively less than unity.

The dynamics of precursor fate is complex because of the heterogeneous nature of the pipe environment and the minute kinetics of all interactions. It may be self-evident that humic substances were adsorbed to biofilm surfaces, since scrapings of biofilm resulted in a visible characteristic brown colour. In this work, THM formation in the absence of any organic matter in the bulk water was observed, supportive of the theory of transfer of precursors from the bulk water phase to the pipe environment. The unrealized formation potential, masked by the chlorine limited state of in-situ water, of those THM precursors that remain in the bulk water phase may have several possible outcomes. Re-chlorination in the form of distribution system booster stations may provide favorable condition for increased THM production beyond conventional SDS prediction (i.e. PE >1.00). Extracellular polysaccarides (EPS), produced by the biofilm, is believed to be a factor in precursor capture; they can create a reservoir for subsequent spread downstream of DBP control, following biofilm detachment or "sloughing". Finally, precursors may remain not susceptible to chlorine in the bulk water state indefinitely.

6.2   Conclusions

Biofilm growth on PVC pipes influences the formation of chloroform in chlorinated drinking water. Based on the present work, the following conclusions are drawn:

1) Although low turbidity and some reduction of chloroform formation were achieved by the ultrafiltration membrane system installed, the treatment did not selectively remove THM precursors.

2) Chlorinated organic free water, incubated in PVC pipe containers, showed an increase in chloroform concentrations, as a result of release of previously adsorbed humic substances onto biofilm surfaces; this was in response to chlorination and subsequent haloform reactions in the bulk water phase.

3) The release of precursors from the biofilm was in association with bacterial regrowth, due to cell damage under chlorination.

4) Chloroform formation took place exclusively in the bulk water phase and was dependent on the relative abundance of slow- and fast-reacting precursors in the finished water.

5) The MS-SDS test proved an extremely useful tool in the assessment of pipe effects on THM production.

6) The increased chloroform production of a finished water, during short contact with PVC pipe walls, may be attributed to the adsorption of a proportionate majority of slow-reacting precursors to the pipe surface, thereby leaving more available chlorine for rapid aqueous haloform reactions.

7) In longer incubation (4 days) at relatively low chlorine application (2-3 mg/L), chloroform yield exceeded that of the same water in contact with PVC pipe walls. In shorter incubation (5 hours), chloroform yield, associated with the water in contact with the pipe walls, sometimes exceeded that of the same water in clean glass bottles.

8) In long incubation MS-SDS, contact with the pipe walls decreased THM production efficiency in terms of lower chloroform formation and higher free chlorine consumption.

9) Free chlorine application at 2 mg/L readily inactivated bioactivity and was effective in reducing biofilm growth in PVC pipes.

10) The heterotrophic mix of nonTHM yielding chlorine demand present in the finished water may reduce chlorine to concentrations insufficient to penetrate the biofilm; bacterial proliferation can occur without utilizing the adsorbed humic substances and subsequent release.

11) Chloroform concentrations were poorly correlated with heterotrophic plate counts of biofilm sampling, likely due to the many interactions involved in chloroform formation within a pipe environment

12) Re-chlorination promoted a more favorable condition for slow chlorine-precursor reactions (including direct formation and formation due to biofilm repair mechanism); a chlorine booster effect was more influential in water with a lower proportion of nonprecursor chlorine demand.

13) The consequences of chlorine dosage on chloroform formation in waters, in contact with the internal PVC pipe environment, was identified and described in this research program.

6.3   Recommendations

1) It is recommended that the MS-SDS test be adopted as a supplemental tool to the more conventional SDS test, currently in use.

6.4   Practical Implication

From a utility's point of view, the practice of residual chlorination in a PVC distribution system is to be cautioned. Specific to the finished water quality, even a relatively low chlorine application (2 to 3 mg/L, approximately) may increase THM exposure to the public, particularly if the travel time to end user is less than 5 hours. Biofilm, associated with PVC pipes, promotes "rapid" formation, that leads to a higher chloroform level than that associated with the finished water alone. In addition, additional chloroform production is suspected to be associated with biofilm regrowth. Thus, SDS assessment tends to underestimate the prediction of THM occurrence. Chlorine booster stations may or may not promote pipe effect, dependent on the *in-situ* internal pipe environment. Only relatively long residence time (days), in generating a chlorine limited situation, nets a decrease in the overall chloroform level.

The quality of tap water is determined by how water treatment affects the raw water and how distribution affects the treated water. New synthetic material, such as PVC, appears to have a low biofilm formation potential (on-line HPC counts $\sim$ 450 cfu/cm^2 ; readily inactivated by 1 mg/L free chlorine), and advanced water treatment technology, such as ultrafiltration membrane, appears to provide finished water with excellent quality (<0.25 NTU) at a consistent performance level. Under these conditions, a different approach to safe drinking water may be considered: good engineering practices and biostable finished water and pipe material may help avoid disinfection and its associated disadvantages, while ensuring the quality of drinking water during distribution.

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APPENDIX A

Sampling data (SDS)

direct filtration

Cl$_2$	chloroform mean	yield / Cl$_2$ mean	Cl$_2$ raw	chloroform mean	yield / Cl$_2$ mean	normalized production yield mean	normalized yield mean	factor level	sampling date	units
0.3	18.91	63.0	1	22.41	22.4	0.84	2.81	15h	10-Jan	Cl$_2$
1	6.2	6.2	0.9	6.77	7.5	0.92	0.82	3h	8-Feb	mg/L
2.64	18.13	6.9	2.5	17.91	7.2	1.01	0.96	5d	8-Feb	
2	6.41	3.2	2.65	10.45	3.9	0.61	0.81	4d	16-Feb	chloroform
1.3	3.77	2.9	2.1	17.04	8.1	0.22	0.36	4d	17-Feb	ug/L

zenon filtration

Cl$_2$	chloroform mean	yield / Cl$_2$ mean	Cl$_2$ raw	chloroform mean	yield / Cl$_2$ mean	normalized production yield mean	normalized yield mean	factor level	sampling date	sampling yield / Cl$_2$ (ug/mg)
0.8	7.45	9.3	0.9	8.06	9.0	-7.57	1.04	5h	23-Feb	n
0.9	5.56	6.2	1	7.64	7.6	-27.23	0.81		24-Feb	
0.9	7.88	8.8	1.2	8.96	7.5	-12.05	1.17		1-Mar	
0.7	5.73	8.2	1	8.28	8.3	-30.80	0.99		2-Mar	
0.8	21.88	27.4	0.9	23.81	26.5	-8.11	1.03		4-Apr	
0.9	22.95	25.5	1	22.52	22.5	1.91	1.13		7-Apr	
0.8	29.29	36.6	1	31.16	31.2	-6.00	1.17		27-Apr	
0.9	27.62	30.7	1.1	29.25	26.6	-5.57	1.15		20-Apr	
2	26.78	13.4	2.1	27.46	13.1	-2.48	1.02		2-May	
mode 0.9	**mean 17.24**	**mean 18.44**	**mode 1**	**mean 18.57**	**mean 16.90**	**mean -10.88**	**mean 1.06**	**factor level 5h**		**n 9**
1.4	7.94	5.7	1.94	13.14	6.8	-39.57	0.84	4d	23-Feb	
1.86	8.6	4.6	2	12.81	6.4	-32.86	0.72		24-Feb	
1.7	14.81	8.7	2	10.3	5.2	43.79	1.69		1-Mar	
1.8	13.06	7.3	2	16.06	8.0	-18.68	0.90		2-Mar	
1.78	63.08	35.4	2.1	63.35	30.2	-0.43	1.17		3-Apr	
1.9	59.39	31.3	2.1	62.07	29.6	-4.32	1.06		6-Apr	
mode 1.8	**mean 27.81**	**mean 15.49**	**mode 2**	**mean 29.62**	**mean 14.35**	**mean -8.68**	**mean 1.06**	**factor level 4d**		**n 6**

77

APPENDIX B

Sampling data (MS-SDS)

PIPES Cl₂	chloroform	yield / CL₂	CL₂ raw	chloroform	yield / CL₂	normalized production yield	normalized yield		sampling date	units
direct filtration										Cl₂ mg/L
1	5.04	5.0	1	5.61	5.6	0.90	0.90	17h	10-Jan	
3	6.18	2.1	3	4.54	1.5	1.36	1.36	5d	10-Jan	
3	8.63	2.9	3	7.24	2.4	1.19	1.19	5d	8-Feb	chloroform
3	7.41	2.5	3	8.61	2.9	0.86	0.86	4d	16-Feb	ug/L
3	5.57	1.9	3	10.65	3.6	0.52	0.52	4d	17-Feb	
										yield / Cl₂
zenon filtration										ug/mg
Cl₂	chloroform	yield / CL₂	CL₂ raw	chloroform	yield / CL₂	normalized production yield	normalized yield		sampling date	
1.68	8.25	4.9	1.7	8.83	5.2	0.93	0.95	5h	23-Feb	
2.38	4.28	1.8	2.48	4.24	1.7	1.01	1.05		2-Mar	
1.24	32.03	25.8	1.26	36.66	29.1	0.87	0.89		4-Apr	
1.1	42.4	38.5	0.9	40.29	44.8	1.05	0.86		7-Apr	
1.8	27.4	15.2	0.3	46.29	154.3	0.59	0.10		27-Apr	
2.5	31.8	12.7	2.38	16.19	6.8	1.96	1.87		20-Apr	
1.1	27.13	24.7	1.42	25.89	18.2	1.05	1.35		2-May	
				mean		1.07	1.01		7 total	
2	9.86	4.9	1.97	10.8	5.5	0.91	0.90	4d	23-Feb	n
2	8.04	4.0	2	8.6	4.3	0.93	0.93		24-Feb	n
2	10.25	5.1	2	11.31	5.7	0.91	0.91		1-Mar	n
2	10.91	5.5	2	9.8	4.9	1.11	1.11		2-Mar	n
1.7	44.34	26.1	1.8	40.56	22.5	1.09	1.16		3-Apr	y
1.76	47.11	26.8	1.72	47.23	27.5	1.00	0.97		4-Apr	y
1.78	35.27	19.8	1.76	40.69	23.1	0.87	0.86		6-Apr	y
1.8	68.21	37.9	1.8	62.94	35.0	1.08	1.08		7-Apr	y
2.46	51.58	21.0	2.44	30.91	12.7	1.67	1.66		27-Apr	y
2.48	66.92	27.0	2.5	36.24	14.5	1.85	1.86		20-Apr	y
				mean		1.26	1.26		10 total	

78

Organic-free water data (SDS and MS-SDS)

incubation time	PE		Cl₂ remain-pipe	chloroform-MS-SDS	Cl₂ remain-SDS	chloroform-SDS	HPC	date	
4d	PE = 1.0	1.0	0.05	1.33	1.8	1.34	0	23-Feb	4d
	PE = 4.3	4.3	0	8.93	1.8	2.06	0?	1-Mar	
	PE = 15.0	15.0	0.02	32.87	2	2.19	60/1000	27-Apr	
	PE = 7.8	7.8	0	11.32	2	1.46	200/1000	16-Feb	
	PE = 4.0	4.0	0	9.37	3	2.36	no data	8-Feb	
		2.0	0	10.18	1.5	5.22		10-Jan	reject
		4.2	1.6	41.43	1.8	9.8	370/400	3-Apr	reject

incubation time	PE		Cl₂ remain-pipe	chloroform-MS-SDS	Cl₂ remain-SDS	chloroform-SDS	HPC	date	
5h	PE = 2.3	2.3	1.1	2.82	1.9	1.25	0	23-Feb	5h
	PE = 1.3	1.3	0.5	2.2	1.8	1.65	0?	2-Mar	
	PE = infinit	#DIV/0!	1.6	7.88	1.8	0	10/340	4-Apr	
	PE = infinit	#DIV/0!	1.0	2.76	1.8	0	0	20-Apr	
		7.0	0	13.02	1	1.87		10-Jan	reject

units: Cl₂ (mg/L)   chloroform (ug/L)   HPC (cfu / ml)

79