Reduction of Trihalomethanes Using Ultrasound as a Disinfectant

by

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ABSTRACT

The emergence of pathogens that are more difficult to inactivate than bacteria, such as *C. parvum* and *G. lamblia,* has led to the enactment of more stringent drinking water regulations. The Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR), promulgated in January 2006, requires increased inactivation of *C. parvum*. However, increasing the disinfectant dose to enhance inactivation, especially when using chlorine, increases production of carcinogenic disinfection by-products (DBPs) such as trihalomethanes (THMs). As a result of the risks posed by DBPs, the Stage 2 Disinfectants and Disinfection By-Products Rule (Stage 2 D/DBP) was promulgated to limit exposure to DBPs by requiring systems to monitor concentrations at the worst cases locations in the distribution system. The purpose of this study was to evaluate sonication as an alternate disinfection strategy to reduce THM formation. Prior research has demonstrated the inactivation kinetics of sonication. Therefore, if sonication also reduces THM formation, this disinfection technology could help water utilities simultaneously comply with the Stage 2 D/DBP Rule and the LT2ESWTR.

Water samples were prepared with varying concentrations of natural organic matter (NOM). THM formation potential reduction was evaluated by treating the water samples with sonication at 20 kHz for 0 seconds, 30 seconds, 60 seconds, 5 minutes and 10 minutes. After treatment, the samples were chlorinated and incubated at 20°C to form THMs. After incubation times from 1 to 7 days, THMs were extracted, and gas chromatography with electron capture detection was used to quantify THM concentrations in treated and control samples. For experimental water with an NOM concentration of 1 mg/L that was dosed with 6 mg/L of NaOCl, the average THM formation potential reduction was 40% for sonication times of 30 seconds, 5 minutes and 10 minutes. The data for 60 seconds of treatment do not follow the same trends as the other data. Additional study is necessary to increase precision of the experimental data; however, this study supports sonication as a potential method of THM reduction.

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Chapter 1: Introduction

Since the enactment of the Safe Drinking Water Act (SDWA) in 1974, regulation of drinking water supplies has become more stringent due to the emergence of pathogens that are more difficult to inactivate than many bacteria such as the protozoa *Giardia lamblia* and *Cryptosporidium parvum.* Most recently, the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) requires at least 99 % percent removal of *Cryptosporidium* for public water treatment systems with additional removal or inactivation based on source water concentrations. Simultaneously, the Stage 2 Disinfectants and Disinfection By-Products Rule (Stage 2 D/DBP) was promulgated, which sets a maximum contaminant level (MCL) for total trihalomethanes (TTHMS) of 80 μg/L, considering a location running annual average at the distribution system locations with the highest levels.

Over 260 million people are exposed to disinfection by-products (DBPs) through public drinking water supplies in the U.S. (U.S EPA, 2007b). There are concerns about possible links between DBP exposure and some types of cancer as well as reproductive problems. In addition to the Stage 2 D/DBP Rule, future regulations are expected to address requirements for specific compounds (instead of groups such as TTHMs) because toxicological data indicates that each compound poses different health risks (Kitis *et al.,* 2001). For example, toxicological studies have reported that bromodichloromethane causes a higher risk of cancer than chloroform (Krasner *et al.,* 1989).

Efforts to reduce DBP formation without compromising disinfection effectiveness have shown some potential options. Chlorine dioxide, a disinfectant more commonly used in Europe than in the U.S. has been shown to produce 97% less DBPs than free chlorine. In addition DBP precursors can be removed from water prior to disinfection by filtration or membranes, thereby averting possible DBP formation. Various coagulants applied prior to the sedimentation process can increase removal of DBP precursors. Finally, treatment with ultrasound, or sonication, is an option for disinfection that may increase disinfection effectiveness *and* decrease DBP formation.

Increased inactivation of microbial pathogens can be achieved with sonication alone or in combination with disinfectants such as chlorine and ozone. Studies have

evaluated various power and frequency settings for ultrasound treatment, the required contact time, and the order of treatment when sonication was applied in combination with other disinfectants. To date, there is a lack of literature discussing the possible effects of sonication on disinfection by-product formation. With a need for increasingly effective disinfection processes to inactivate more resistant pathogens such as *Cryptosporidium*, the potential for disinfection by-product production must be addressed.

This study evaluated the effect of sonication at 20 kHz frequency on THM formation under conditions similar to drinking water treatment processes. Reagent grade water was prepared with natural organic matter (NOM) extracted from the Suwannee River. Two concentrations of NOM were used: 1 mg/L and 2.5 mg/L. The solutions were treated with sonication for times of 30 seconds, 1 minute, 5 minutes or 10 minutes. In addition, control samples that were not treated with sonication were analyzed. After treatment, the solutions were dosed with concentrations of sodium hypochlorite.

A review of background information related to drinking water regulations and disinfection processes as well as prior research on the use of sonication for disinfection is presented in Chapter 2. A detailed description of the experimental design and analytical methods used for this research is presented in Chapter 3. Following the methodology, Chapter 4 discusses the results of these experiments followed finally by the conclusions and recommendations in Chapter 5.

Chapter 2: Literature Review

In the United States, surface waters used as drinking water sources are required to be filtered and disinfected. The majority of treatment plants use free chlorine for primary disinfection and a chlorine based compound for secondary disinfection. While effective for inactivating many pathogens, chlorine has several limitations. First, some pathogens such as *Cryptosporidium* are relatively resistant to chlorination. Second, chlorination forms disinfection by-products (DBPs) that are regulated by the U.S. Environmental Protection Agency. Therefore, alternative disinfection options are desirable.

The following sections present background information and relevant literature on several topics. First, the major U.S. drinking water treatment regulations are discussed with particular relevance to pathogens and DBPs. Next, widely used and alternative disinfection methods are presented. Finally, there is a literature review of sonication as a disinfectant for aqueous systems.

2.1 Drinking Water Regulations

Prior to the 1900s, drinking water quality was determined mostly by aesthetic properties such as taste, odor and color. An epidemiologist, Dr. John Snow, was the first scientist to directly link cholera outbreaks in London to drinking water sources in the 1850s. Later, in the 1880s, the theory of disease transmission by microscopic waterborne pathogens was introduced by Louis Pasteur. These advancements in the understanding of drinking water quality led to the expansion of water quality characteristics to include contaminants other than those which can be assessed visually and to the development of federal regulations for drinking waters in the U.S. and other countries. In the U.S., the first drinking water regulations were established in 1914 and set a limit 100 cfu per mL for total bacterial plate count. This regulation also stipulated that no more than one in five 10 mL portions of each sample could contain *E. coli*. This rule applied to water supplied to the public by interstate carriers. These standards were revised in 1942, 1946 and 1962. The 1962 standards were the most comprehensive drinking water regulations

prior to the Safe Drinking Water Act (SDWA) and applied to 28 constituents (AWWA, 1999).

A 1969 study conducted by the Public Health Service revealed that 40% of drinking water systems were not meeting the existing drinking water regulations. In response to the inadequacy of a large portion of the nation's drinking water supply, SDWA was passed in 1974 (U.S. EPA, 1999a). The SDWA regulations that are relevant to this study are regulations on surface water treatment and DBPs. Continued development of more stringent drinking water regulations provides the motivation to develop new disinfection technologies.

2.1.1 Surface Water Treatment

Approximately one fifth of drinking water systems in the U. S. use surface water sources (U.S. EPA, 1999a). These systems serve 195 million people or approximately 65% of the U. S. population (U.S. EPA, 2003). Since surface waters are considered to be particularly vulnerable to microbial contamination, the 1989 SDWA Amendments included the Surface Water Treatment Rule. This rule has been revised by the Interim Enhanced Surface Water Treatment Rule (IESWTR), the Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR) and the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) (U.S. EPA, 1999a). The following sections discuss each of these regulations with relevance to drinking water disinfection.

2.1.1.1 Surface Water Treatment Rule

The Surface Water Treatment Rule (SWTR) mandates that all surface water systems are filtered and disinfected. These treatments must achieve a minimum of 99.9% combined removal and inactivation of *Giardia lamblia* and 99.99% removal and inactivation of viruses. In addition, the rule set maximum contaminant levels (MCLs) for THMs, filtered water turbidity limits and the maximum contaminant level goal (MCLG) for *Giardia* and viruses at zero due to the health risk of any exposure to these pathogens. The rule also set maximum filter effluent turbidity standards. Figure 2.1 illustrates the subsequent reduction of waterborne disease outbreaks after the passage of the SWTR in

1989. Since there were still up to 10 outbreaks per year in the 1990s, the SWTR was reviewed and revised in subsequent legislation to further minimize risk of known and unknown contaminants (U.S. EPA, 1999a).

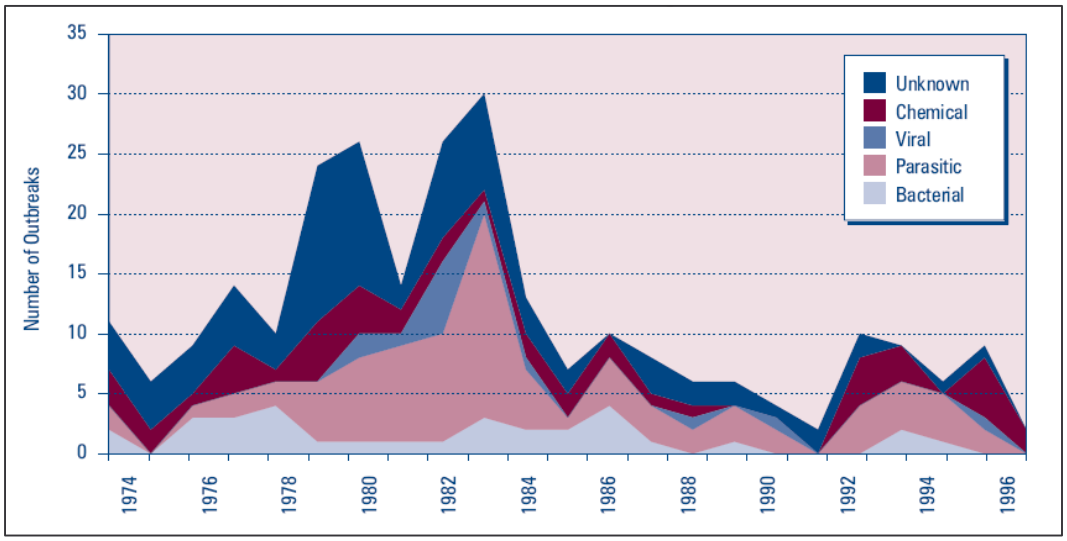

Figure 2.1: Waterborne Disease Outbreaks and Their Causative Agents (U.S. EPA, 1999a)

2.1.1.2 Interim Enhanced Surface Water Treatment Rule

A 1993 outbreak of *Cryptosporidium* that caused approximately 400,000 illnesses and 50 deaths in Milwaukee was instrumental in the eventual development of additional regulations. The 1996 amendments to the SDWA required the U.S. EPA to develop rules to strengthen protection against pathogens such as *Cryptosporidium*. At the time of the Milwaukee outbreak, the drinking water system was in compliance with all regulations, including regulations on microorganisms. However, *Cryptosporidium*, a protozoan pathogen, is more difficult to inactivate than bacterial pathogens. *Cryptosporidium* has been found to be present in over 50% of surface waters sources in the United States (HDR Engineering, 2001).

The Interim Enhanced Surface Water Treatment Rule (IESWTR) was established in 1998 with the specific goal of controlling microbial pathogens in drinking water. This required assessment of the risks posed by pathogens such as *Cryptosporidium* and by DBPs. The IESWTR rule took effect in 1999 and applied to all systems that use surface water as well as all those that use ground water under the direct influence of surface

water (GWUDI) and serve greater than 10,000 individuals (U.S. EPA, 1998a). Compliance with the rule was required by 2002. Some of the specific provisions of this rule are:

- Maximum contaminant level goal (MCLG) of zero for *Cryptosporidium*
- 2-log *Cryptosporidium* removal requirements for systems that filter
- More stringent combined filter effluent standards for turbidity
- Systems using (GWUDI) must comply with new rules for *Cryptosporidium*
- Addition of *Cryptosporidium* watershed protection requirements for unfiltered water systems (U.S. EPA, 1998a)

2.1.1.3 Long Term 1 Enhanced Surface Water Treatment Rule

The Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR), promulgated in 2002, builds on the requirements of the IESWTR. As with prior rules, this rule seeks to prevent risk of exposure to pathogens when DBP reduction efforts are made. The requirements of this rule are parallel to the requirements of the IESWTR, but now apply to systems serving fewer than 10,000 people. All systems were required to meet these regulatory standards in early 2005 (U.S. EPA, 2002).

2.1.1.4 Long Term 2 Enhanced Surface Water Treatment Rule

Further augmentation of the SWTR includes the Long Term 2 Enhanced Surface Water Treatment Rule (LT2). This rule applies to all surface water systems and GWUDI systems, thereby including public water systems that collectively serve approximately 180 million people. Under this rule, all systems must monitor for *Cryptosporidium* in their source waters. Based on the results, each system is classified into one of four treatment categories. Those that are in the lowest category will not be required to meet any additional regulations. The systems that are classified into the 3 remaining categories will be required to meet 1.0 to 2.5 additional log reduction or inactivation of *Cryptosporidium* beyond the 2 log removal required under the IESWTR and LT1. For unfiltered systems, 99 or 99.9 percent (2 or 3 log) of *Cryptosporidium* inactivation is required.

Public water systems serving greater than 100,000 individuals began monitoring for source water *Cryptosporidium* concentrations in October 2006. Systems that serve fewer than 10,000 people will not begin monitoring for this rule until October 2008. After the monitoring stage is complete, systems will have three years to comply with the LT2ESWTR removal and inactivation requirements. This rule is projected to reduce the illnesses associated with *Cryptosporidium* by as many as 1,459,000 cases per year (U.S. EPA, 2007a).

2.1.2 Disinfection By-Product Regulations

Disinfection by-products (DBPs) were first regulated under the SWTR. In November of 1979, the MCL for total trihalomethanes (TTHMs), chloroform, bromoform, dibromochloromethane and bromodichloromethane, was set at 100 μg/L. All public drinking water facilities serving populations greater than 10,000 individuals were required to comply with this limit (U.S. EPA, 1998a). Many other developed nations require even lower MCLs for TTHMs. The European Union limits TTHMs to 30 μg/L and Germany, an EU member, enforces an even stricter 10 μg/L limit (Lin *et al.,* 2006). All of these regulations are similar in that they limit only total THMs. Studies have demonstrated that individual DBPs may have varying health risks. For example, bromodichloromethane may be more carcinogenic than chloroform. Therefore, future regulations may limit individual DBPs rather than groups (Kitis *et al.,* 2001)

The Information Collection Rule was promulgated in 1996. This rule was intended to gather information on the presence of pathogens in source waters, the formation of DBPs and the ability of various treatments such as membrane separation processes and adsorption processes to control DBPs and pathogens. This rule applied to public drinking water utilities that used surface water sources that had an average annual total organic carbon (TOC) concentration of greater than 4.0 mg/L and served greater than 100,000 citizens. The Information Collection Rule also applied to drinking water utilities that used a ground water source with an average annual TOC concentration of greater than 2.0 mg/L and that served at least 50,000 citizens (U.S. EPA, 1997). The

results of the Information Collection rule were used to develop further regulations to simultaneously control pathogen and DBP risk.

In December of 1998, the Stage 1 Disinfectants and Disinfection By-Products Rule (D/DBP) set a new MCL of 80 μg/L for TTHMs and 60 μg/L for five haloacetic acids (HAA5: monochloro-, dichloro-, trichloro-, monobromo- and dibromo-acetic acids). Drinking water treatment facilities were required to comply with these regulations by December 2003. This rule was expected to improve protection from DBPs for 140 million people (Kitis *et al.,* 2001)

Stage 2 of the D/DBP Rule was promulgated on December 15, 2005 and set more stringent regulations for TTHMs and HAA5, respectively (U.S. EPA, 2006b). Previous legislation required only that the average TTHMs concentrations from all sampling locations within a distribution system meet the MCL. The Stage 2 D/DBP rule introduces the locational running annual average (LCAA) concept. This determines compliance by calculating the running annual average TTHMs concentration of samples from each individual monitoring locations within a distribution system. If the LCAA for *any* of the sampling locations does not meet the MCL, then the system is not in compliance and must take steps to reduce the LCAA. In addition, systems are required to identify locations with the highest DBP concentrations, and use these in their monitoring programs.

There are several actions that systems must take in order to comply with this rule. As shown in Table 2.1, the largest public drinking water systems were required to begin a monitoring plan in 2006 and will be required to comply with the rule in April of 2012. All other systems will be required to comply by late 2013. Also, this rule sets MCLGs for each THM separately. For bromoform and bromodichloromethane, the MCLG is zero. For chloroform, the MCLG is 70 μg/L and for dibromochloromethane, the MCLG is 60 μg/L (U.S. EPA, 2006b).

Table 2.1: Schedule for compliance with Stage 2 D/DBP Rule (U.S. EPA, 2006b)

PUBLIC WATER SYSTEMS*	ACTIONS			
	Submit IDSE monitoring plan, system specific study plan, or 40/30 certification	**Complete an initial distribution system evaluation (IDSE)**	**Submit IDSE Report**	**Begin subpart V (Stage 2) compliance monitoring**
CWSs and NTNCWSs serving at least 100,000	October 1, 2006	September 30, 2008	January 1, 2009	April 1, 2012
CWSs and NTNCWSs serving 50,000 - 99,999	April 1, 2007	March 31, 2009	July 1, 2009	October 1, 2012
CWSs and NTNCWSs serving 10,000 - 49,999	October 1, 2007	September 30, 2009	January 1, 2010	October 1, 2013
CWSs serving fewer than 10,000	April 1, 2008	March 31, 2010	July 1, 2010	October 1, 2013
NTNCWSs serving fewer than 10,000	NA	NA	NA	October 1, 2013

*CWS = community water system; NTNCWS = non-transient non-community water system

2.2 Removal and Inactivation of Pathogens

As required by the SWTR and its subsequent amendments, pathogens are controlled in drinking water sources by a combination of clarification, filtration and disinfection. The purpose of these treatment processes is to reduce the risk of waterborne illnesses. Advancements in treatment processes are aimed at increasing efficiency, decreasing costs and meeting more stringent regulations including limitations of DBP production. The following sections discuss current disinfection technologies, physical pathogen removal methods and alternative technologies for inactivation of microorganisms in drinking water sources.

2.2.1 Physical Removal Methods

Treatment processes used for pathogen removal include conventional filtration and membrane filtration. The Surface Water Treatment Rules requires a combination of

removal and inactivation to meet pathogen reduction limits. Therefore, removal processes reduce the dependence on disinfection to meet regulatory limits. With growing concern about DBP levels, the use of filtration methods for pathogens is desirable (U.S. EPA, 2006a).

2.2.1.1 Sedimentation and Filtration

Sedimentation is a water clarification process that has been used since ancient times. Modern improvements to the sedimentation process include technologies such as flotation, adsorption clarifiers and enhanced solids contact. There are two typical applications of sedimentation in water treatment: plain sedimentation and sedimentation of coagulated and flocculated waters. Particles such as gravel, sand and silt will naturally settle without chemical treatment as in plain sedimentation. Chemicals such as alum can be used to enhance the natural settling process through coagulation and flocculation (HDR Engineering, 2001).

Water filtration is both a physical and chemical process that removes colloids and suspended solids from water via passage through granular material. Filters are typically 1.5 to six feet deep and remove solids by two steps. The physical filtration step removes solids through size exclusion and the chemical filtration step removes particles by attachment to the filter media surface and other particles previously retained by the filter. The two main types of filtration systems are gravity filtration systems and pressure filtration systems. The most widely used filtration method for water treatment is rapid gravity filtration. Slow-sand filters, a type of gravity filter system, can provide 3-log removal of *Giardia* and *Cryptosporidium* (HDR Engineering, 2001).

2.2.1.2 Membrane Processes

The U.S EPA defines membrane filtration as a pressure or vacuum driven separation process in which particulate matter larger than 1 micrometer is contained by a barrier through a size exclusion mechanism. If membranes are used to meet the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR), the filtration efficiency must be measurable using a target microorganism. Pathogens of interest are referred to as target organisms for the purpose of evaluating whether a membrane filtration system

meets treatment objectives. Therefore, a target organism is chosen based on treatment objectives. If, for example, a membrane filtration system is used for compliance with the LTSESWTR, then *Cryptosporidium* is an appropriate target organism.

The four types of membrane processes, in order from smallest to largest pore size, are reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF) and microfiltration (MF) (U.S. EPA, 2006a). In addition, electrodialysis (ED) and electrodialysis reversal (EDR) are classified as membrane processes; however, these processes do not meet the definition of membrane filtration as set out by the LT2ESWTR.

Reverse osmosis (RO) membranes have been used in water desalination since the 1960s and nanofiltration (NF) membranes have been used for softening and total organic carbon (TOC) removal since the 1980s. These processes are used primarily for dissolved contaminant removal. Although RO and NF membranes are often called "filters" for dissolved solids, the removal mechanism is actually a semi-permeable membrane that does not have distinguishable pores. RO membranes exclude particles greater than 0.0001 μm and NF membranes exclude particles greater than 0.001 μm or 200 Daltons molecular weight. Viruses, the smallest pathogens, are typically between 0.01 and 0.1 μm in size. Both processes remove dissolved solids through osmosis and also provide a barrier to particulate matter which contributes to their use as a "filtration" process (U.S. EPA, 2006a).

Microfiltration (MF) and ultrafiltration (UF) are the processes most closely associated with membrane filtration. These processes are able to remove suspended solids and colloids through a sieving mechanism, thereby reducing turbidity and microorganisms. Key parameters for MF and UF processes are membrane pore sizes and the sizes of the particulate matter to be removed. The commercialization of MF and UF processes in the early 1990s has resulted in wider acceptance and regulation of all existing types of membrane treatment processes for drinking water. MF membranes usually have pores ranging from 0.1 to 0.2 μm and UF membrane pore sizes typically range from 0.01 to 0.05 μm (U.S. EPA, 2006a). Studies have found both MF and UF membranes to be successful in removing *Cryptosporidium* and *Giardia* to below

detectable levels. Depending on the feed water concentration of protozoa, up to 7-log removal has been documented (AWWA, 1999).

2.2.2 Inactivation

Common disinfectants used in drinking water treatment include chlorine compounds, ozone and ultraviolet irradiation. More recently, research has explored the use of multiple disinfectants and unconventional technologies such as sonication. The following sections discuss the fundamentals and some advantages and disadvantages of each disinfection method, beginning with a section on how disinfection processes are regulated.

2.2.2.1 CT Concept

The effectiveness of disinfection is dependent on the quality of the water to be treated, the disinfectant used, and the microorganism being inactivated. With regard to water quality, pH, temperature, turbidity and dissolved organics in source water can all affect disinfection processes. For disinfection with chlorine compounds, the pH can affect the reactions that take place. Ozone and UV disinfection are not affected by pH. The most important parameters for determining disinfection effectiveness are the concentration and contact time (U.S. EPA, 1999c). These parameters are called "CT," where C is the concentration of the residual disinfectant in mg/L and T is the contact time in minutes (HDR Engineering, 2001). The CT value is used for regulatory compliance.

As shown in Table 2.2, the EPA specifies necessary CT values to achieve different levels of virus inactivation using various disinfectants including chlorine, chloramines, ozone and UV. For a given disinfectant, a higher log inactivation requires a larger CT value. When comparing different disinfectants for a given inactivation level, the CT values vary widely among the disinfectants. For example, 2-log inactivation of viruses with ozone requires a CT of 0.5 mg-min/L whereas chloramines require a CT of 643 mg-min/L, three orders of magnitude higher.

Table 2.2: Example CT Values for inactivation of viruses (HDR Engineering, 2001)*

Disinfectant	Units	CT for Virus Inactivation		
		2-log	3-log	4-log
Chlorine**	mg•min/L	3	4	6
Chloramine†	mg•min/L	643	1067	1491
Chlorine Dioxide‡	mg•min/L	4.2	12.8	25.1
Ozone	mg•min/L	0.5	0.8	1
UV	mW•s/cm2	21	36	Not Available

*All values are based on temperature of 10°C.
**Values are based on a pH range of 6-9, and a free chlorine residual of 0.2 to 0.5.
†Values are based on a pH of 8.
‡ Values are based on a pH range of 6-9.

Table 2.3 shows CT values for the inactivation of *Giardia* for 1-log, 2-log and 3-log inactivation with several disinfectants. As with inactivation of viruses, there is broad variation in CT values among disinfectants and log removal. Chlorine, for example, requires a CT of 104 to achieve 3-log inactivation of *Giardia*, whereas chloramines require a CT value of 1850 to achieve the same effect.

Table 2.3: Example CT Values for inactivation *Giardia* (HDR Engineering, 2001)*

Disinfectant	Units	CT for *Giardia* Inactivation		
		1-log	2-log	3-log
Chlorine**	mg•min/L	35	69	104
Chloramine†	mg•min/L	615	1230	1850
Chlorine Dioxide††	mg•min/L	7.7	15	23
Ozone	mg•min/L	0.48	0.95	1.43

*All values are based on temperature of 10°C.
**Values are based on a free chlorine residual of less than or equal to 0.4 mg/L and a pH of 7.
†Values are based on a pH range of 6-9
††Values are based on a pH of 7.

Different pathogens exhibit different resistance to inactivation. Therefore, they require varying CT values for inactivation. As illustrated by Table 2.4, cysts such as *Giardia* are more resistant to chlorine disinfection than viruses. For example, 2-log inactivation of *Giardia* requires 69 mg-min/L, compared to 3 mg-min/L for viruses.

Table 2.4: CT values for different pathogens by chlorine disinfection (HDR Engineering, 2001)

Pathogen	CT for Inactivation			
	1-log	2-log	3-log	4-log
Viruses	Not available	3	4	6
Giardia	35	69	104	Not available

CT values are derived from kinetic models of chemical reactions in which the microorganisms and the disinfectant are the reactants and the reaction is characterized by a rate constant as shown in Equation 1.

Equation 1

$$r = -kN$$

where r = inactivation rate $\left(\frac{\text{organisms inactivated}}{\text{volume} - \text{time}}\right)$

k = rate constant (1/time)
N = concentration of viable organisms (organisms/volume)

This relationship was developed by H. Chick in 1908 and is known as Chick's Law (AWWA, 1999). Chick's Law states that the number of organisms inactivated with respect to time is proportional to the number of organisms. This relationship is shown in Equation 2.

Equation 2

$$\frac{dN}{dt} = -kN$$

Chick's Law is applicable under ideal conditions. Poor distribution of microorganisms, poor distribution of the disinfectant, or declining disinfectant concentration can cause inactivation rates to depart from those predicted by Chick's model. For this reason, the CT values for regulatory compliance were developed to account for realistic conditions (Davis and Masten, 2004).

2.2.2.2 Chlorine

An early application of chlorine was for waste treatment in France in 1825 and later as a prophylactic in Europe during the cholera epidemic of 1831. The first use of

chlorine to disinfect drinking water was in 1908 in Chicago, Illinois, and within two years it was introduced in New York City, Montreal, Milwaukee, Cleveland, Nashville, Baltimore, and Cincinnati and small cities throughout the U.S. As chlorine disinfection spread, typhoid outbreaks dramatically decreased and by 1918 the cities utilizing chlorine disinfection numbered over 1000 (AWWA, 1999). In Wheeling, West Virginia, for example, the rate of typhoid fever was 150 -200 cases per 100,000 population in 1917 – 1918. In 1918, when chlorination was added, the rate of typhoid infection decreased to 25 per 100,000 population (HDR Engineering, 2001).

The three forms of chlorine that are used for drinking water disinfection are chlorine gas, sodium hypochlorite and calcium hypochlorite. When chlorine gas reacts with water, it forms hypochlorous acid (HOCl) as in Reaction 1. Sodium hypochlorite is formed by dissolving chlorine gas in a solution of sodium hydroxide. The reaction that takes place when sodium hypochlorite is added to water is shown in Reaction 2. The third option for chlorination is calcium hypochlorite, which is purchased in a solid form and when dissolved in water it reacts to form HOCl as shown in Reaction 3.

Reaction 1 $Cl_{2(g)} + H_2O \Rightarrow HOCl + H^+ + Cl^-$

Reaction 2 $NaOCl + H_2O \Rightarrow HOCl + Na^+ + OH^-$

Reaction 3 $Ca(OCl)_2 + 2H_2O \Rightarrow 2HOCl + Ca^{2+} + 2OH^-$

Production of the hydroxyl ion as shown in Reaction 2 and Reaction 3 causes an increase in the pH of the water. Conversely, chlorine gas reduces the pH by forming hydrogen ions (HDR Engineering, 2001). Regardless of the form of chlorine added, the HOCl produed in water can dissociate to OCl^-. If the pH is below 6.5, the HOCl will not dissociate. If it is between 6.5 and 8.5, there will be partial dissociation of HOCl resulting in the presence of both HOCl and OCl^-. When the pH is over 8.5, HOCl will completely dissociate to OCl^-. Disinfection is more efficient at lower pH levels because of the relative strength of HOCl compared to OCl^- (HDR Engineering, 2001).

Chlorine is the most commonly used disinfectant in the U.S., and is also used as an oxidant for drinking water treatment. The primary advantage to using chlorine is its effectiveness in inactivating a wide range of pathogens. In addition, it is economical and has a history for successful use as a drinking water disinfectant (U.S. EPA, 1999c). Chlorine can be used not only for primary disinfection, but also for secondary disinfection as it provides an easily measurable residual for distribution system protection.

There are also several disadvantages to chlorine disinfection. First, chlorine reacts with organic and inorganic compounds in water to produce undesirable DBPs. Higher doses can cause poor taste and odor in treated water (U.S. EPA, 1999c). Finally, the use of chlorine is resulting in the evolution of resistant microorganisms such as Aeromonas and *Cryptosporidium* (Furata *et al.*, 2004).

2.2.2.3 Chloramines

Chloramination is achieved by sequential or simultaneous application of chlorine and ammonia. This method was first used in Ottawa, Canada and Denver, Colorado in 1917 (AWWA, 1999). Originally, chloramines were used to improve the taste and odor of drinking water (HDR Engineering 2001). This process decreased in popularity during World War II due to limited availability of ammonia (AWWA, 1999). Around this time, it was acknowledged that free chlorine is a superior primary disinfectant. However, chloramination is an effective secondary disinfectant and can prevent bacterial growth in distribution systems more effectively than free chlorine (HDR Engineering, 2001). Lastly, chloramines produce fewer DBPs than free chlorine because they are less reactive with organic matter (HDR Engineering, 2001; AWWA, 1999).

Chloramines are formed when hypochlorous acid reacts with ammonia in water to form monochloramine (NH_2Cl), dichloramine ($NHCl_2$) and nitrogen trichloride (NCl_3) as show in Reactions 4 through 6.

Reaction 4: Monochloramine $NH_3 + HOCl \Rightarrow NH_2Cl + H_2O$

Reaction 5: Dichloramine $NH_2Cl + HOCl \Rightarrow NHCl_2 + H_2O$

Reaction 6: Nitrogen Trichloride $NHCl_2 + HOCl \Rightarrow NCl_3 + H_2O$

The pH, chlorine to ammonia nitrogen ratio, temperature and contact time all influence which types of chloramines will be present. At lower pH levels, monochloramine and dichloramine will dominate, while trichloramine will dominate at pH levels over 8 (HDR Engineering, 2001).

2.2.2.4 Chlorine Dioxide

Chlorine dioxide was first used to disinfect water in a spa in Belgium in the early twentieth century. This chlorine compound is highly soluble in water and disinfects by oxidation. Chlorine dioxide is reduced to chlorite by the transfer of one electron in an oxidation reaction. Disinfection by-products produced by chlorine dioxide include chlorite, chlorate and chloride. Less effective than ozone and more effective than chlorine, chlorine dioxide inactivates pathogens effectively in a wide range of pH levels. An advantage of chlorine dioxide with regard to DBPs is that it does not react to form THMs (U.S. EPA, 1999f)

2.2.2.5 Ozone

Although ozone has been used for drinking water treatment in Europe since the late nineteenth century, it has gained popularity it the United States slowly. Between 1991 and 1998, the number of American water treatment plants using ozone treatment rose from 40 to 264. Ozone is used in a gaseous form for disinfection and is a very strong oxidant. During treatment, the ozone spontaneously decomposes through a series of complex reactions that are summarized in Figure 2.2. The aqueous ozone can either directly oxidize compounds or it can decay to produce hydroxyl radicals which then oxidize compounds. An advantage of ozone is that it decays to oxygen during water treatment so there is no need to remove the disinfection chemical once treatment is complete.

There are a few disadvantages of using ozone for disinfection. Some of the ozone will form compounds that can react with chlorine disinfectants and create chlorinated aldehydes and ketones. Also, ozone is not always effective in preventing bacterial

regrowth in distribution systems because ozone can increase biodegradability of organic matter thus increasing potential for regrowth if the degradable organics are not removed after ozonation (U.S. EPA, 1999b).

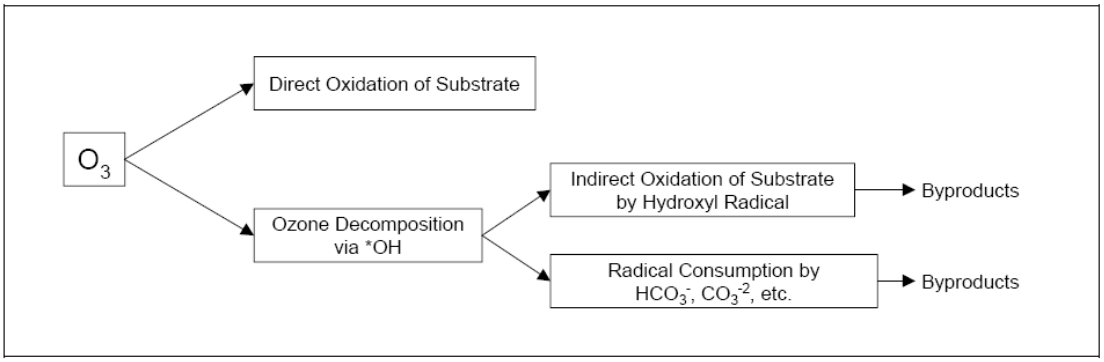

Figure 2.2: Oxidation reactions of compounds during ozonation of water (U.S. EPA, 1999b)

2.2.2.6 Ultraviolet Irradiation

While the disinfection methods previously discussed inactivate microorganisms by chemical reactions, ultraviolet radiation inactivates microorganisms by altering cellular components that are integral to the cell's functions. Ultraviolet irradiation has been found to be effective for bacterial inactivation, as well as inactivation of protozoa including *Giardia* and *Cryptosporidium*. The most resistant organism is Adenovirus. UV radiation is electromagnetic waves with wavelengths from 100 to 400 nm. For disinfection, the ideal UV range is between 245 and 285 nm.

UV doses are calculated using Equation 3:

Equation 3

$$D = I \bullet t$$

where D = UV Dose ($mW \cdot s/cm^2$)
I = intensity (mW/cm^2)
t = exposure time (s) (U.S. EPA, 1999e)

The survival of coliforms given a particular UV dose can be calculated using Equation 4. This equation can be used to measure the log reduction of coliforms when water is treated with UV radiation.

Equation 4

$$N = f \bullet D^n$$

where N = effluent coliform density (cfu/100mL)
D = UV dose ($mW \cdot s/cm^2$)
n = empirical coefficient related to dose
f = empirical water quality factor (U.S. EPA, 1999e)

2.2.2.7 Peroxone

Peroxone is a drinking water treatment method that uses ozone and usually hydrogen peroxide. By adding hydrogen peroxide, the decomposition of the ozone is accelerated, subsequently increasing the oxidation rate. As discussed in Section 2.2.2.5, ozone oxidizes compounds directly and indirectly. Using peroxone increases indirect oxidation. An important disadvantage of using peroxone is that there is no measurable disinfection residual. This prohibits the calculation of CT values which are needed to compare peroxone to other disinfects (U.S. EPA, 1999g).

2.2.2.8 Synergistic Disinfection

Synergistic disinfection refers to the use of multiple disinfectants either sequentially or simultaneously with greater combined disinfection effectiveness than the sum of the effects of the individual disinfectants used. Synergistic disinfection is sometimes referred to as interactive disinfection (U.S. EPA, 1999h) In the past twenty five years, disinfection practices have continually been evaluated for possible means of improvement with synergistic disinfection gaining more interest.

In 1991, Koume and Haas reported greater inactivation of *E. coli* when exposed to a combination of free chlorine and chloramine than would be expected from each treatment individually. A decade later, Li *et al.* (2001) reported synergy in the use of ozone and free chlorine for inactivating *C. parvum* oocysts. In addition, Rennecker *et al.* (2000) studied synergy for the combinations of ozone and free chlorine as well as ozone

and monochloramine. In both studies, the synergistic effects were more significant at lower temperatures. Also, Dreidger *et al.* (2001) found the required doses and contact times for monochloramine were reduced from 70,000 mg-min/L to 2000 mg-min/L when ozone pretreatment was used. Consistent with previous studies, Corona-Vasquez *et al.* (2002) observed synergistic effects when ozone and free chlorine were used to inactivate *C. parvum*. In 2005, Biswas *et al.* added that while synergy is present when using ozone followed by monochloramine for oocyst inactivation, the efficacy of this treatment was affected by turbidity, organic carbon concentration and temperature.

2.2.2.9 Sonication

A method traditionally used for disinfecting laboratory equipment, sonication is being investigated as a possible alternative for drinking water treatment alone and in combination with other disinfectants such as chlorine and ozone. Sonication uses high frequency sound waves that disrupt cells causing inactivation. A more detailed discussion of sonication research is provided in Section 2.4.

2.3 Disinfection By-Products

One of the most important characteristics of chlorinated organic compounds is their resistance to biological and environmental degradation. This quality has made these compounds exceptionally useful while also making them difficult to rid from the environment. The first concerns about chlorinated compounds arose in the 1960s with regard to chlorinated pesticides. Later, solvents such as trichloroethylene (TCE) became pervasive groundwater contaminants. In 1974, the Dutch chemist Johannes Rook linked chlorine to the production of chlorinated and brominated by-products called trihalomethanes (THMs) in treated drinking waters. Shortly after, halogenated organic, nonhalogenated organic and inorganic halogen oxide compounds were added to the list of DBPs (HDR Engineering, 2001; Rook, 1974).

2.3.1 DBP Precursors

When chlorine reacts with organic matter in water, by-products are formed. These by-products are generally referred to as disinfection by-products (DBPs) and are considered a risk to public health. The organic materials, or organic precursors, that react with chlorine to form DBPs are characterized as natural organic matter (NOM) and are comprised of humic substances and nonhumic substances (Marhaba *et al.*, 2000).

Humic substances in NOM are hydrophobic, heterogeneous, polyfunctional polymers that form through decomposition of organism tissues and through other chemical and biological processes. Between one third and one half of the dissolved organic carbon (DOC) in natural waters is humic substances (Kim & Yu, 2006; Marhaba *et al.,* 2000). These substances are not very well understood or characterized due to their complex polymeric structures and properties (Kim & Yu, 2006). Non humic substances are characterized as hydrophilic substances and can be hydrophilic acids, proteins, amino acids and carbohydrates (Marhaba *et al.*, 2000). It has been observed that humic substances have a higher DBP formation potential than nonhumic precursors (Kim & Yu, 2005). Additionally, NOM that absorbs UV light has higher bromine incorporation in disinfection reactions. This UV absorbing fraction of NOM is a major contributor to DBP formation (Kitis *et al.,* 2001.).

Humic substances are a major constituent of NOM in surface waters and can be divided into two major categories: humic acids (HA) and fulvic acids (FA). Although humic substances are hydrophobic, FA are considered to be less hydrophobic than HA and FA typically are of lower molecular weight. Using adsorption chromatography, researchers have fractionated HA and FA and found that HA are insoluble and FA are soluble at pH 2. Also, FA are humic substances that are soluble in bases and acids, while HA are humic substances that are soluble in bases and insoluble in acids (Marhaba *et al.,* 2000).

2.3.2 Types of DBPs

There are many chemical residuals and by-products produced from chlorine disinfection. Table 2.5 shows the various types of disinfection residuals, inorganic by-products, organic oxidation by-products and halogenated organic by-products that result from chlorine disinfection (U.S. EPA, 1999c). More specifically, the halogenated organic by-products are trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles (HANs) and haloketons (HKs) (Chang *et al.*, 2000). These by-products are also called total organic halides (TOX). In 2005, less than 50% of TOX had been identified. Of the identified TOX 10-20% are THMs (Sorlini & Collivignarelli, 2005).

Table 2.5: Disinfectant residuals and disinfection by-products (U.S. EPA, 1999c)

DISINFECTANT RESIDUALS	HALOGENATED ORGANIC BYPRODUCTS
Free Chlorine	Trihalomethanes
Hypochlorous Acid	Chloroform
Hypochlorite Ion	Bromodichloromethane
Chloramines	Dibromochloromethane
Monochloramine	Bromoform
Chlorine Dioxide	Haloacetic Acids
INORGANIC BYPRODUCTS	Monochloroacetic Acid
Chlorate Ion	Dichloroacetic Acid
Chlorite Ion	Trichloroacetic Acid
Bromate Ion	Monobromoacetic Acid
Iodate Ion	Dibromoacetic Acid
Hydrogen Peroxide	Haloacetonitriles
Ammonia	Dichloroacetronitrile
ORGANIC OXIDATION BYPRODUCTS	Bromochloroacetonitrile
Aldehydes	Dibromoacetonitrile
Formaldehyde	Trichloroacetonitrile
Acetaldehyde	Haloketones
Glyoxal	1,1-Dichloropropanone
Hexanal	1,1,1-Trichloropropanone
Heptanal	Chlorophenols
Carboxylic Acids	2-Chlorophenol
Hexanoic Acid	2,4-Dichlorophenol
Heptanoic Acid	2,4,6-Trichlorophenol
Oxalic Acid	Chloropicrin
Assimilable Organic Carbon	Chloral Hydrate
	Cyanogen Chloride
	N-Organochloramines
	MX*

Factors that affect DPB formation are chlorine dose, chlorine contact time and water quality parameters such as organic content, bromide concentration, temperature and

pH (HDR Engineering, 2001, Marhaba *et al.,* 2000). The most common types of DBPs are THMs and HAAs (HDR Engineering, 2001). As shown in Table 2.5, there are four types of trihalomethanes: chloroform, bromoform, bromodichloromethane and dibromochloromethane. The naturally occurring organic precursors that form THMs are humic species and some amino acids (HDR Engineering, 2001). In water that has less than 10 μg/L of bromine the principle THM formed is chloroform (Sorlini & Collivignarelli, 2005). Additionally, more THMs are produced by HA than FA; therefore, testing for this report was done using HA (Reckhow *et al.,* 1990). The persistence of THMs in environmental waters varies greatly depending on the site characteristics. Chloroform is the most persistent of the four compounds. The half-lives of the THMs vary by more than 2 orders of magnitude with some being as low as one day and others being greater than 120 days (Pavelic *et al.,* 2006)

2.3.3 Health Effects

As previously mentioned, the balance between effective inactivation of microbial pathogens and minimizing DBP formation is delicate and of foremost concern in the development of new regulations This is partly because chlorinated compounds such as THMs are difficult to degrade, but more importantly because they pose a public health risk. The growing body of literature on the health risks posed by DBPs was reviewed in the development of the Stage 2 D/DBP Rule. These studies found evidence that THMs are carcinogens and teratogens—chemicals causing reproductive defects (Chang *et al.*, 2000).

The basic known health effects of each THM are summarized in Table 2.6. In addition, the pervasiveness of each chemical is evidenced by the number of National Priorities List (NPL) sites that have been found to contain each chemical.

Table 2.6: THM health effects (ATSDR, 1997, 1999 and 2005)

Chemical	Health Effects	National Priorities List
Bromodichloromethane	Not known to cause adverse health effects in people. Animal studies show that high concentrations can affect the brain as well as damage the liver and kidneys.	5
Bromoform	High levels are known to cause liver and kidney damage and to affect the brain.	40
Chloroform	Short term effects are dizziness fatigue and headaches. Long term effects are liver and kidney damage.	717
Dibromochloromethane	High levels are known to cause liver and kidney damage and to affect the brain.	174

The EPA classifies all four THMs as either possible (C) or probable (B) human carcinogens. These classifications are based on studies which indicate that there are links between cancer occurrence and THMs in drinking water. According to the U.S. EPA classifications, Group A are human carcinogens, Group B are probable human carcinogens and Group C are possible human carcinogens. Interestingly, the U.S. EPA classifies dibromochloromethane as class C, which is less serious than B. However, according to the Agency for Toxic Substances and Disease Registry, bromodichloromethane has no known adverse health effects in humans, while dibromochloromethane is described as similar to chloroform and bromoform (ATSDR, 1997, 1999 and 2005). This small inconsistency highlights the lack of concrete knowledge about the health effects of these chemicals.

2.3.3.1 Reproductive Studies

There is evidence that THM exposure can lead to reproductive problems including birth defects (BD), low birth weight (LBW), premature delivery (PMD) and spontaneous abortion (SAB). Table 2.7 summarizes four studies that found evidence of reproductive problems associated with THM exposure. In order to understand this summary, some background information is needed. These studies were both qualitative

and quantitative in nature. Low birth weight (LBW) was considered to be birth weight < 2500 g and premature delivery was defined as < 37 weeks. Intrauterine growth retardation was defined as a fetus having less than the 5th percentile for weight at a particular gestational age. All newborns with a body length of less than 36 cm were classified as "small for age or short body length." A small cranial circumference was defined as less than 50 cm. Other defects observed included major cardiac defects, neonatal jaundice, neural tube defects and oral clefts. These studies included women who were exposed to municipal water supplies, as well women drinking private and bottled water.

Table 2.7: Summary of studies linking THMs to reproductive defects (Calderon, 2000)

	Study by (author)			
Characteristic	Bove *et al.*	Kanitz *et al.*	Savitz *et al.*	Waller *et al.*
Study design	qualitative	qualitative	quantitative	quantitative
Disinfectant	unknown	chlorine dioxide	chlorine	chlorine
Outcome*	BD, LBW	LBW	LBW, SAB, PMD	SAB
Exposure	population	population	individual	individual
Water consumption	not obtained	not obtained	obtained	obtained

*BD = birth defects; LBW = low birth weight; PMD = premature delivery; SAB = spontaneous abortions.

Two qualitative studies that showed associations between chlorine disinfectants and birth defects were reported by Bove *et al.* (1992) and Kanitz *et al.* (1996). Bove *et al.* (1992) studied birth outcomes on 80,938 live births and 594 fetal deaths between 1985 and 1988 in the state of New Jersey. Multiple births and fetuses with chromosomal defects were excluded from this study. Exposure to THMs during pregnancy was estimated for mothers using tap water sample data. Total THM levels which exceeded 100 ppb were found to reduce birth weight among full term fetuses by 70.4 g (Bove *et al.*, 1992).

A two year study of 676 births in Liguria, Italy was conducted by Kanitz *et al.* (1996). Pregnant women in this area were exposed to water disinfected with either sodium hypochlorite, chlorine dioxide or both. The THM concentrations in treated water

were 1-3 ppb for water treated with chlorine dioxide and 8-16 ppb for water treated with sodium hypochlorite. A higher frequency of small body length and small cranial circumference was found in infants whose mothers drank water disinfected with chlorine compounds as compared to a control group of women who drank well water that was not disinfected. Additionally, neonatal jaundice was almost twice as common in newborns whose mothers were exposed to water treated with chlorine dioxide compared to women whose drinking was not disinfected or was disinfected with sodium hypochlorite (Kanitz *et al.*, 1996).

Additionally, two quantitative studies, as reported by Savitz *et al.* (1995) and Waller *et al.* (1998), are summarized in Table 2.7. Cases of miscarriage, low birth weight and preterm delivery were studied by Savitz *et al*. (1995) in North Carolina. This study found little evidence that LBW, PMD and miscarriage were caused by exposure to THMs in drinking water, except that there was an increased risk of miscarriage for women who ingested water with a THM concentration greater than 100 ppb (Savitz *et al.,* 1995).

Similarly, Waller *et al.* (1998) found that women who drank at least 5 glasses per day of cold tap water containing at least 75 ppb of TTHMs have an adjusted odds ratio of 1.8 for spontaneous abortion. When taken individually, bromodichloromethane was found to be the most likely to increase risk for spontaneous abortion at concentrations as low as 18 ppb. For this study 5,144 pregnant women were participants and THM sampling data from seventy-eight drinking water utilities was obtained. Drinking water THM concentrations were estimated using the sampling data provided by local water utilities (Waller *et al.,* 1998).

2.3.3.2 Cancer Studies

Another possible health effect caused by THM exposure that isn't represented by the ATSDR chemical descriptions is cancer. As with reproductive effects of THM exposure, the studies assessing the cancer risks associated with THM exposure are growing in number. King and Marrett (1996) examined the relationship between bladder cancer and exposure to DBPs in public drinking water. This study, conducted in Ontario, Canada, estimated individual exposure to THMs using data from public water utilities over a forty year period. The control group included 1545 individuals who were exposed

to public drinking water supplies for at least 30 years and the experimental group was comprised of 696 individuals diagnosed with bladder cancer. Individuals who were exposed to chlorinated drinking water for greater than 30 years were found to be more likely to be diagnosed with bladder cancer than those who were exposed for 10 years or less. More specifically, those who were exposed to THM concentrations of at least 50 ppb for greater than 35 years had 1.63 times the risk of being diagnosed with bladder cancer. Researchers concluded from this study that the risk of bladder increases with both concentration and duration of exposure to THMs in drinking water (King & Marrett, 1996).

Another study conducted by Canadian researchers came to similar conclusions (Kasim *et al.,* 2005). This study included 3,420 control individuals and 686 cases of chronic myeloid leukemia. At least 30 years of water quality data were available for all participants. For a THM concentration of 40 μg/L with the longest exposure duration, the adjusted odds ratio was 1.72. Kasim *et al.* (2005) concluded that increased years of exposure is associated with increased risk of chronic myeloid leukemia. Additionally, the researchers found increased risk of kidney, pancreas and brain cancers in individuals exposed to chlorinated drinking water supplies (Kasim *et al.*, 2005).

In conclusion, the health effects of THM exposure are clearly cause for concern, but are not well understood at this time. A recent development in toxicology studies of THMs has been the idea that people are exposed to DBPs by multiple routes. Exposure to chlorinated water occurs while showering, bathing, swimming, and washing dishes and clothes in addition to ingestion through drinking tap water. Thus, people are exposed to THMs through inhalation and dermal contact as well as ingestion. Initial studies on the significance of exposure by dermal contact and inhalation indicate that these routes may be more detrimental to human health than ingestion (Wang *et al.*, 2006).

2.3.4 Minimizing DPB Formation

Many modes to minimize DBP formation have been suggested. As described in Section 2.1.2, the Information Collection Rule required public water systems to conduct treatment studies with membrane separation processes or adsorption processes. More

specifically, the membrane processes studies were nanofiltration (NF) and reverse osmosis (RO), while adsorption refers to granular activated carbon (GAC) (U.S. EPA, 1996a). These processes reduce DBP formation by removing DBP precursors. Other options for removing DBP precursors include enhanced coagulation and ion exchange (Kitis *et al*., 2001; Gopal *et al.,* 2006). Additionally, humic substances are more reduced by conventional water treatment processes than non humic substances (Kim & Yu, 2006). In addition to DBP precursor removal, another option is to vary when and where disinfectants are applied in the treatment plant.

Chlorine dioxide is a widely used disinfectant in Europe. This disinfectant has been found to produce 97% less THMs than chlorination (Sorlini & Collivignarelli, 2005). Chlorine dioxide also produces fewer THMs than ozonation. This disinfectant requires smaller doses and a shorter reaction time to produce the same inactivation effect (Chang *et al.,* 2000). More information about chlorine dioxide is presented in Section 2.2.2.4.

2.4 Sonication

This research examined disinfection of drinking water using ultrasound treatment. The fundamental mechanisms of sonication are explained in Section 2.4.1, followed by a detailed discussion of research and applications of sonication to aqueous systems including drinking waters and wastewaters.

2.4.1 Cavitation Theory

Cavitation is the formation of partial vacuums in a liquid that result from the application of ultrasound waves. Sound waves at frequencies too high for most humans to hear are called ultrasound waves or ultrasonic waves. Frequencies higher than 16 kHz or 16,000 cycles per second are generally considered to be ultrasonic. When liquids are exposed to these high frequency waves, alternating compression and rarefaction takes place, which leads to cavitation. In the rarefaction phase, the liquid is stretched which results in the formation of microbubbles of reduced pressure. Gases dissolved in the

liquid vaporize and fill the microbubbles. As shown in Figure 2.3, bubbles increase and decrease in diameter during expansion and compression waves, respectively. The radii of the bubbles are on the order of 100 μm, and a bubble life is typically a fraction of a second.

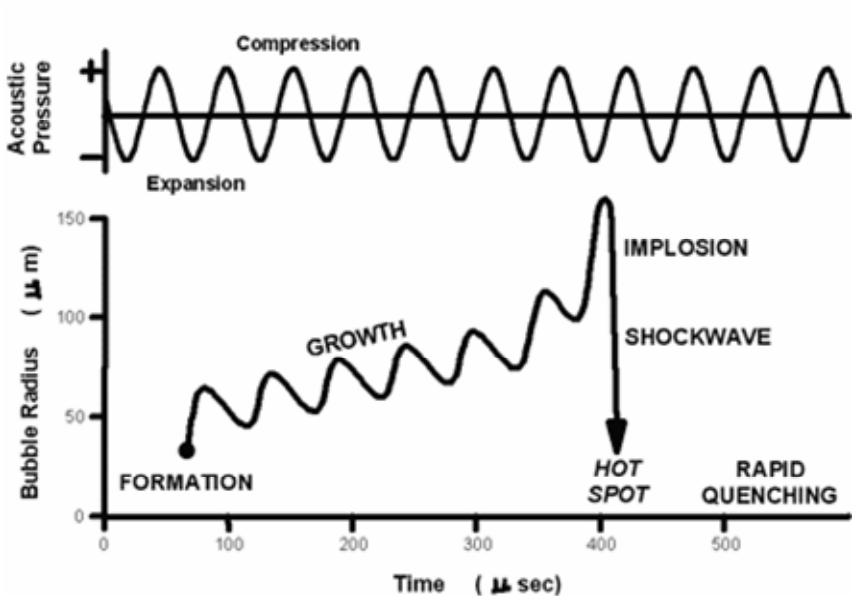

Figure 2.3: Microbubble formation during cavitation (Suslick, 1994)

As the alternating cycle continues, the bubbles implode during a compression cycle. The size at which bubbles collapse varies depending on the liquid and the ultrasound frequency being applied (Adewuyi, 2001). For example, at 20 kHz, the radius at which bubbles will collapse is in the range of 100 – 170 μm. Cavitation causes extreme heating and pressure differentials within the liquid on the microscopic scale. The temperature in microbubbles can rise to 5000 K or 8540° F. In addition, the pressure in the voids can reach 1000 atmospheres. The temperature and pressure changes induced by cavitation can last for as short as a millionth of a second.

The wavelength of ultrasonic waves is too large compared to molecules to directly effect molecular reactions. Instead, the cavitation caused by ultrasound waves has an effect at the molecular level. The collapse of the microbubbles releases large amounts of energy that can cause high-energy chemical reactions to take place. Increased reactivity of hydrogen with unsaturated organic compounds has been reported. For example,

hydrogenation of alkenes by nickel powder is enhanced about 100,000 fold by ultrasonic waves (Suslick, 1994). It has been documented that ultrasound can increase reactivity by almost a million fold.

While increased reactivity due to cavitation is most often referred to in terms of chemical reactions, this project was also concerned with how cavitation can affect biological molecules and reactions. If cell functions can be sufficiently altered to inactivate cells, then this process will be a successful method to treat drinking water sources and decrease the necessity of chlorine disinfection.

2.4.2 Prior Sonication Research

Studies of different possible applications of ultrasound irradiation to microbial water contaminants have yielded a variety of results. Ultrasound treatment has increased inactivation of both viruses and bacteria (Scherba *et al.*, 1991). The following sections provide information on sonication research for water treatment and wastewater treatment.

2.4.2.1 Water Treatment Applications

The majority of research on the use of sonication for drinking water disinfection has focused on inactivation of bacteria, typically *E. coli*. Several relevant studies quantified the influence of different parameters for *E. coli* inactivation by sonication. The parameters varied in the studies were frequency, power, contact time, temperature, squeeze film thickness and presence of gases.

Various researchers have tested sonication powers ranging from 1 to 140 watts. Intensity is a parameter that relates power to liquid volume. When applying ultrasound treatment, the frequency of the sound waves as well as the intensity of the power output can be varied. Hua and Thompson (2000) tested the inactivation of *E. coli* at sound wave frequencies of 20, 205, 358, 618 and 1071 kHz. Table 2.8 shows the inactivation rates that were achieved when treating *E. coli* with these frequencies. The power setting was 128 to 140 Watts and the power to volume values ranged from 0.43 to 0.46 W/mL. The log inactivation rates ranged from 2 to 4.4, with 205 kHz frequency yielding the highest inactivation at 4.4-log.

Table 2.8: Ultrasound frequencies and *E. coli* inactivation rates (Hua and Thompson, 2000)

Frequency	Inactivation	
kHz	Log	Rate min^{-1}
20	2.8	0.031
205	4.4	0.078
358	3.5	0.064
618	2.5	0.041
1071	2.0	0.030

To evaluate the importance of power and intensity, Hua and Thompson (2000) tested the 20 kHz frequency with intensities varying from 4.6 to 74 W/cm^2. Throughout these experiments, cooling water at a temperature of 12° C was used to maintain the 300 mL samples at temperatures of 20-29° C. All samples were sonicated for 70 minutes. For these intensities, the corresponding power values were 140 to 80 Watts and 0.46 to 0.27 Watts/mL. This testing showed that the higher intensity (74 W/cm^2) was the most effective for inactivating *E. coli.* This intensity yielded a 3-log inactivation of *E. coli* in 70 minutes with an inactivation rate of 0.046 min^{-1}. This rate is faster than the 0.031 min^{-1} rate that was achieved at the same frequency, but with a lower power intensity (see Table 2.8; Hua and Thompson, 2000).

Synergistic disinfection using sodium hypochlorite and sonication has been tested for inactivation of *E. coli*. Duckhouse *et al.* (2004) tested both under two conditions: simultaneous application of hypochlorite and sonication, and sonication followed by hypochlorite. Table 2.9 summarizes the ultrasound parameters for this study. All experiments were performed at 20°C. Chlorine dose information was not provided in the article. For experiments with sequential application of sonication and sodium hypochlorite, the sonication was applied for 0.5, 1, 2 and 5 minutes and immediately followed by chlorine dosing.

Table 2.9: Sonication parameters for Duckouse *et al.* (2004)

Frequency (kHz)	Power (Watts)	Intensity (W/cm^2)
850	1.22	0.029
20	22.34	16.92

Figure 2.4 shows a comparison of *E. coli* inactivation in the sequential and simultaneous disinfection experiments. For the sequential experiments, sonication pretreatment was applied for 1 minute and followed by chlorination for 20 minutes. For simultaneous application, the chlorine and sonication were both applied for 1 minute and then chlorination continued in silent conditions for the remainder of the 20 minutes. For simultaneous application, 20 kHz was more effective than 850 kHz. Simultaneous sonication and chlorination at 20 kHz achieved approximately 4-log reduction in *E. coli*. For one minute of 850 kHz sonication followed by chlorination, 4-log inactivation was achieved.

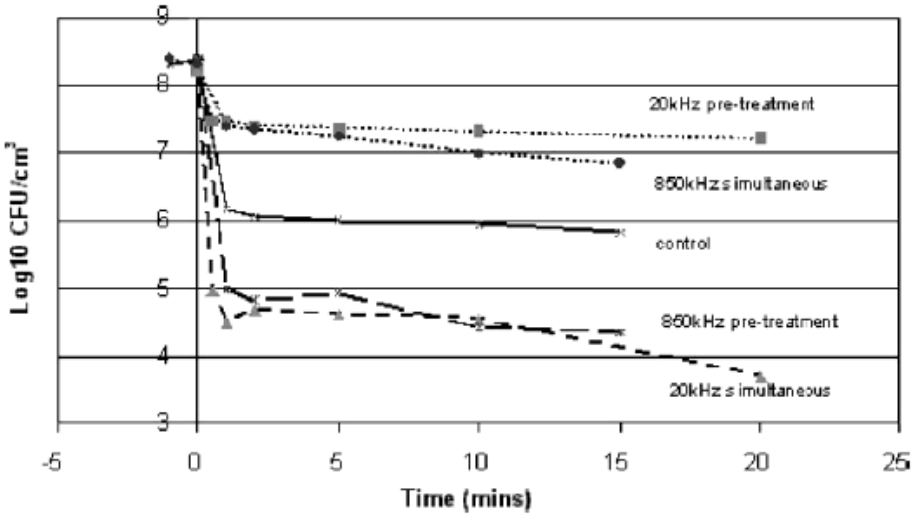

Figure 2.4: A comparison of inactivation by sequential (1 minute pretreatment with sonic) and simultaneous treatments with 850 and 20 kHz (Duckhouse *et al.*, 2004)

Experiments were also conducted with simultaneous ultrasound and chlorine treatment for 5 minutes. One possible explanation for why 850 kHz caused greater inactivation of *E. coli* as a 1 minute pretreatment than as a 5 minute simultaneous

application is that it can cause agglomeration of dead cells. These bacterial clumps formed *after* the first minute of sonication and may have protected still living cells from inactivation. Therefore, the higher frequency is more effective for a short exposure time (1 minute). For a longer exposure time (5 minutes or more), a lower frequency of 20 kHz inactivates *E. coli* without causing bacterial agglomeration. Conversely, the low frequency of 20 kHz was not effective as a 1 minute pretreatment. For that experimental setup, *E. coli* concentrations were only reduced by about 1-log. The control experiment that used only hypochlorite reduced *E. coli* by only 2-log (Duckhouse *et al.*, 2004).

Finally, varying the squeeze film thickness has been found to affect the inactivation of *E. coli* treated with sonication. As shown in Figure 2.5, the rate of inactivation is approximately 3 times greater with a squeeze film that is 2 to 4 mm thick as opposed to a film of 1 mm thickness (Furata *et al.*, 2004). Conditions for this study were 27.5 kHz frequency and 42 W/mL power. Sonication treatment was applied for 5 minutes. The 2, 3 and 4 mm thick squeeze films yielded 6-log inactivation of *E. coli.* According to Figure 2.5, squeeze film thicknesses of 2-4 mm yielded inactivation rates of over 0.03 per second or 1.8 per minute. The highest inactivation rate in the study by Hua and Thompson (2000) was 0.078 per minute. This is a significant difference in results. One possible cause of the higher inactivation rate could be that Furata *et al.* (2004) used a power to volume ratio of 42 W/mL while Hua and Thompson (2000) used a power to volume ratio of about 0.45 Watts/mL which is two orders of magnitude smaller.

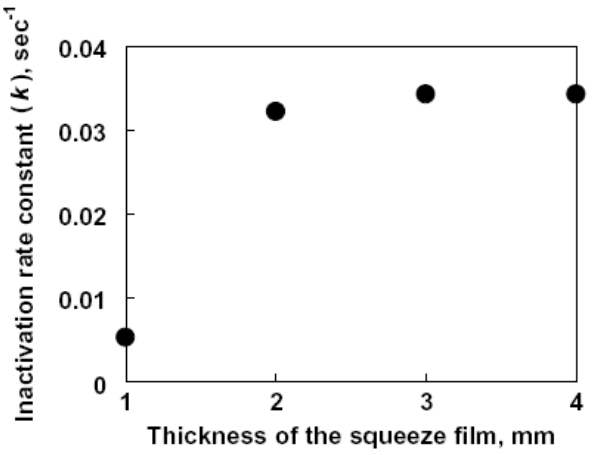

Figure 2.5: Inactivation rate constant as a function of squeeze film thickness (Furata *et al.*, 2004)

Although numerous experiments have been conducted on *E. coli* inactivation with sonication, *E. coli* are not the most resistant organisms of interest in drinking waters. For example, some bacterial species are able to agglomerate into clusters, which helps to protect the cells from disinfection methods. Studies have been performed on bacteria such as *Bacillus subtilis*, yeast such as *Saccharomyces cerevisiae* and protozoa such as *Cryptosporidium*.

It has been suggested that ultrasound waves can aid in breaking apart clusters of bacteria to make them more susceptible to disinfection methods such as UV irradiation and chlorination. Joyce *et al.* (2003) tested the effects of sonication on *Bacillus subtilis* (a clumping bacteria) with different frequencies, intensities and times to learn whether there is an optimal way to declump and inactivate bacteria with ultrasound. Frequencies from 20 kHz to 850 kHz were tested as shown in Table 2.10. All samples were sonicated for 15 minutes. As with Duckhouse *et al.* (2004), the 20 kHz frequency was more effective for bacterial inactivation, however the exact rate of reduction and log removal were not quantified in the literature.

Table 2.10: Ultrasonic frequencies and power values for Joyce *et al.* (2003)

Frequency	Power
kHz	Watts/mL
20	0.18 0.24
512	0.071
850	0.064

Tsukamoto *et al*. (2004) tested the effects of ultrasound and sodium hypochlorite, separately, to inactivate *Saccharomyces cerevisiae*. The frequency used was 27.5 kHz with a 2 mm squeeze film at a maximum of 176 watts of power. Sonication was applied for 10 minutes. Figure 2.6 shows less than 1-log reduction of *S. cerevisiae* was observed for powers from 30 to 60 Watts, while greater than 2-log reduction was obtained for 180 Watts. The triangles represent bacterial numbers estimated using the colony counting method and the circles represent bacterial numbers estimated with calorimetric methods

(Tsukamoto *et al.*, 2004). These log reduction numbers are less than were found by Furata *et al.* (2004) using a 2 mm squeeze film and 27 kHz. This indicates the resistance of yeast cells compared with *E. coli*.

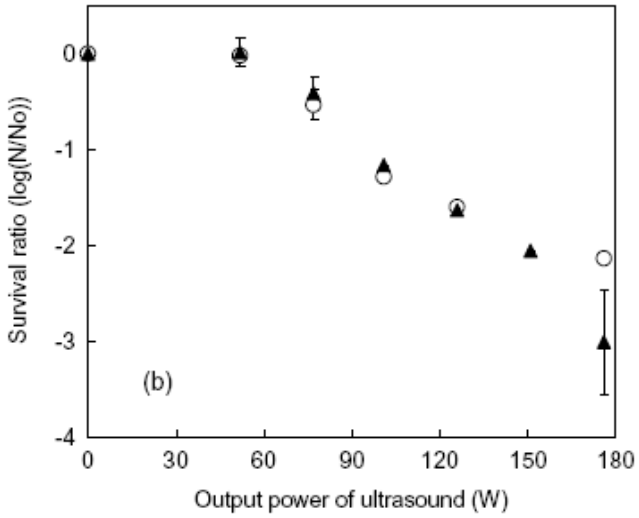

Figure 2.6: Log inactivation of *S. cerevisiae* as a function of ultrasound power (Tsukamoto *et al.*, 2004)

Nakanishi *et al.* (2001) tested the effect of sonication on *Cryptosporidium*. This study tested varying frequencies and varying exposure time. For 28, 45 and 100 kHz, all samples were exposed for 10 minutes. The oocysts were not affected by sonication at 100 kHz for 10 minutes. At a frequency of 45 kHz, 13% of oocyst nuclei were destroyed. The lowest frequency was most effective at inactivating *Cryptosporidium*, and therefore this frequency was used in evaluating exposure times. The samples were sonicated at 28 kHz for 2, 10 and 20 minutes. When sonicated for 2 minutes, 40% of oocyst nuclei were destroyed. A 10 minute exposure yields 97.3% inactivation. For a 20 minute exposure time, 99% of *Cryptosporidium* oocysts were inactivated (Nakanishi *et al.*, 2001).

2.4.2.2 Wastewater Treatment Applications

Sonication is a potentially useful technology for wastewater treatment processes as well. As with drinking water treatment, some pathogens are more difficult to

inactivate with UV irradiation and chlorine disinfection. In wastewater, some bacteria of concern are fecal coliforms, *E. coli* and streptococci.

Burleson *et al.* (1975) studied inactivation of several bacteria: *Staphylococcus aureus, Pseudomonas fluorescens, Salmonella typhimurium*, *E. coli.*, *Vibrio cholerae* and *Shigella flexneri.* This study included ozone only treatment and ozone and sonication combined treatment for treatment of secondary wastewater effluent. Sonication was applied at 40 kHz frequency and 150 Watts power. Ozone was applied at 152.4 cm^3 per hour. The fecal coliform concentration of the secondary effluent was 80 cfu/mL. All experiments were conducted for 10 minutes. Sonication alone did not inactivate bacteria in secondary effluent. Compared to ozone alone, sonication with ozone achieved 7-log inactivation of *E. coli* in approximately 30 seconds less contact time. For example, 7-log inactivation of *E. coli* with ozone alone required 1 minute and 15 seconds. With combined sonication and ozone, the contact time was reduced to 30 seconds (Burleson *et al.*, 1975)

Blume quantified the energy savings due to the synergy of ultrasound and UV irradiation (2004). The frequency for this study was 20 kHz, the power was 41 to 154 watts and the intensities ranged from 1.7 to 60.8 watts per square centimeter. These values are comparable to some of those used in drinking water treatment studies (Blume, 2004). The UV dose was 3 Watts applied at 254 nm. Thirty seconds of UV treatment alone reduced fecal coliforms by 3.7 log. The same reduction was achieved with five seconds of ultrasound followed by five seconds of UV irradiation. The energy consumption was reduced by 57% percent for the combined disinfection compared to the energy consumption of UV alone. In this study, sonication is believed to have enhanced the power of UV irradiation by breaking up bacterial clusters as in Joyce (2003).

Madge and Jensen (2002) evaluated the effect of water quality on ultrasonic treatment of wastewater using a 20 kHz probe. Treatment for 6 minutes with 700 Watts/L can achieve 4-log inactivation of fecal coliform. This study found little effect in disinfection effectiveness caused by the following water quality parameters: pH, total suspended solids (TSS), volatile suspended solids (VSS), biochemical oxygen demand (BOD), alkalinity and total organic carbon (TOC). Additionally, pulsed ultrasound was

found under these conditions to have a disinfection rate 2.8 times slower than continuous application of ultrasound treatment (Madge & Jensen, 2002).

2.5 Conclusion

This chapter summarizes the literature and regulations relavant to this study. The Surface Water Treatment Rules and disinfection by-products rules are discussed. Physical and chemical methods for the removal and inactivation of pathogens are described. Also included are the mechanisms by which disinfection by-products are formed and the known health effects of these compounds. The section on health effects of disinfection by-products reveals uncertainty about the exact risks posed by exposure to THMs through drinking water. Finally, the theory of sonication and literature presenting research on the use of sonication for pathogen inactivation are reviewed. Sonication has been shown to be effective for inactivation of many pathogens as a sole or combined disinfectant. However, the effects of sonication treatment on disinfection by-product formation are not well studied.

Chapter 3: Methodology

The goal of this research was to evaluate the impact of sonication on disinfection by-product formation. This chapter provides the methods used to quantify the trihalomethane formation potential of natural organic matter exposed to ultrasound treatment. First, an overview of the experimental design is presented. Then, the procedures for conducting the experiments are detailed. Lastly, the analytical methods for measuring chlorine and trihalomethanes are provided.

3.1 Experimental Design

The goal of this research was to determine the impact of sonication on DBP formation potential. A series of experiments were conducted to evaluate the resulting trihalomethane formation potential (THMFP) after treatment with sonication compared to control samples that were not sonicated. In brief, prepared waters with natural organic matter (NOM) were sonicated, dosed with chlorine and allowed to react to form THMs under controlled pH and temperature conditions. Then, THMs were extracted and quantified. Control samples were also run which were not sonicated. The variables tested included the sonication. The experimental design is show in Table 3.1. Each experiment was done in triplicate and a power to volume ratio of 180 Watts per liter was used throughout.

Table 3.1: Experiments summary

Time (min)	NOM (mg/L)	NaOCl mg/L
0	1 2.5	6 10
0.5	1 2.5	6 10
1	1 2.5	6 10
5	1 2.5	6 10
10	1 2.5	6 10

3.2 Experimental Procedures

This section describes in detail the procedures used in preliminary experiments as well as in sonication experiments. One set of preliminary experiments was carried out with a range of chlorine and NOM doses to determine the combination of doses that would yield the desired chlorine residual concentration. Sonication experiments were then completed to determine the impact of sonication on THM formation potential.

3.2.1 Natural Organic Matter

The natural organic matter used in this research was Suwannee River Natural Organic Matter (RO Isolation) purchased from the International Humic Substances Society (Catalog #1R101N, IHSS, St. Paul, MN). This NOM has been well studied as a DBP precursor.

3.2.2 Experimental Water

Experimental water was prepared using E-pure water (Barnstead E-Pure Water System, Barnstead International Dubuque, Iowa), a buffer solution and NOM. For each

liter of experimental water prepared, 20 mL of buffer was added. Reagent grade water was prepared using a Barnstead ROPure® ST reverse osmosis (RO) water purification unit followed by a Barnstead E-pure water system. The E-pure water system produces Type I reagent-grade bacteria-free water. E-pure water is treated by a pretreatment cartridge to remove colloids and bacteria, ion exchange and granular activated carbon. These treatments are followed by filtration through an 0.2 μm cartridge filter.

To make buffer for the experimental water, 11.7 grams of NaOH and 68.1 g KH_2PO_4 were dissolved in E-pure water in a 1 liter volumetric flask. The volume in the flask was brought up to the 1 liter mark with E-pure water. The pH of the buffer was verified and adjusted if it was not approximately 7. pH measurements were done according to Standard Methods 4500-H+ B (APHA *et al.* 1998) using an Orion 420A pH meter (Orion Research Inc., Beverly, MA) calibrated using standard buffers of 4, 7 and 10. Between measurements, the pH probe was stored in electrode storage solution purchased from Fisher Scientific (Catalog #13-641-908)

To prepare the experimental water, the appropriate mass of NOM was weighed on an analytical balance. Then a volumetric flask was partially filled with E-pure water and the NOM was added. The volumetric flask was brought up to the mark with E-pure water and the solution was mixed. Lastly, 20 mL of buffer was added.

3.2.3 Preliminary Experiments

Preliminary experiments were conducted to determine the appropriate chlorine dose to use for determining the THMFP. According to Standard Method 5710 A and B (APHA *et al.,* 1998), formation potentials are measured by dosing samples with chlorine and allowing the chlorine to react for 7 days at 25 degrees Celsius. The residual concentration of chlorine after 7 days should be 3-5 mg/L. For the preliminary experiments, experimental water was prepared with five different concentrations of NOM. Each concentration was dosed with 5 different chlorine doses in headspace free BOD bottles, as shown in Table 3.2. The samples were incubated in the dark at 25°C and

free chlorine residuals were measured at days 1, 2, 4 and 7. The results were used to determine appropriate chlorine doses for the subsequent experiments.

Table 3.2: Chlorine dose and organic matter concentration experiment summary

TOC mg/L	Cl_2 / TOC	Cl_2 Dose mg/L
1	1	1
	3.00	3
	5.00	5
	7.00	7
	10.00	10
2.5	0.80	2
	3.60	9
	6.40	16
	9.20	23
	12.00	30
5	1.00	5
	3.00	15
	5.00	25
	7.00	35
	10.00	50
7.5	1.33	10
	4.00	30
	6.00	45
	8.67	65
	10.67	80
10	1.33	10
	4.00	30
	6.67	50
	9.33	70
	13.33	100

3.2.4 Sonication Experiments

This section describes the procedures used for all sonication and control experiments. First, the experimental water was prepared as described in Section 3.2.2. After the experimental water was prepared, the water was treated with sonication, or left untreated (control). For samples that were sonicated, a 500 mL volume of experimental water was used. The sonication system consisted of a probe-type sonicator (Sonicator

3000, Misonix Inc., Farmingdale, NY). A constant ultrasonic frequency, 20 kHz, was used throughout testing. The sonicator output power intensity ranged from 0.0 to 10.0, with the highest intensity yielding a power output of approximately 90 Watts. The power to volume ratio was varied by adjusting the intensity on the sonicator.

All sonication experiments were conducted at room temperature (~25 ° C). The experimental water was placed in a 500 mL beaker and the beaker was positioned so as to center the sonication probe within it. The probe was lowered into the water to a depth of approximately 1 inch below the surface of the sample water. The sonicator was started and the power output was monitored. The intensity was adjusted to maintain a constant power output, if necessary. After the appropriate sonication time was reached, the sonicator was turned off and the sample water was chlorinated to determine formation potential as described in Section 3.2.5.

3.2.5 Chlorination and Incubation

For each of the samples (sonicated samples and control samples that received no sonication), the experimental water was poured into a 300 mL biochemical oxygen demand (BOD) bottle until the bottle was approximately half full. The appropriate chlorine dose, as determined by the preliminary experiments, was added. All samples with the same NOM concentration were chlorinated with the same chlorine dose. In this way, it was possible to directly compare the disinfection by-product production in sonicated samples with that of the control samples. The bottle was filled with sample water and capped such that it was headspace free. The bottles were incubated in the dark at 25°C for 1, 2, 4 or 7 days. After the reaction period, the samples were removed from the incubator and processed as described in Section 3.2.6.

3.2.6 Chlorine Residual and THM Extraction

After samples were incubated, the free and total chlorine residuals were measured as described in Section 3.3. Total chlorine was measured only for the first set of experiments. These results indicated that all chlorine present was free chlorine, therefore

free chlorine measurements were sufficient for measuring chlorine residual. Following chlorine residual measurement, samples were quenched with sodium thiosulfate to stop the formation of THMs. The THMs were extracted and quantified on a gas chromatograph (GC) with an electron capture detector (ECD). The detailed procedures for these methods are described in Sections 3.4. Results from treated and untreated samples were compared to determine the impact of sonication on THMFP.

3.3 Chlorine Methods

The chlorine stock for experimentation was NaOCl purchased from Fisher Scientific. The stock solution had an approximate concentration of 5% chlorine by weight (50 mg/mL). In order to prevent decomposition of the chlorine stock solution over time, the stock NaOCl bottle was wrapped in aluminum foil and stored in a refrigerator at 4°C. Approximately 30 mL of NaOCl stock solution was transferred to a 40 mL vial for regular use in experiments. This prevented contamination of the stock bottle. The 40 mL vial was also wrapped in aluminum foil and stored in the refrigerator when not in use. In cases where the volume of NaOCl stock required for dosing was too small to accurately measure, a diluted chlorine solution was used. Specifically, a 10% chlorine stock was prepared by using 1 part NaOCl stock and 9 parts E-pure water, and stored in a separate aluminum wrapped vial.

3.3.1 Chlorine Demand Free Glassware

All glassware utilized in chlorine measurements was chlorine demand free (CDF). CDF glassware was prepared by soaking in a 100 mg/L chlorine bath for a minimum of 1 hour. Prior to use, the glassware was removed from the bath and rinsed three times with E-pure water. The glassware was prepared on the day of use.

3.3.2 Free Chlorine Calibration Curve

A free chlorine calibration curve was prepared using Standard Method 4500-Cl F, the DPD Ferrous Titrimetric Method and Standard Method 4500-Cl G, the DPD Colorimetric Method (APHA *et al.,* 1998). This curve was used to determine the chlorine stock concentration via titration and the chlorine residual concentration of samples by measuring their absorbance. The chlorine stock concentration was measured once per week to ensure that it had not degraded.

The CDF glassware required for this method includes five 150 mL Erlenmeyer flasks and five 100 mL volumetric flasks (labeled numbers 1-5). Each of the volumetric flasks was filled to the 100 mL mark with E-pure water. Next, 5 mL of DPD buffer solution (see Section 3.3.6), followed by 5 mL of DPD indicator solution (see Section 3.3.5) were added to each Erlenmeyer flask. A 10 μL syringe dedicated to chlorine transfers was used to transfer approximately 1 μL of chlorine stock using the solvent flush technique into the volumetric flask labeled #1. The contents of the volumetric flask were then transferred to Erlenmeyer flask #1 using a swirling technique to ensure the chlorine was mixed uniformly in the water. The solution in the Erlenmeyer flask turned pink which indicated chlorine was present. A small volume of the pink solution was transferred to a 10 mm quartz spectrophotometer cell to rinse the cell. This volume was poured back into the Erlenmeyer flask to retain the volume and then the cell was again filled with the pink solution. The absorbance was then measured on the spectrophotometer at a wavelength of 515 nm (Cary 50 Scan, Varian Australia Pty Ltd., Mulgrave, Victoria, Australia).

Immediately after the absorbance was measured, the solution in the spectrophotometer cell was poured back into the Erlenmeyer flask. A magnetic stir bar was placed in the flask and the flask was placed on a magnetic stirrer. FAS titrant solution (see Section 3.3.7) from a buret was used to titrate the pink solution until it turned clear as described in Method 4500-Cl F in Standard Methods (APHA *et al.*, 1998). The burette readings before and after the titration were recorded, and the volume of FAS titrant used was calculated.

The procedure just described was repeated for chlorine stock additions of 2, 3, 4 and 5 μL. After all five measurements were completed, several calculations were completed. First, the chlorine concentration in the NaOCl stock was computed using Equation 5. The results from all five measurements were averaged to determine the chlorine stock concentration. Next, the chlorine concentration in each volumetric flask was calculated. This was computed using the average NaOCl stock concentration and Equation 5.

Equation 5

$$C_{stock}\left(\frac{mg}{mL}\right)=\left(\frac{V_{titrant}(mL)}{V_{chlorine}(\mu L)}\right)\times 100$$

Equation 6

$$C_{Flask}\left(\frac{mg}{L}\right)=\frac{C_{stockavg}\left(\frac{mg}{mL}\right)\times V_{added}(\mu L)}{100(mL)}\times\frac{1(mL)}{1000(\mu L)}\times\frac{1000(mL)}{1(L)}$$

Lastly, the five data points were used to create a free chlorine standard curve with the chlorine concentration in the volumetric flasks (mg/L) on the y-axis and the absorbance readings (1/cm) on the x-axis. The equation for the trend line and R^2 value were calculated using Microsoft Excel.

3.3.3 Residual Chlorine Measurements

The DPD colorimetric method (Standard Method 4500-Cl G) was used to measure chlorine residuals for all experiments (APHA *et al*., 1998). First, free chlorine residuals were measured. For each sample, one CDF test tube was prepared. Next, 0.5 mL DPD buffer solution and 0.5 mL DPD indicator were added to the test tube in that order. Then 10 mL of the sample was added to the test tube. The solution was poured into the 10-mm quartz spectrophotometer cell. The cell was rinsed with the solution from the test tube, and then refilled with the same solution. The cell was placed into the spectrophotometer and absorbance at 515 nm was measured. The free chlorine residual concentration was calculated using the equation of the trend line from the free chlorine

calibration curve. This process was repeated to determine the free chlorine residual for each sample. The same method was used to measure total chlorine except that 0.1001 g of potassium iodide (KI) was added to the test tube first. After the buffer, indicator and sample were added; the sample was allowed to sit for 2 minutes before measuring the absorbance.

3.3.4 Chlorine Quench

After the free and total chlorine concentrations were measured on the samples, the chlorine was quenched using a sodium thiosulfate ($Na_2S_2O_3$) solution. The quench solution is described in Standard Method 9060A (APHA *et al.*, 1998) for anhydrous sodium thiosulfate. A 3% solution is prepared by dissolving 3 g of sodium thiosulfate in 100 mL of E-pure water. For this research, $Na_2S_2O_3 \cdot 5H_2O$ was used. In this case, 4.7069 grams of the solid chemical was required to make a 3% solution. The solution was stored at 4°C. According to Standard Methods, 0.1 mL of the 3% sodium thiosulfate solution will quench up to 5 mg/L of residual chlorine in 120 mL of sample. This quantity was adjusted to account for the 300 mL samples and the maximum concentration of chlorine that could be present based on the chlorine doses used. Therefore, 0.3 mL was used to quench samples dosed with 6 mg/L of chlorine and 0.5 mL was used to quench samples dosed with 10 mg/L of chlorine.

3.3.5 DPD Indicator Solution

DPD indicator solution turns pink in the presence of chlorine, with the color development proportional to the chlorine concentration in the sample. By either titrating back to a clear color or measuring the absorbance of the colored sample, the chlorine concentration was determined. This solution was purchased from Fisher Scientific and stored in the refrigerator for 2 months before being replaced (Catalog #265516).

3.3.6 DPD Buffer Solution

When measuring free and total chlorine, DPD buffer solution as described by Standard Method 4500-Cl F was required (APHA *et al*., 1998). The solution was used to

buffer samples to a pH of 6.2 to 6.5. To prepare this solution, 24 g of anhydrous Na_2HPO_4 and 46 g of anhydrous KH_2PO_4 were measured on an analytical balance and dissolved in approximately 200 mL of E-pure water. A separate 100 mL volume of E-pure water was measured and then 800 mg of disodium ethylenediamine tetrascetate dihydrate (EDTA) was dissolved in this volume. Next, these two solutions were combined in a 1 L volumetric flask and the entire solution was diluted to a total volume of 1 L with E-pure water. This solution was stored at 4°C in the refrigerator. The shelf life of this solution is approximately 3 months.

3.3.7 Ferrous Ammonium Sulfate (FAS) Titrant

The titrant for the titrimetric measurement of free and total chlorine is ferrous ammonium sulfate (FAS), which was prepared in accordance with Standard Method 4500-C F (APHA *et al.*, 1998). To prepare this solution, 1 mL of 1+3 H_2SO_4 was mixed with approximately 100 mL E-pure water. Next, 1.106 g of $Fe(NH_4)_2(SO_2)_4 \bullet 6H_2O$ was dissolved in the solution containing 1+3 H_2SO_4 and the solution was diluted up to 1 L with E-pure water. The FAS titrant was stored in the refrigerator at a temperature of 4°C for no more than 1 month.

3.4 Analytical Methods for THMs

The following sections detail the methods used to extract, preserve and analyze THMs in chlorinated samples. All glassware used in THM analyses was bathed for at least 1 hour in a 20% sulfuric acid bath. The glassware was then rinsed in E-pure water prior to use. Acid washed glassware included 40 mL vials, beakers of various sizes and flasks.

3.4.1 Trihalomethane Measurement by EPA Method 551

EPA Method 551 as modified by Reckhow (2006) at the University of Massachusetts Amherst was used to quantify THM concentrations in the experimental

waters. In this method, THMs are extracted into pentane and analyzed using gas chromatography with electron capture detection.

As stated previously, experimental waters were chlorinated and incubated for 1, 2, 4 or 7 days. After incubation, the free and total chlorine residual were measured and the samples were quenched to prevent further chlorine reactions. At this time, 20 mL of each sample was placed into an acid-washed 40 mL vial using an Eppendorf pipet (Catalog # 2137925). A repeater pipette was used to add 4 mL of pentane with internal standard (IS) (See Section 3.4.3) to each vial (Fisher Scientific, Catalog # 03692178). Next, approximately 15 g of Na_2SO_4 was added to each vial and the vials were capped with screw caps. The vials were shaken vigorously by hand for 15 minutes. Following shaking, Pasteur pipettes were used to transfer 2 mL of the top layer (pentane with IS and extracted THMs) within each vial to GC autosampler vials. One time use GC vials and screw caps were used for all GC analyses (Agilent Technologies, Catalog # 5182-0714 and 5182 0718). All vials were labeled with a black marker and placed in a freezer for no less than 3 hours. Since pentane will not freeze at 0°C, any frozen material in the GC vials after 3 hours was water. If water was found in any GC vials, they were allowed to thaw and the pentane was again drawn off the top and transferred into a new GC vial. This was done to prevent water from being taken up by the GC during analysis.

In addition to the samples, standards to create a calibration curve were also prepared. Standards from 0 to 250 μg/L were used. To prepare THM standards, 20 mL of E-pure water was first added to each 40 mL vial. Then, the appropriate volume of THM Stock II was added to each vial using a glass syringe (see Section 3.4.2). After this step, the standards were extracted in the same manner as experimental waters by adding pentane with internal standard and Na_2SO_4, shaking and transferring the top layer to GC vials.

The extracted THMs (samples and standards) were quantified by an Agilent Technologies 6890N gas chromatograph. The temperature program is shown in Table 3.3 and the parameters used for GC analysis are summarized in Table 3.4. Pentane with IS was placed prior to samples in the GC autosampler sequence to ensure that the GC was stable before reading any samples.

Table 3.3: GC Temperature program

Temperature Program				
Ramp	**°C per Minute**	**Next °C**	**Hold Min**	**Run Total**
Initial		27	10	10
1	3.00	41	6	20.67
2	5.00	81	0	28.67
3	25.00	180	3	35.63
Post		50		

Table 3.4: Instrument parameters for THM analysis

Parameter	**Units**	**Values**
Injection volume	mL	4
Purge flow to split vent	mL/min	35.8
Heater	°C	175
Pressure	kPa	175
Total flow	mL/min	40.8
Oven set point	°C	27
N_2 Flow		
Pressure	kPa	175
Flow	mL/min	3.4
Average velocity	cm/s	58
Detector		
Heater	°C	275
Makeup flow	mL/min	60
Minimum peak width	min	0.04
Washes	**Pre**	**Post**
Solvent	3	
Solvent A	2	2
Solvent B	2	2
P	4	

3.4.2 THM Stock Solutions

The THM standards were prepared from a commercially available mix: EPA 551A Commercial Mix (Supelco #48046). This mix contains chloroform, bromoform, bromodichloromethane and dibromochloromethane. The concentration of each

compound is 2000 μg/mL. From this mix, THM Stock II solution was prepared. First, about 5 mL of acetone was placed in a 10 mL volumetric flask. Next, 100 μL of 551A commercial THM mix was added and then acetone was added to bring the volume in the flask up to 10 mL. The stock solution was stored in a heavy-walled vial (Supelco Catalog #33299). The Stock II solution had a concentration of 20 μg/mL. This stock solution was used to prepare THM standards for the calibration curve. Equation 7 was used to calculate the required volume of Stock II to create a standard of a particular concentration. For example, a 50 μg/L standard was made by adding 50 μL of Stock II solution to 20 mL of E-pure water.

Equation 7

$$V_{\text{Stock II}} = \frac{C_{\text{std}} \times V_{\text{std}}}{C_{\text{Stock II}}}$$

$$5 \times 10^{-5}\,\text{L or } 50\,\mu L = \frac{50\left(\frac{\mu g}{L}\right) \times 0.02(L)}{20{,}000\left(\frac{\mu g}{L}\right)}$$

3.4.3 Pentane with Internal Standard

The THMs formed in the experimental waters were extracted into pentane. 1, 2 dibromopropane was used as an internal standard as described below, and was added to the pentane as a quality control measure. Since the internal standard concentration was known, its peak on GC analysis outputs always appeared at the same retention time and magnitude. An internal standard stock solution (IS Stock) was used to create the pentane with internal standard solution. To create this stock solution, a 10 mL volumetric flask was partially filled with MtBE and placed on an analytical balance. The reading on the balance was then set to zero. Approximately 5 drops of 1, 2 dibromopropane was added and the weight recorded. The IS Stock was determined using Equation 8. The concentration of the IS stock should be approximately 10 mg/mL. The IS Stock was stored in a heavy-walled extract vial in the refrigerator.

Equation 8

$$\text{C}_{\text{IS Stock}} = \frac{\text{weight IS}\,(g)}{10mL} \times 1000\left(\frac{mg}{g}\right)$$

The IS Stock was then used to prepare the pentane with Internal Standard (IS). A 1 L volumetric flask was first rinsed with HPLC Grade pentane. Next, the flask was filled to 2/3 capacity with HPLC grade pentane. At this point, the required IS Stock needed to prepare a 300 μg/L concentration solution was calculated using Equation 9 and added to the flask. The flask was filled to the mark with HPLC grade pentane, mixed and stored in an amber bottle in a refrigerator.

Equation 9

$$C_{\text{IS Stock}} V_{\text{IS Stock}} = C_{\text{Pentane with IS}} V_{\text{Pentane with IS}}$$

$$V_{\text{IS Stock}} = \frac{0.3\left(\frac{mg}{L}\right) \times 1(L)}{10{,}000\left(\frac{mg}{L}\right)} = 3 \times 10^{-5} \text{ L or } 30\ \mu\text{L}$$

3.4.4 THM Calibration

THM calibration curves were created using the THM standards described in Section 3.4.1. Each time samples were analyzed on the GC new, THM standards were prepared and analyzed. To minimize error in peak areas for standards, several adjustments were made to calibration curve calculations. First, for any THM that the zero standard had a peak area greater than zero, this peak was divided by the peak for the internal standard for the zero standard. This ratio was then used to adjust all of the standards so that the calibration curves would go through the origin (0, 0). For all remaining standards, the peak area was divided by the internal standard peak area and then the ratio calculated for the zero standard was subtracted from this value. Calibration curves were plotted using Microsoft Excel. The concentration of each THM was on the x-axis and the ratio of the THM peak area to the IS peak area was plotted on the y-axis. A trend line was fit to the data points and the equation for the line was used to calculate the concentrations of THMs in experimental samples.

Chapter 4: Results

This section of the report contains the results of laboratory analyses for the quantification of chlorine residuals and THMs. First, the calibration curves for free chlorine and chloroform are presented. Next are the results of the preliminary experiments to determine the appropriate chlorine doses for the experimental waters. Following the preliminary results are the experimental results for sonication experiments. This includes chloroform formation over time for the control and sonicated samples, a comparison of chloroform reductions from sonication and the averages and standard deviations for three data sets, and a statistical analysis of the results.

4.1 Calibration Curves

Chlorine and chloroform concentrations were determined based on comparison to standard curves. Therefore, this section provides a free chlorine calibration curve and a calibration curve for chloroform, as well as information on use of the curves.

4.1.1 Free Chlorine Calibration

Figure 4.1 provides a chlorine calibration curves that was developed on April 5, 2001. Chlorine curves were used to determine free chlorine residuals in experimental waters based on the regression line that was fit to the data in Microsoft Excel, where y is the absorbance and x is the chlorine concentration. As shown in Figure 4.1, a high R^2 value (>0.99) indicates a high degree of linearity in the data. A new set of calibration standards and a calibration curve were created approximately once per week throughout experimentation. The curves were used to measure chlorine concentrations from 0.5 mg/L to 3 mg/L. All chlorine calibration curves and corresponding raw data are presented in Appendix A.

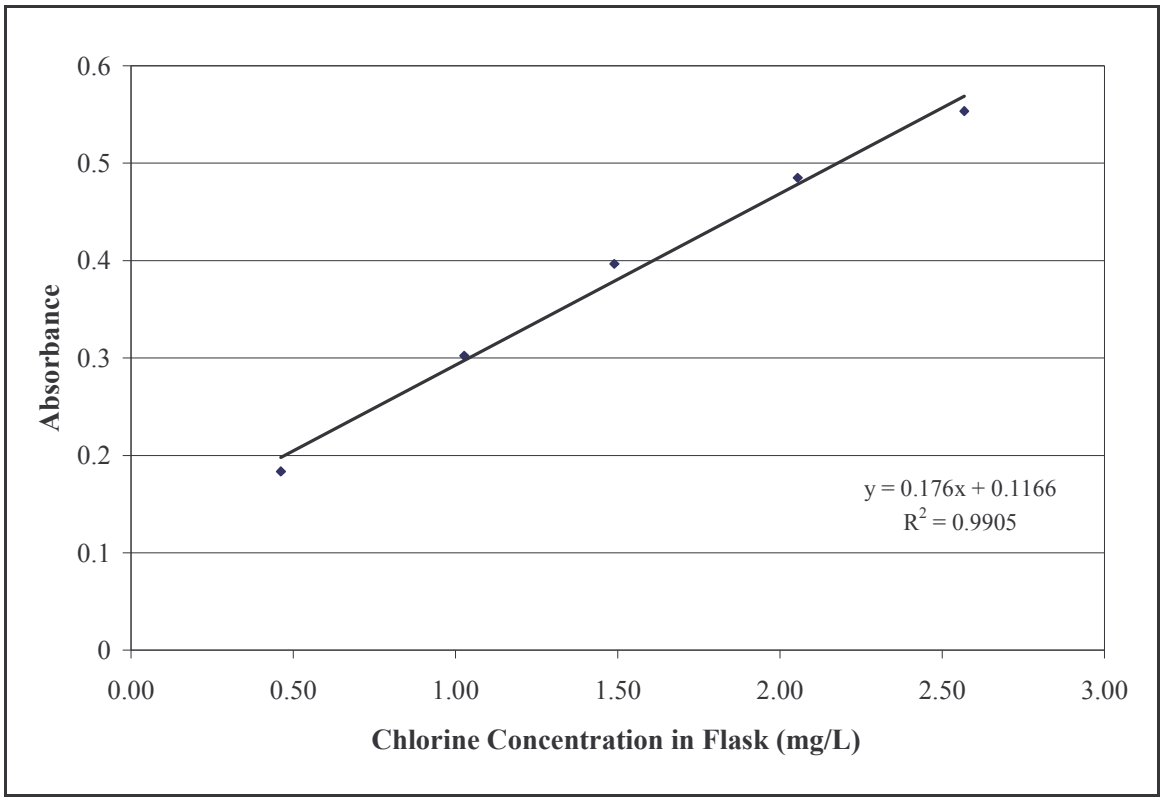

Figure 4.1: Example free chlorine calibration curve from April 5, 2007

4.1.2 Trihalomethane Calibration

As described in Chapter 3, a commercial mix containing all four THMs was used to create THM standard curves. A new set of THM standards was analyzed each time GC analysis was done. A sample standard curve for chloroform is shown in Figure 4.2. The concentration of the chloroform is shown on the x-axis and the peak area ratio of chloroform to the internal standard is plotted on the y-axis. For this calibration curve, the equation for the linear regression line and the R^2 value are displayed on the chart. Figure 4.2 shows the standard curve from April 12, which was completed using seven standards from 0 to 200 μg/L. The high R^2 value (0.9976) demonstrates the linearity of the data. Additional standard THM curves are shown in Appendix B. There is one example curve for each of the three THMs that did not form at detectable levels, dibromochloromethane, bromoform and bromodichloromethane, and all the chloroform curves that were used other than the one from April 12.

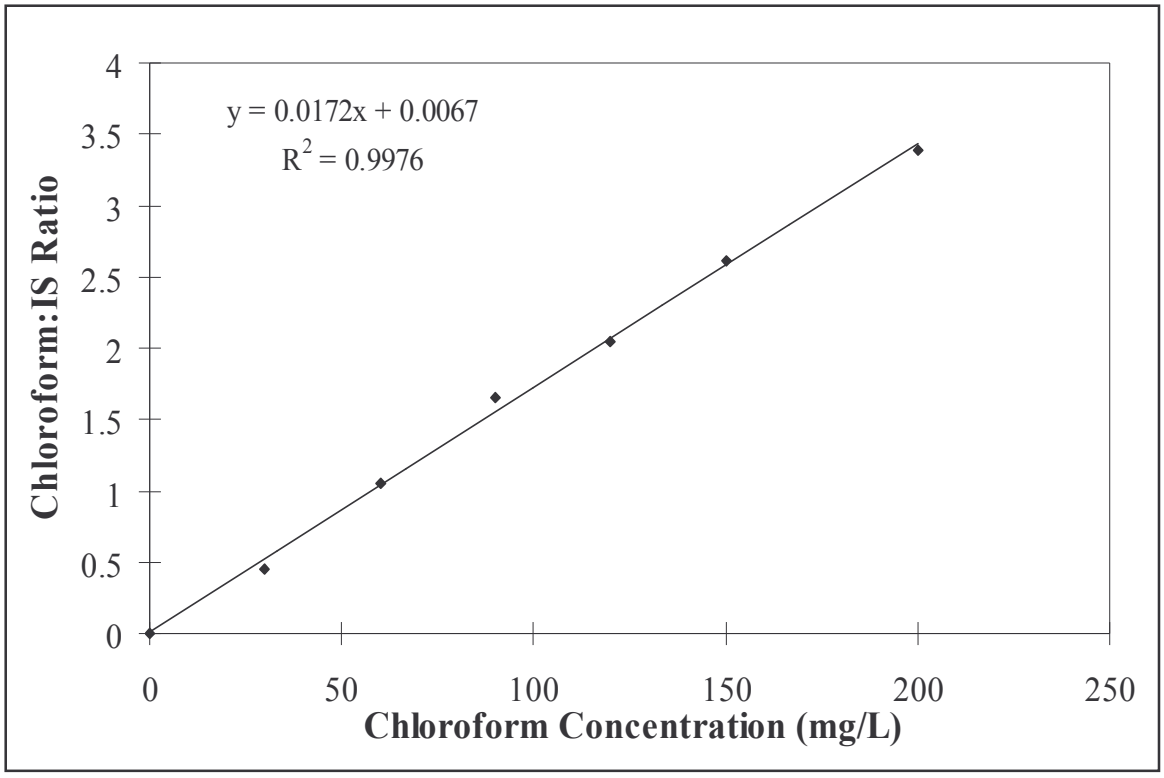

Figure 4.2: Chloroform calibration curve from April 12, 2007

4.2 Preliminary Chlorine Dose Experiments

As described in Chapter 3, preliminary dose experiments were carried out for 25 combinations of NOM concentration and NaOCl dose. Free chlorine residuals were measured for these samples four and seven days after application of the chlorine dose. The residual chlorine measurements showed the 1 mg/L and 2.5 mg/L NOM concentrations to be the most likely to yield a chlorine residual in the range of 2 to 5 mg/L with the chlorine doses that were used. The chlorine residuals at seven days for these two NOM concentrations are shown in Table 4.1. Additional data for NOM doses of 5, 7.5 and 10 mg/L for four and seven days are shown in Appendix A.

For a 1 mg/L NOM concentration with 3 and 7 mg/L chlorine doses, the results have been shaded. The seven day free chlorine residual for a 1 mg/L NOM solution dosed with 3 mg/L of NaOCl is 1.78 mg/L and the seven day free chlorine residual for a 1 mg/L NOM solution dosed with 7 mg/L is 4.82 mg/L. The results for 2.5 mg/L NOM

dosed with 9 mg/L NaOCl has also been shaded. This free chlorine residual is 4.82 mg/L after seven days. These NOM and chlorine dose combinations had results in the desired chlorine residual range. Thus, the appropriate NaOCl to NOM ratio ranged from three to seven. Based on these results, the doses used for sonication experiments were 1 mg/L NOM and 6 mg/L NaOCl with an NaOCl/NOM ratio of 6, and 2.5 mg/L NOM and 10 mg/L NaOCl with an NaOCl/NOM ratio of 4.

Table 4.1: Preliminary chlorine dose results for 1 mg/L and 2.5 mg/L NOM doses

NOM Concentration (mg/L)	NaOCl Dose (mg/L)	Ratios NaOCl/ NOM	Free Chlorine Residual (mg/L)
1	1	1.0	0.00
	3	3.0	1.78
	5	5.0	-0.32
	7	7.0	4.82
	10	10.0	5.44
2.5	2	0.8	0.00
	9	3.6	4.82
	16	6.4	0*
	23	9.2	22.94
	30	12.0	30.00

*Data is missing due to incorrect chlorine dose

4.3 Sonication Experiment Results

This section details the results of sonication experiments. Trihalomethane formation potential was quantified in control samples (0 seconds) and in samples sonicated for 30 seconds to 10 minutes. In all experiments, the only THM produced in detectable concentrations was chloroform. This was an expected result as the experimental water contained no bromide. Since bromide is necessary to form bromoform, bromodichloromethane and dibromochloromethane, chloroform was expected to be the primary compound formed.

Experimental results are presented as follows. First, sample data for chloroform formation over time is presented for both NOM concentrations and for all sonication times tested. This is one complete set of experimental data rather than replicates averaged. Next, selected data from this single data set are used to illustrate the percent reduction in chloroform concentration for sonicated samples versus control samples. Next, the average chloroform formation for each NOM concentration with each sonication time is presented, using all three sets of replicate data. Standard deviations were calculated for the various incubation times, NOM concentrations and sonication times. Lastly, statistical analysis of the replicate data sets is provided.

4.3.1 Experimental Data Set #1—Chloroform Formation

Figure 4.3 shows the chloroform concentration over time for samples with 1 mg/L NOM and 6 mg/L NaOCl. This is for one entire set of experimental data, rather than several averaged together. This graph is shown with the same y-axis scale as Figure 4.4 to show the relative chloroform formation for the two NOM doses tested. This set best shows the THM formation trends that would be expected out of the three data sets.

As would be expected the majority of the chloroform was formed in the first two days of incubation for all data sets. After 1 day of incubation the highest chloroform formation is found in the 60 second sample, 80 μg/L. The other samples are consistent with the idea that increased sonication time results in decreased THM formation. The control sample formed 78 μg/L of chloroform. The samples treated for 30 seconds, 5 minutes and 10 minutes formed 42, 32 and 25 μg/L, respectively. After seven days of incubation, the data do not exhibit this trend so clearly. The control sample final chloroform concentration is 140 μg/L. The 30 second, 5 minute and 10 minute sonication samples formed 52, 45 and 60 μg/L of chloroform respectively. In this case the 10 minute sample has a higher concentration than might be expected; however it is a similar level of reduction to the other sonicated samples (except for 60 seconds). Again the 60 second sample shows less reduction than the other sonicated samples with 115 A more detailed discussion of the relative reduction of chloroform formation among

sonication times is shown in Section 4.3.2. Raw data for Figure 4.3 is available in Appendix C.

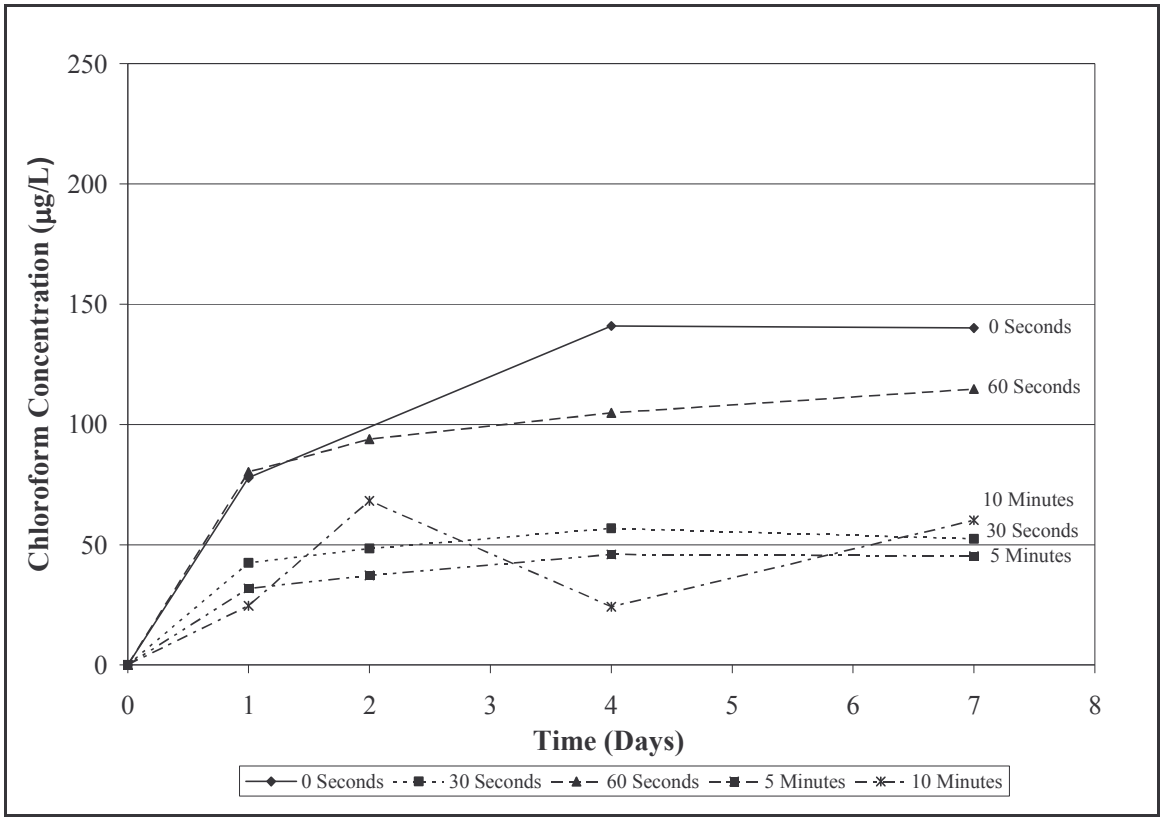

Figure 4.3: Chloroform formation potential with and without sonication for 1 mg/L NOM and 6 mg/L NaOCl

Figure 4.4 shows the chloroform formation over seven days of incubation for samples with 2.5 mg/L NOM and 10 mg/L NaOCl. After one day of incubation, the control concentration for chloroform is 139 µg/L. As with the 1 mg/L NOM dose, the 60 second sample had a high concentration of chloroform at 143 µg/L. The 30 second, 5 minute and 10 minute sonication samples showed reduction from the control concentration with concentrations of 82, 87 and 45 µg/L, respectively. The seven day results for this data show a similar trend to the 1 mg/L NOM samples shown in Figure 4.6 in that the overall reduction of chloroform concentration in the 30 second, 5 minute and 10 minute samples is similar. The seven day concentrations for these samples were 116, 112 and 108 µg/L, respectively, with the seven day control sample concentration being 140 µg/L.

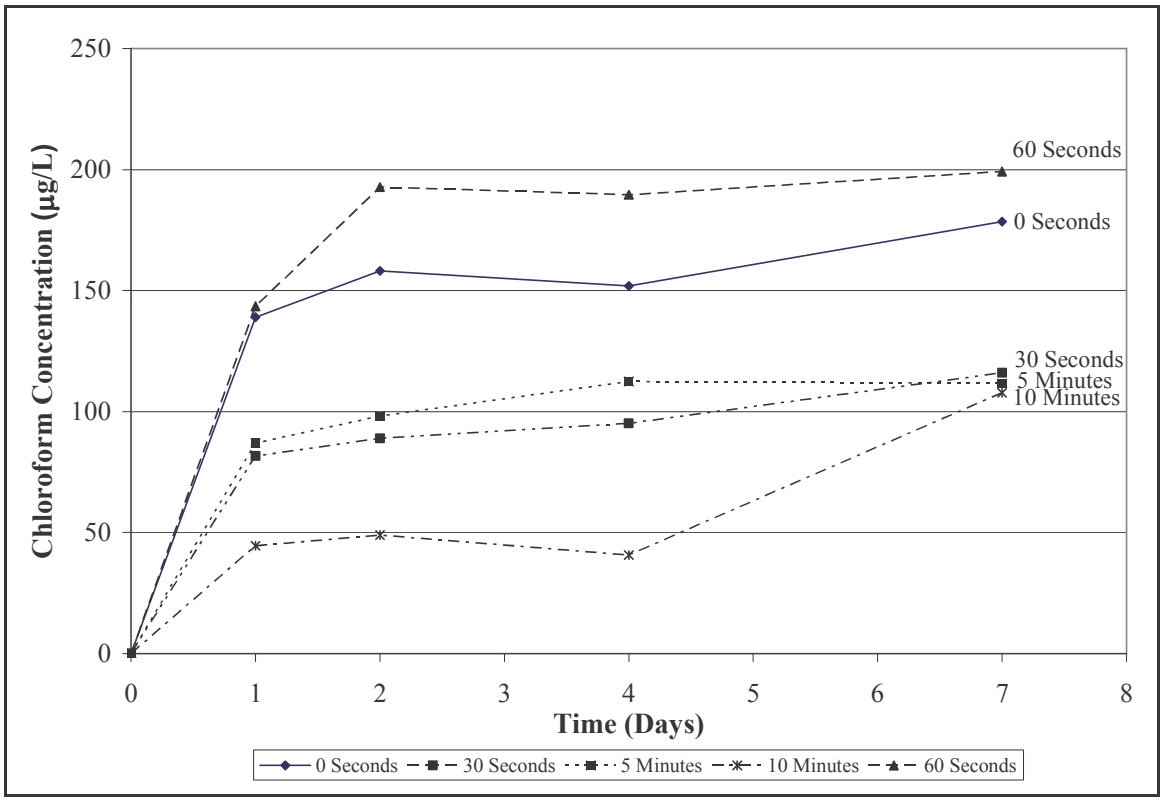

Figure 4.4: Chloroform formation potential with and without sonication for 2.5 mg/l NOM and 10 mg/L NaOCl

It is important to ask why the 60 second samples show less reduction than other sonicated samples. Experimental error is an unlikely cause for this trend as several solutions of experimental water were made separately for these samples. Therefore, an error in NOM concentration or NaOCl concentration is unlikely to appear throughout the data. For both NOM concentrations, sonicating with the 30 seconds, 5 minutes or 10 minutes resulted in decreased chloroform formation potential.

4.3.2 Reduction in Chloroform Formation Potential Using Sonication

The percent reduction in chloroform formation for each sonication time compated to the control was calculated for an incubation time of 1 day. These calculations were completed for data set #1 only (average percent reduction is shown and discussed in Section 4.X). The percent reduction for the 1 mg/L NOM concentration and 6 mg/L NaOCl dose after 1 day of incubation is shown in Table 4.2. The percent reduction from

the control sample for 30 seconds of sonication treatment is 45%. For 5 minutes of sonication, there was a 59% reduction in chloroform formation and for 10 minutes of sonication treatment the reduction is 68%. For 60 seconds of sonication time, the chloroform concentration of 80 μg/L is actually higher than the control concentration of 78 μg/L. These data show the possible reduction in chloroform formation using sonication treatment.

Table 4.2: Percent reduction in chloroform formation by sonication time for 1 mg/L NOM concentration and 6 mg/L NaOCl dose at time = 1 day

Sonication Time (minutes)	Chloroform Concentration (μg/L)	Reduction from Control (%)
0.0	78	0
0.5	42	45
1.0	80	-3
5.0	32	59
10.0	25	68

The same data for a dose of 2.5 mg/L NOM and 10 mg/L NaOCl are shown in Table 4.3. As with the 1 mg/L NOM dose, the reduction in chloroform concentration for 10 minutes of sonication treatment is 68%, and there was no reduction for 60 seconds of treatment. Approximately 40% reduction from the control was shown in the 30 second and 5 minute samples. These data were shown because the percent reductions for 1 mg/L NOM doses and 2.5 mg/L doses were comparable.

Table 4.3: Percent reduction in chloroform formation by sonication time for2.5 mg/L NOM concentration and 6 mg/L NaOCl dose at time = 1 day

Sonication Time (minutes)	Chloroform Concentration (mg/L)	Reduction from Control (%)
0.0	139	0
0.5	82	41
1.0	143	-3
5.0	87	37
10.0	45	68

4.3.3 Averages and Standard Deviations

All experiments were conducted in triplicate. These data were used to calculate average chloroform concentrations for all treatment conditions. In addition, the standard deviations of all data were found. Finally, the average percent reduction by time was found for each day and NOM concentration.

Figure 4.5 shows the average chloroform concentrations for all sonication times after one day of incubation. The dark blue bars represent samples with 2.5 mg/L NOM and 10 mg/L NaOCl while the light blue bars represent samples with 1 mg/L NOM and 6 mg/L NaOCl. The average chloroform concentrations for the 1 mg/L NOM and 2.5 mg/L NOM controls were 43 μg/L and 85 μg/L, respectively. The average data for all three sets showed an increase in chloroform formation when samples were treated with 60 seconds of sonication. The average chloroform concentration for samples with 1 mg/L NOM was 49 μg/L and the average chloroform concentration for samples with 2.5 mg/L NOM was 85 μg/L. However, all other sonication times caused a decrease in chloroform concentration. For the 1 mg/L concentration, there was an average chloroform formation reduction of 23%, 42% and 21% from the control for 30 seconds, 5 minutes and 10 minutes of sonication, respectively. For samples with 2.5 mg/L NOM, the percent reductions for 30 seconds, 5 minutes and 10 minutes were very similar at 33%, 27% and 32%, respectively.

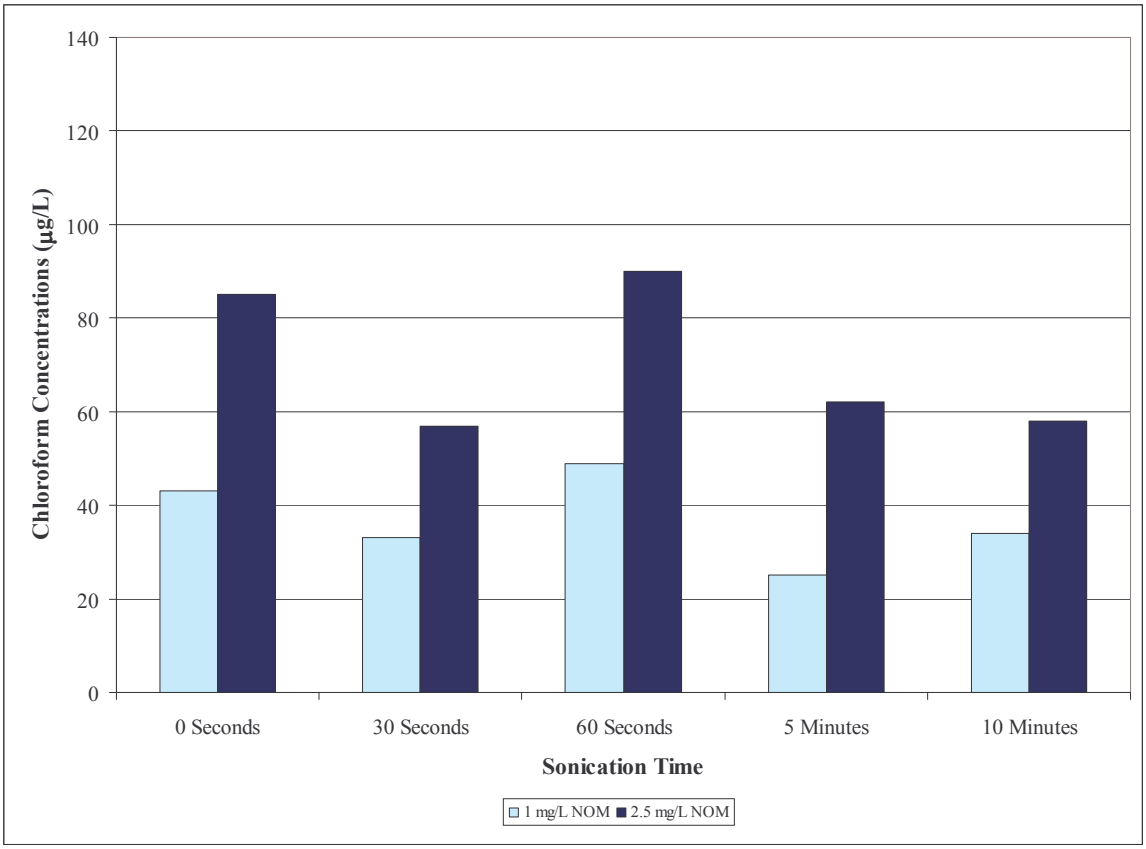

Figure 4.5: Average chloroform concentrations with and without sonication at t = 1 day

Figure 4.6 shows the average chloroform concentrations after seven days of incubation. This figure is shown with the same y-axis scale as the one day results so that it is easy to view the additional chloroform formation between day one and day seven. The chloroform concentrations for 1 mg/L NOM samples in order of increasing sonication time are 87, 53, 69, 48 and 51 mg/L. The chloroform concentrations for 2.5 mg/L NOM samples in order of increasing sonication time are 130, 101, 128, 105 and 105 µg/L. The samples treated with 60 seconds of sonication showed little reduction of chloroform formation compared to the control. All other sonication times (30 seconds, 5 minutes and 10 minutes) yielded similar results, showing approximately 30% reduction in chloroform formation compared to the control sample with no sonication.

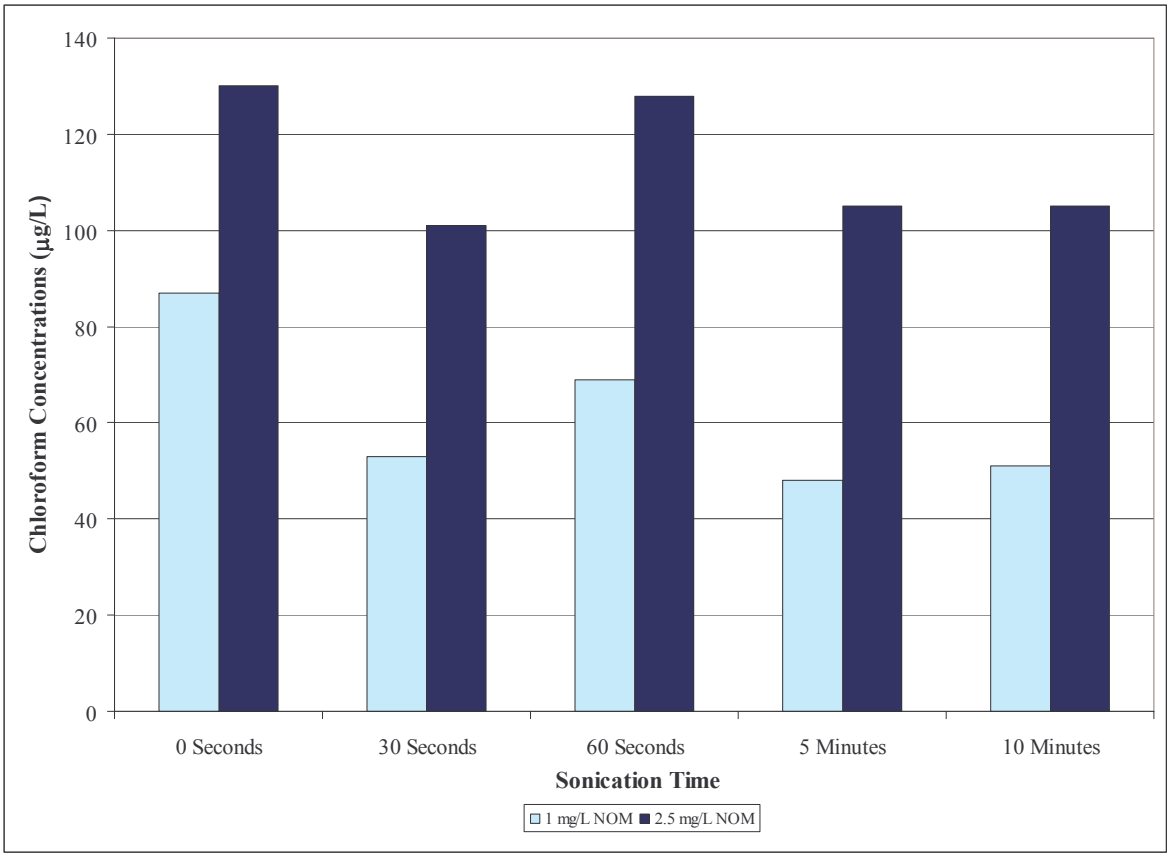

Figure 4.6: Average chloroform concentrations with and without sonication at t = 7 days

Standard deviations for the three data sets were calculated. The average standard deviation for the control data is 67 µg/L. The average standard deviations for 30 second, 5 minute and 10 minute sonication times were 28 µg/L, 32 µg/L and 33 µg/L, respectively. These standard deviations, while higher than desired, are consistent with each other. The standard deviation of the 60 second sonication data was 59 µg/L.

Microsoft Excel was used to perform ANOVA on the data sets to determine statistical differences, if any, between the various treatment times. Results showed that there was no statistical difference among the controls and sonicated samples. The p value for the 1 mg/L NOM concentration data was 0.456. The p value for the 2.5 mg/L NOM concentration data was 0.591. An acceptable p value for this study is 0.05. Lack of statistical differences is likely due to variability in the data resulting in high standard deviations. This result supports the need for more precise replicate data sets. Raw data for these statistical analyses is shown in Appendix D.

Chapter 5: Conclusions and Recommendations

The conclusions are recommendations for future research based on the results of this study are presented in the following sections.

5.1 Conclusions

This research studied the formation of trihalomethanes in water disinfected with sonication, and compared the results to control samples without sonication. Experimental samples with 1 or 2.5 mg/L NOM were sonicated for 30 seconds, 60 seconds, 5 minutes or 10 minutes. After treatment, samples were chlorinated and incubated in the dark at 25 °C and analyzed for THM formation after 1, 2, 4 and 7 days. Based on the experimental results of this study, the conclusions are as follows:

1. Application of ultrasound waves reduces the formation of chloroform compared to control samples without sonication. As dibromochloromethane, bromoform or bromodichloromethane were not formed, no conclusions can be drawn about those three compounds.
2. Sonication when applied for 30 seconds, 5 minutes or 10 resulted in approximately 30, 32 and 31 % reduction of chloroform formation, respectively. Sonication for 60 seconds showed no significant change reduction in chloroform formation compared to control samples.
3. Due to variability in experimental results, statistical differences between control and sonicated samples were not found.

5.1 Recommendations

Due to the limited scope of this research and issues with data precision, it is recommended that additional research is conducted. Specific recommendations are:

1. Utilize sample storage method as described in EPA Method 551 as modified by UMass Amherst. Some experimental error may have resulted from extracting THMs in samples from the same experiment on different days and into different solutions of pentane with internal standard. For more controlled conditions, the

samples for the same experiment for days 1, 2, 4 and 7 should be contained in the same mixture of solvent.

2. Obtain a sonication machine that can apply a consistent power output for several hours of use in one day. As samples were sonicated for 30 seconds or more, the power output began to decrease. Therefore, it was necessary to wait in between sonicating samples and this was time consuming.

3. Repeat all experiments done in this study, with particular concern for the controls and the 60 second samples.

4. Conduct experiments with water containing bromine to analyze the production of all four THMs and their relative concentrations formed in experimental water.

5. Conduct experiments with samples of water from natural drinking water supplies after completion of additional experiments with the laboratory prepared waters.

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Appendices

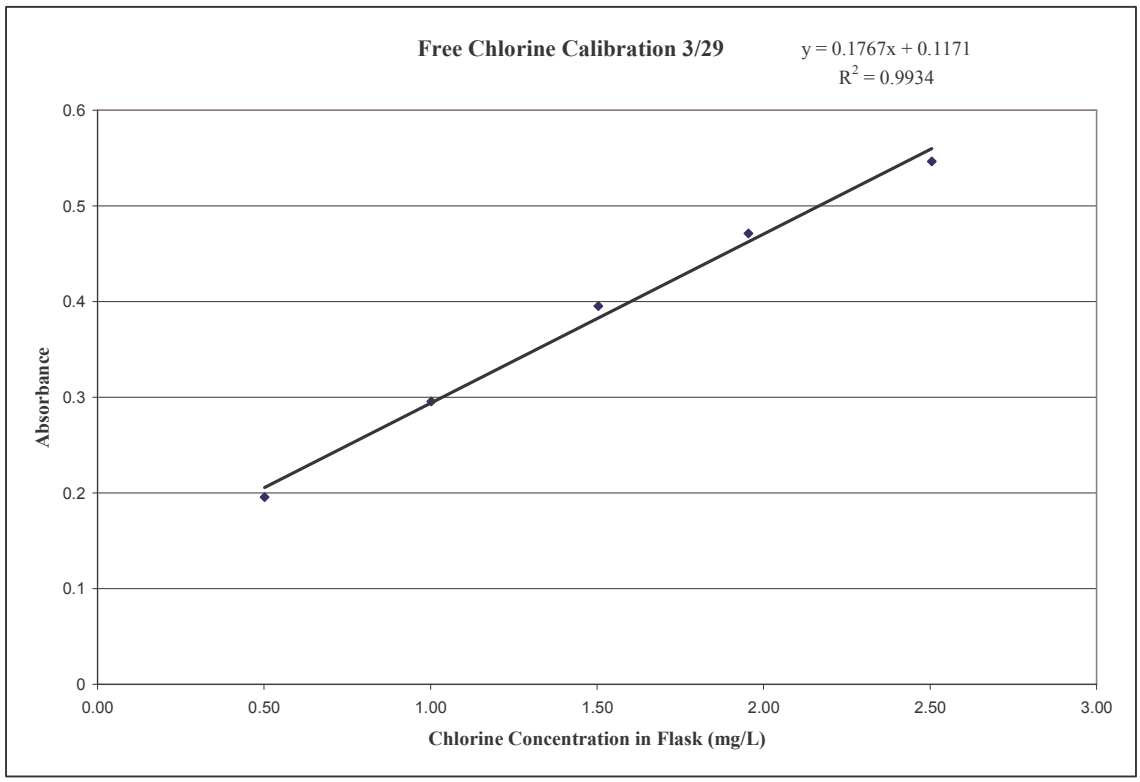
Appendix A: Chlorine Analyses

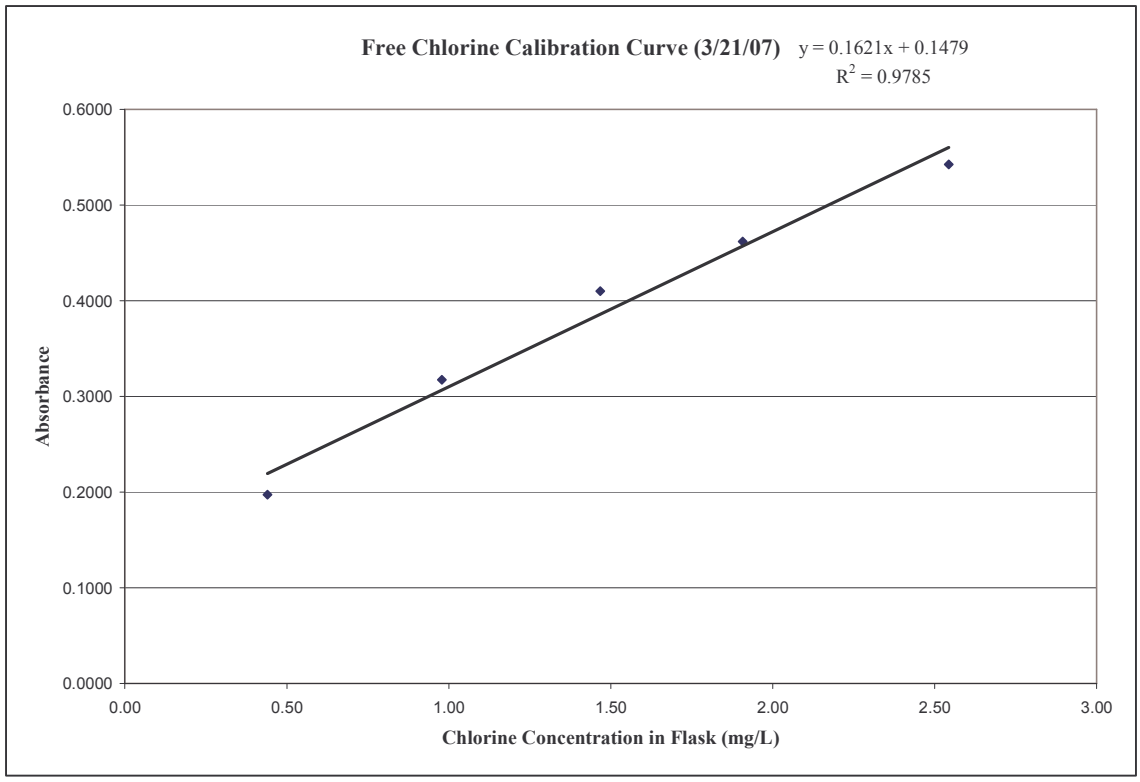
Preliminary Chlorine Dose Experiments Raw Data

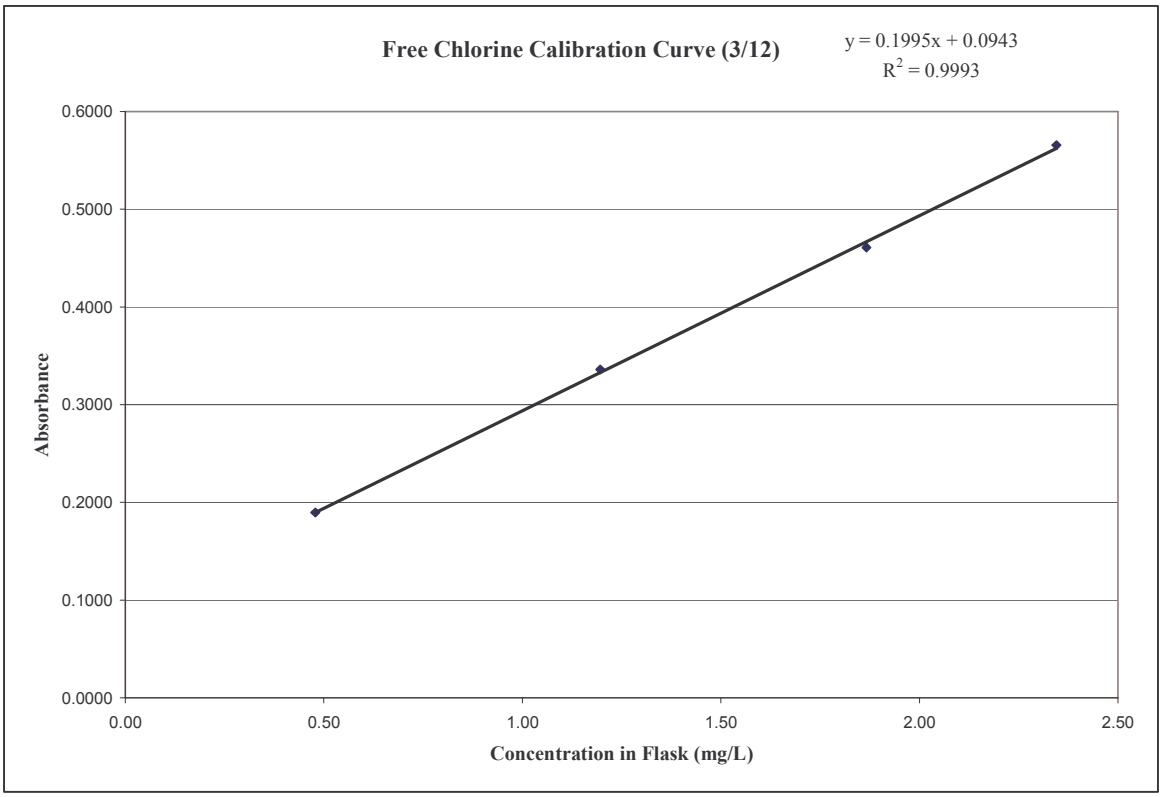
	26-Feb	Free Chlorine		Ratios			1-Mar	Free Chlorine		Ratios
NOM	NaOCl Dose	Absorbance	mg/L	NaOCl/ NOM		NOM	NaOCl Dose	Absorbance	mg/L	
1	1	0.0489	0	1.0		1	1	0.0537	0	1.0
	3	0.2963	1.35	3.0			3	0.3562	1.78	3.0
	5	0.4898	2.73	5.0			5	0.0609	-0.32	5.0
	7	0.7433	4.52	7.0			7	0.7846	4.82	7.0
	10	0.7980	4.91	10.0			10	0.8717	5.44	10.0
2.5	2	0.0527	0	0.8		2.5	2	0.0638	0	0.8
	9	0.6834	4.10	3.6			9	0.7846	4.82	3.6
	16	0.0487	0	6.4			16	0.0648	0	6.4
	23	0.6330	18.71	9.2			23	0.4290	22.94	9.2
	30	0.8160	25	12.0			30	0.5215	30	12.0
5	5	0.1568	0.36	5.0		5	5	0.0794	-0.19	1.0
	15	0.8482	5.27	15.0			15	0.8800	5.49	3.0
	25	0.6735	20.15	25.0			25	0.4341	23.30	5.0
	35	0.5563	32	35.0			35	0.4362	33	7.0
	50	0.7176	43	50.0			50	0.5111	48	10.0
7.5	10	0.2716	1.18	1.3		7.5	10	0.3649	1.84	1.3
	30	0.4347	23.34	4.0			30	0.4301	23.02	4.0
	45	0.6394	38	6.0			45	0.4576	42	6.0
	65	0.6704	57	8.7			65	0.4827	67	8.7
	80	0.7828	69	10.7			80	0.4796	66	10.7
10	10	0.1010	0	1.3		10	10	0.0683	0	1.3
	30	0.4538	2.47	4.0			30	0.0505	-0.39	4.0
	50	0.2944	19.11	6.7			50	0.4587	35.77	6.7
	70	0.6542	56	9.3			70	0.5454	62	9.3
	100	0.8260	51	13.3			100	0.6325	93	13.3

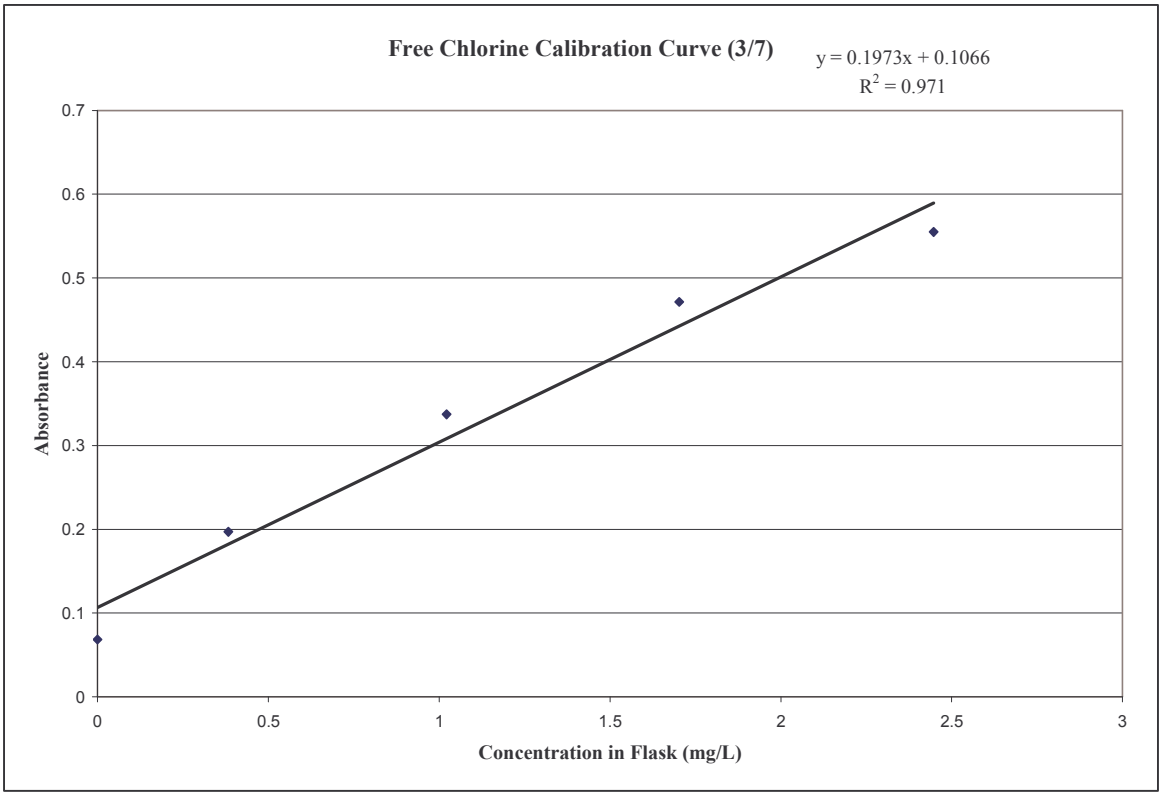
Free Chlorine Calibration Raw Data

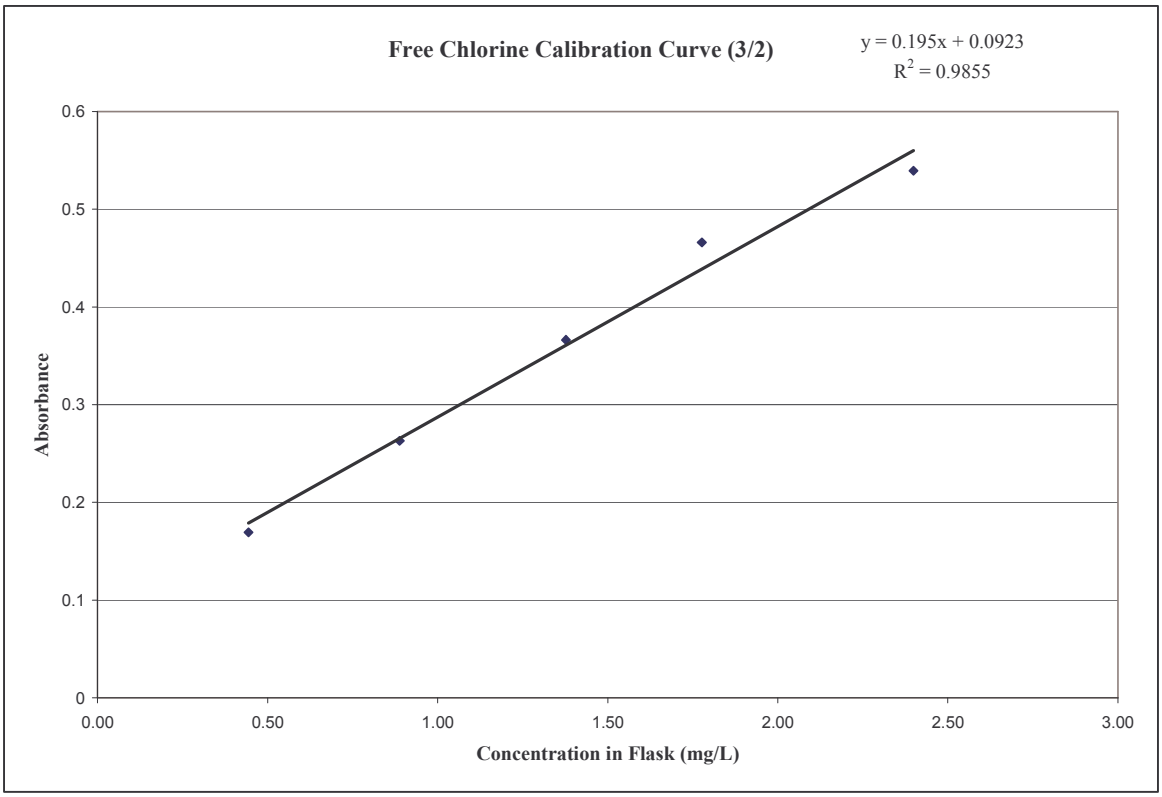
5-Apr	Std	Absorbance	NaOCl Added	Titrant	Concentration	Concentration in Flask
			µL	mL	mg/mL	mg/L
	1	0.1837	0.90	0.50	55.56	0.46
	2	0.3022	2.00	1.05	52.50	1.03
	3	0.3966	2.90	1.50	51.72	1.49
	4	0.4851	4.00	2.00	50.00	2.05
	5	0.5535	5.00	2.35	47.00	2.57
				Average =	51.36	
29-Mar	1	0.1957	1.00	0.55	55.00	0.50
	2	0.2957	2.00	0.95	47.50	1.00
	3	0.3951	3.00	1.50	50.00	1.50
	4	0.4713	3.90	1.95	50.00	1.95
	5	0.5465	5.00	2.40	48.00	2.51
				Average =	50.10	
21-Mar	1	0.1974	0.90	0.75	83.33	0.44
	2	0.3173	2.00	1.15	57.50	0.98
	3	0.4100	3.00	1.50	50.00	1.47
	4	0.4618	3.90	1.75	44.87	1.91
	5	0.5423	5.20	2.25	43.27	2.54
				Average =	48.91	
12-Mar	1	0.1895	1.00	0.55	55.00	0.48
	2	0.3360	2.50	1.15	46.00	1.20
	3	0.4608	3.90	1.80	46.15	1.87
	4	0.5655	4.90	2.17	44.29	2.35
				Average =	47.86	
7-Mar	0	0.0684	0.00	0.00	0	0
	1	0.1971	0.90	0.50	55.56	0.38
	2	0.3372	2.40	0.65	27.08	1.02
	3	0.4713	4.00	1.80	45.00	1.70
	4	0.5550	5.75	2.45	42.61	2.45
				Average =	42.56	
2-Mar	1	0.1693	1.00	0.50	50.00	0.44
	2	0.2630	2.00	0.90	45.00	0.89
	3	0.3662	3.10	1.35	43.55	1.38
	4	0.4660	4.00	1.75	43.75	1.78
	5	0.5394	5.40	2.15	39.81	2.40
				Average =	44.42	
February II	1	0.1953	1.30	0.60	46.15	0.59
	2	0.2648	1.90	0.95	50.00	0.87
	3	0.3422	3.00	1.30	43.33	1.37
	4	0.5145	5.30	2.30	43.40	2.42
				Average =	45.72	

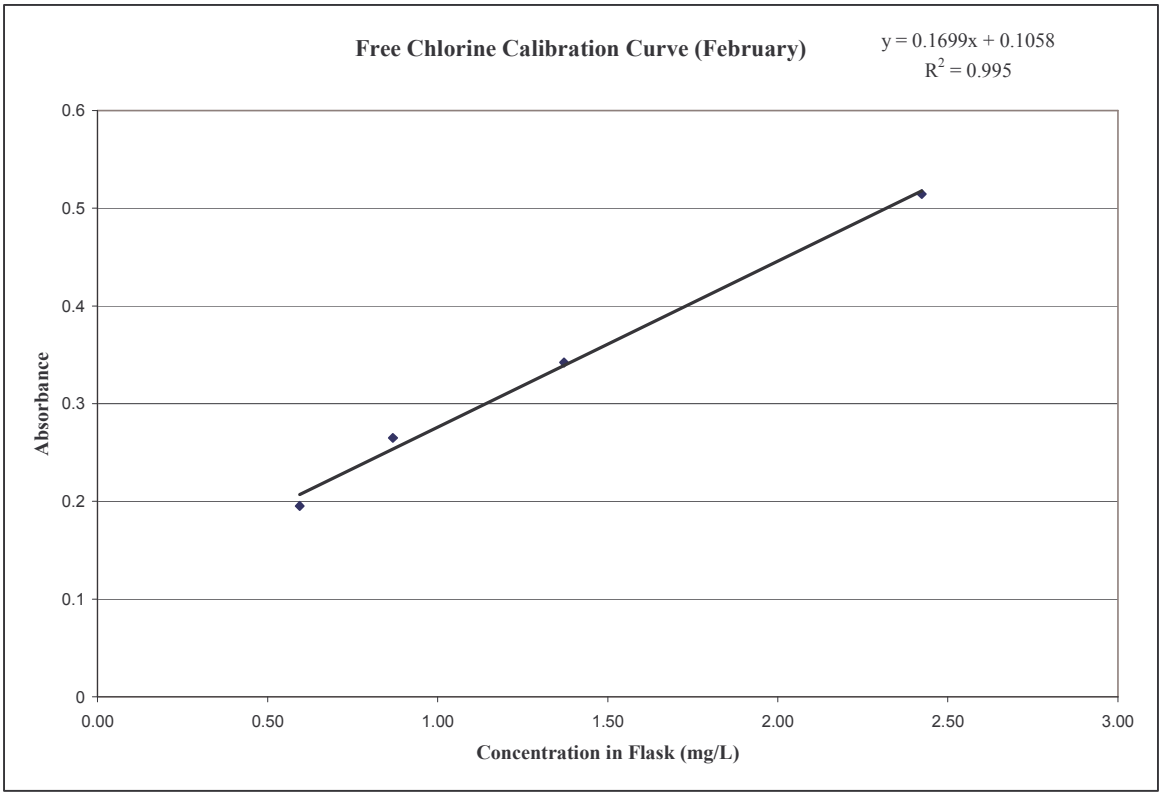

Free Chlorine Calibration 3/29
y = 0.1767x + 0.1171
R2 = 0.9934
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
0.00
0.50
1.00
1.50
2.00
2.50
3.00
Chlorine Concentration in Flask (mg/L)


Free Chlorine Calibration Curve (3/21/07)
y = 0.1621x + 0.1479
R2 = 0.9785
Absorbance
0.6000
0.5000
0.4000
0.3000
0.2000
0.1000
0.0000
0.00
0.50
1.00
1.50
2.00
2.50
3.00
Chlorine Concentration in Flask (mg/L)


Free Chlorine Calibration Curve (3/12)
y = 0.1995x + 0.0943
R2 = 0.9993
Absorbance
0.6000
0.5000
0.4000
0.3000
0.2000
0.1000
0.0000
0.00
0.50
1.00
1.50
2.00
2.50
Concentration in Flask (mg/L)


Free Chlorine Calibration Curve (3/7)
y = 0.1973x + 0.1066
R2 = 0.971
Absorbance
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.5
1
1.5
2
2.5
3
Concentration in Flask (mg/L)

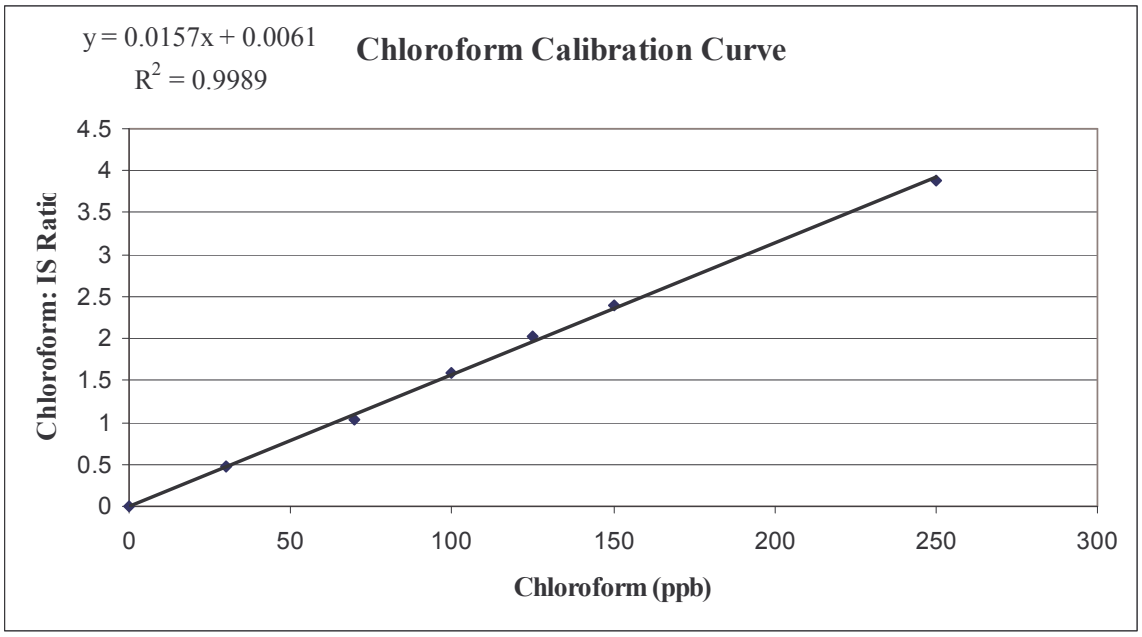

Free Chlorine Calibration Curve (3/2)
y = 0.195x + 0.0923
R2 = 0.9855
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
0.00
0.50
1.00
1.50
2.00
2.50
3.00
Concentration in Flask (mg/L)


Free Chlorine Calibration Curve (February)
y = 0.1699x + 0.1058
R2 = 0.995
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
0.00
0.50
1.00
1.50
2.00
2.50
3.00
Concentration in Flask (mg/L)

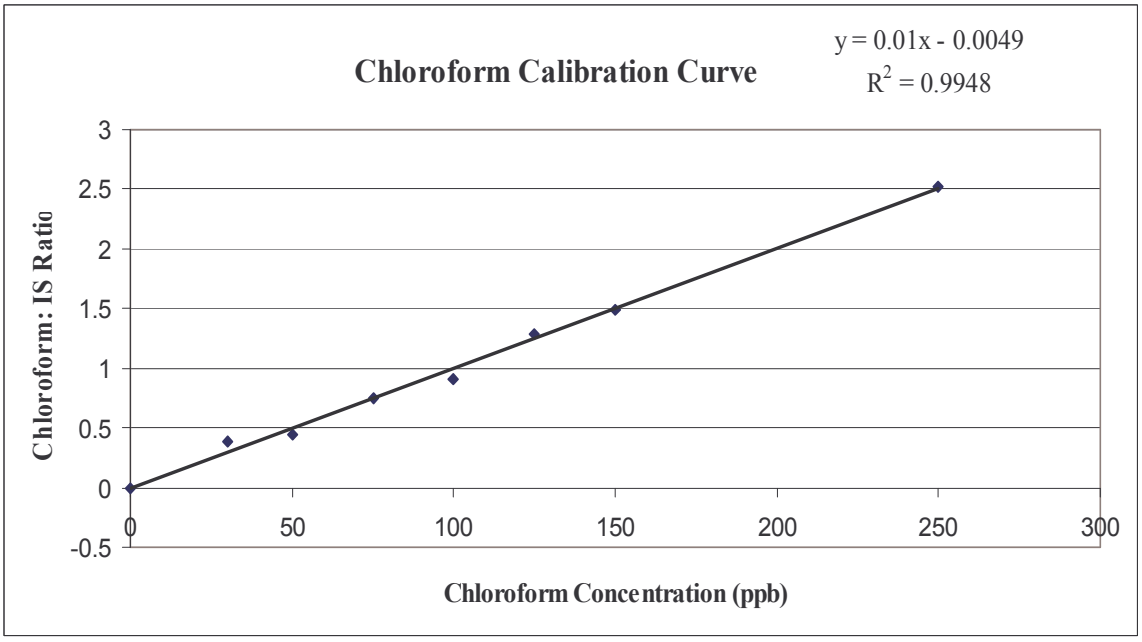
Appendix B: THM Calibration Curves

Chloroform Calibration Curves

March 30

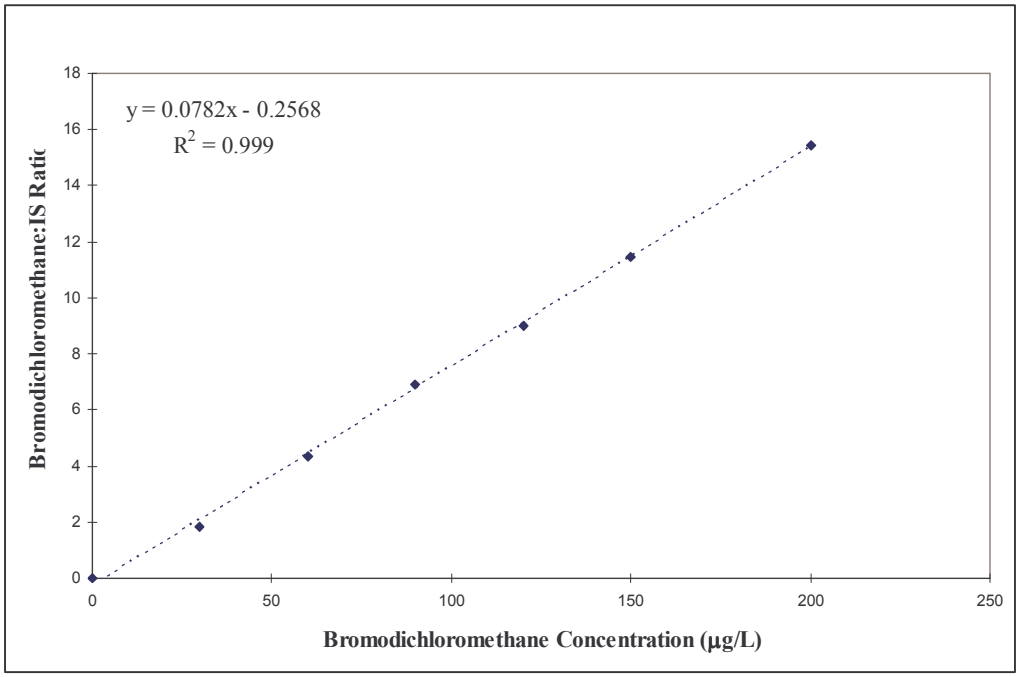


March 23



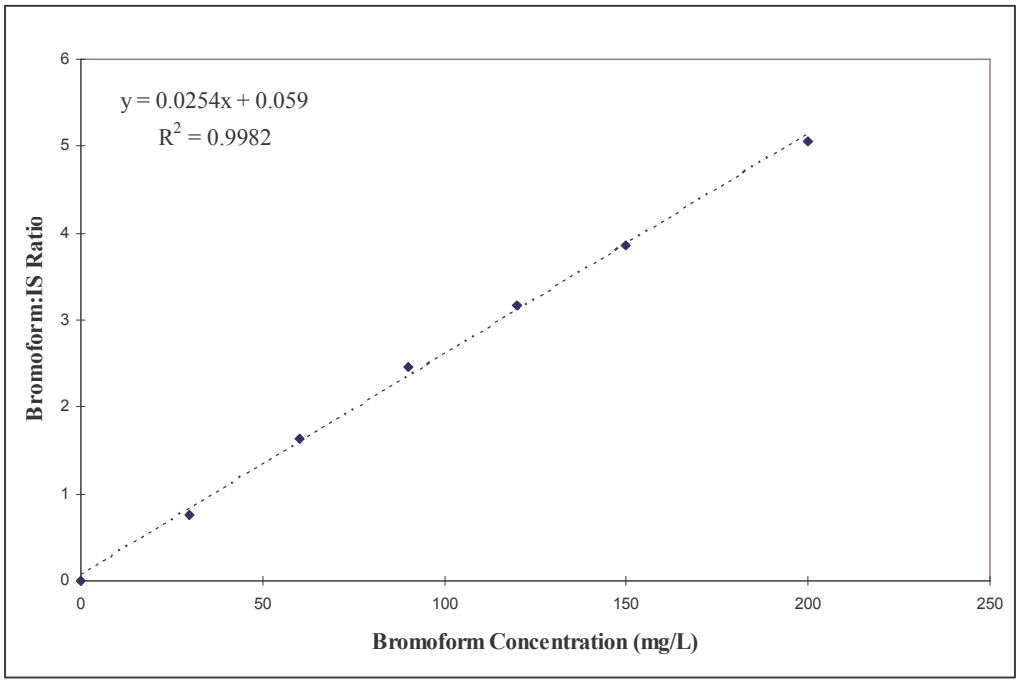
Bromodichloromethane Calibration Curve

April 12



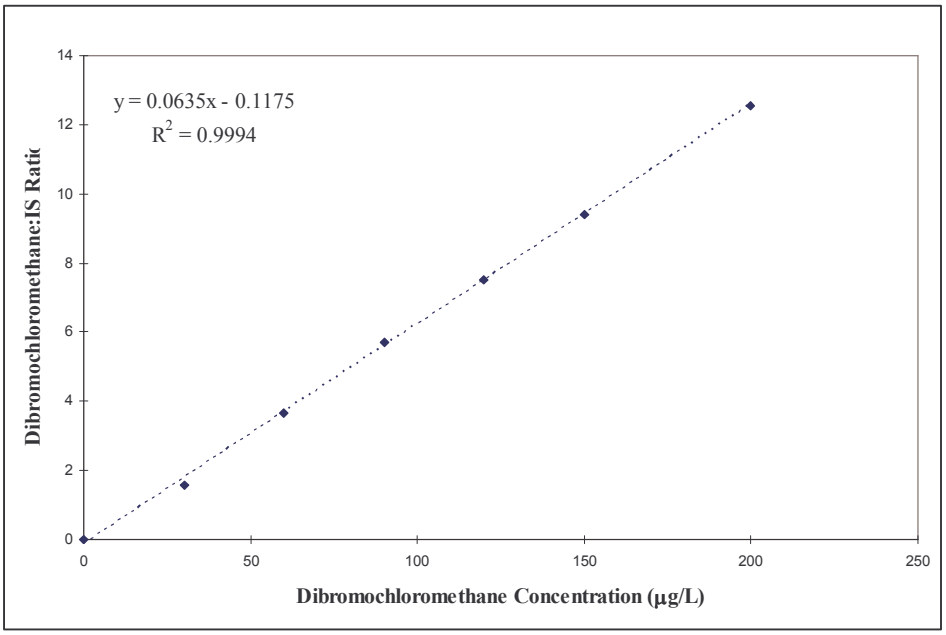
Bromoform Calibration Curve

April 12, 2007



Dibromochloromethane Calibration Curve

April 12, 2007



Appendix C: THM Analyses

Raw Data

Control Data												
		1	**2**	**4**	**7**				**1**	**2**	**4**	**7**
1/6	IS	887.97	-	1014.37	1032.96		**2.5/10**	IS	929.12	929.33	875.94	1032.96
	Chloroform	686.74	-	1424.23	1441.85			Chloroform	1286.93	1464.51	1326.79	1441.85
		0.773382	#VALUE!	1.4040537	1.395843				1.385106	1.575877	1.5147042	1.395843
		78		141	140				139	158	152	140
1/6	IS	1693.79	1918.56	2609.62	**807.18**		**2.5/10**	IS	1654.06	1909.41	2572.28	807.64
	Chloroform	513.17	487.86	598.53	757.16			Chloroform	1169.67	519.53	1156.61	1437.50
		0.30	0.25	0.23	0.94				0.71	0.27	0.45	1.78
		31	26	23	59.36				71	28	45	112.98
1/6	IS	1297.33	1399.98	2013.29	**760.62**		**2.5/10**	IS	1287.95	1413.67	2012.08	**768.71**
	Chloroform	424.62	505.73	717.60	812.24			Chloroform	940.32	1279.06	1593.86	1814.14
		0.33	0.36	0.36	1.07				0.73	0.90	0.79	2.36
		20.46	22.62	22.31	61.70				46.11	57.24	50.07	136.82

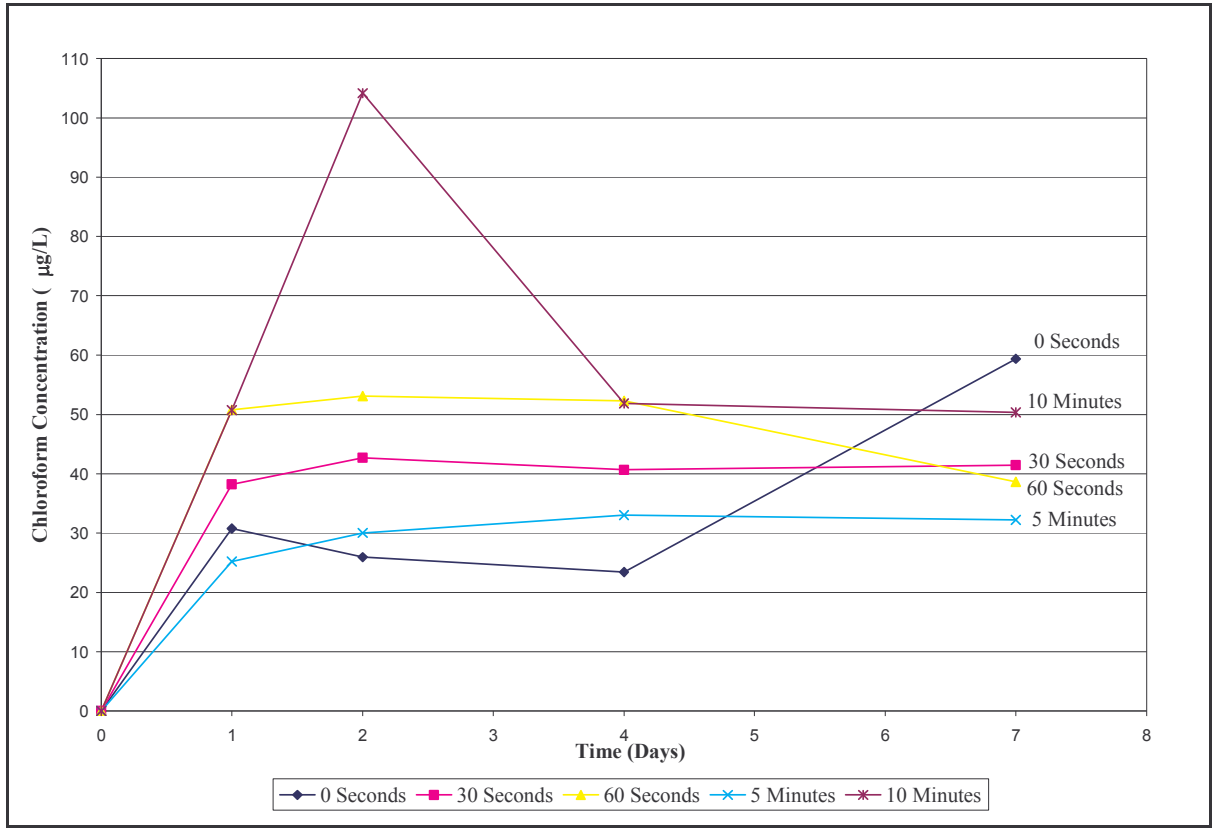
30 Second Data												
		1	**2**	**4**	**7**				**1**	**2**	**4**	**7**
1/6	IS	1208.47	1148.82	1349.7	1417.24		**2.5/10**	IS	1160.68	1222.63	1326.02	1403.99
	Chloroform	507	550.74	760.55	736.19			Chloroform	941.24	1081.56	1254.74	1621.84
		0.419539	0.4793963	0.5634956	0.5194533				0.810938	0.884618	0.9462452	1.1551649
		42	48	57	52				82	89	95	116
1/6	**Peak**	**1**	**2**	**4**	**7**		**2.5/10**	**Peak**	**1**	**2**	**4**	**7**

	IS	780.4	859.46	924.43	1055.82			IS	789.33	829.77	878.83	1064.44
	Chloroform	473.07	581.34	595.64	693.19			Chloroform	704.58	982.85	1205.86	1510.55
		0.606189	0.6764015	0.6443322	0.6565418				0.89263	1.184485	1.3721198	1.419103
		38	43	41	41				56	75	87	90
1/6	**Peak**	**1**	**2**	**4**	**7**		**2.5/10**	**Peak**	**1**	**2**	**4**	**7**
	IS	1507.75	1710.52	**726.05**	**797.92**			IS	1511.60	1722.69	**743.65**	**820.30**
	Chloroform	447.57	584.35	731.73	879.03			Chloroform	759.69	902.70	1222.30	1373.25
		0.30	0.34	1.01	1.10				0.50	0.52	1.64	1.67
		18.52	21.37	58.20	63.66				31.62	32.99	95.17	96.94
60 Second Data												
		1	**2**	**4**	**7**				**1**	**2**	**4**	**7**
1/6	IS	908.13	941.27	1005.01	1073.36		**2.5/10**	IS	900.69	968.06	963.3	1132.44
	Chloroform	724.59	878.86	1048.59	1225.92			Chloroform	1287.94	1861.82	1821.5	2250.04
		0.797892	0.933696	1.0433628	1.1421331				1.429948	1.923249	1.8908959	1.9868956
		80	94	105	115				143	193	190	199
	Peak	**1**	**2**	**4**	**7**			**Peak**	**1**	**2**	**4**	**7**
	IS	750.11	899.12	892.9	1053.33			IS	776.43	849.99	900.27	1063.54
	Chloroform	603.06	754.59	738.67	645.09			Chloroform	1147.75	1319.72	1412.17	1388.18
		0.803962	0.8392539	0.8272707	0.6124292				1.47824	1.55263	1.5686072	1.3052447
		51	53	52	39				94	99	100	83
	Peak	**1**	**2**	**4**	**7**			**Peak**	**1**	**2**	**4**	**7**
	IS	1499.80	1710.22	**720.16**	**815.44**			IS	1554.67	1722.74	**724.50**	**808.86**
	Chloroform	397.65	558.74	751.90	754.97			Chloroform	779.10	942.87	1290.20	1464.98
		0.27	0.33	0.55	0.93				0.50	0.55	1.78	1.81
		16.50	20.42	31.43	53.44				31.53	34.47	103.15	104.91
5 Minute Data												

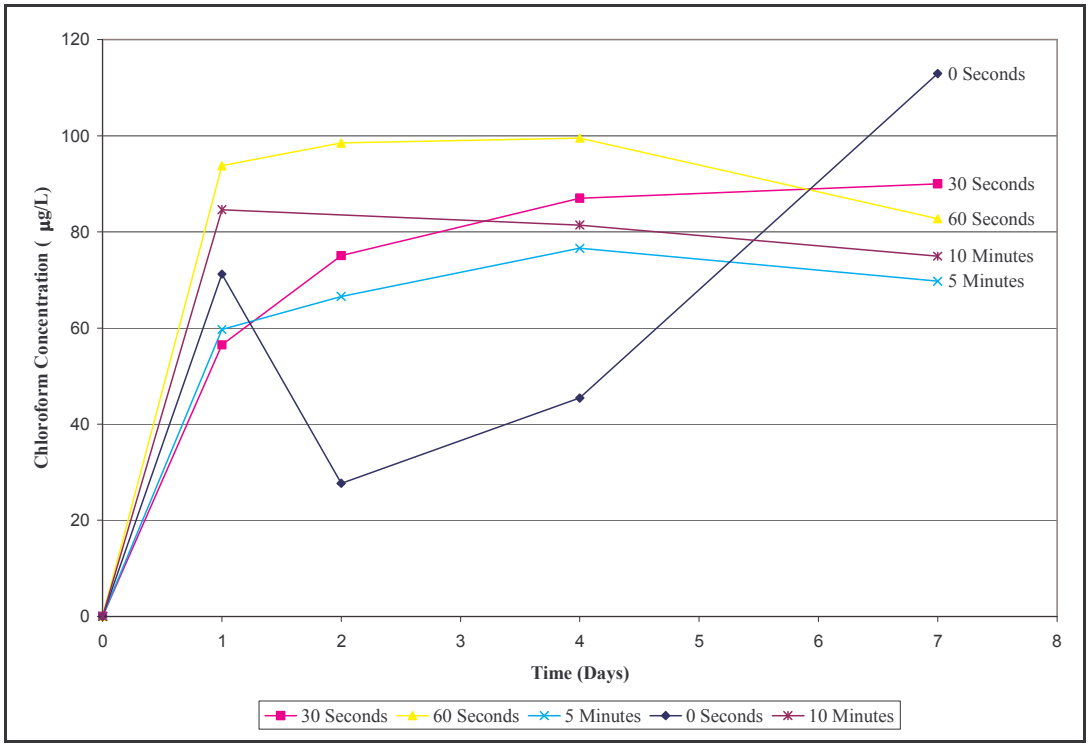
		1	2	4	7				1	2	4	7
1/6	IS	1165.51	1202.1	1295.53	1319		**2.5/10**	IS	1127.89	1186.74	1269.66	1400.91
	Chloroform	365.44	441.84	589.3	591.4			Chloroform	975.28	1159.03	1421.38	1556.03
		0.313545	0.3675568	0.4548718	0.44837				0.864694	0.97665	1.1194966	1.110728
		32	37	46	45				87	98	112	112
1/6	IS	**829.05**	**913.12**	**1027.39**	**1300.30**		**2.5/10**	IS	**818.44**	**920.03**	**1024.00**	**1319.36**
	Chloroform	332.98	436.04	**538.78**	665.95			Chloroform	772.35	967.28	1238.06	1452.72
		0.40	0.48	0.52	0.51				0.94	1.05	1.21	1.10
		25.19	30.03	33.01	32.23				59.72	66.58	76.62	69.74
1/6	IS	1511.94	1702.10	**724.98**	**792.70**		**2.5/10**	IS	1521.51	1729.41	**719.39**	**810.09**
	Chloroform	467.08	512.31	704.69	891.56			Chloroform	952.76	1124.17	1564.11	1855.55
		0.31	0.30	0.97	1.12				0.63	0.65	2.17	2.29
		19.29	18.78	56.12	65.00				39.50	41.01	126.02	132.78
10 Minute Data												
		1	**2**	**4**	**7**				**1**	**2**	**4**	**7**
1/6	IS	1723.8	1889.32	2655.81	**809.39**		**2.5/10**	IS	1667.4	1930.4	2781.49	**812.64**
	Chloroform	416.53	1279.24	629.35	**769.6**			Chloroform	734.61	935.31	1117.8	**1379.28**
		0.241635	0.6770902	0.236971	**0.9508395**				0.440572	0.484516	0.4018709	**1.6972829**
		25	68	24	**60**				45	49	41	**108**
	Peak	**1**	**2**	**4**	**7**			**Peak**	**1**	**2**	**4**	**7**
	IS	766.31	848.3	888.46	1145.41			IS	805.21	-	898.76	1094.81
	Chloroform	615.21	1392.68	728.87	912.16			Chloroform	1074.86	-	1154.57	1295.14
		0.802821	1.6417305	0.8203746	0.7963611				1.334882	-	1.2846255	1.1829815
		51	104	52	50				85	-	81	75
10 Minutes	**Peak**	**1**	**2**	**4**	**7**		**10 Minutes**	**Peak**	**1**	**2**	**4**	**7**

	IS	1333.59	1400.69	2006.11	**776.59**			IS	1318.48	1410.96	2025.36	**771.89**
	Chloroform	579.88	708.84	996.27	1122.82			Chloroform	919.71	1129.31	1454.63	1756.67
		0.43	0.51	0.50	0.72				0.70	0.80	0.72	2.28
		27.31	31.84	31.24	41.37				44.04	50.59	45.36	131.92

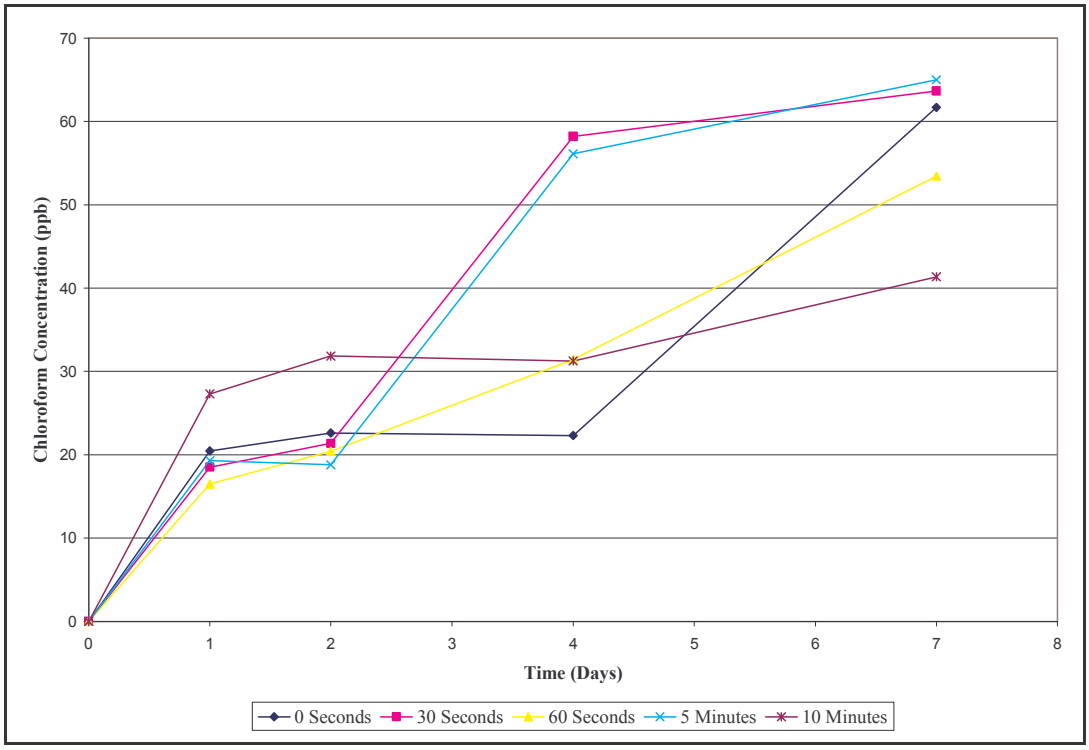
Set 2, 1 mg/L NOM



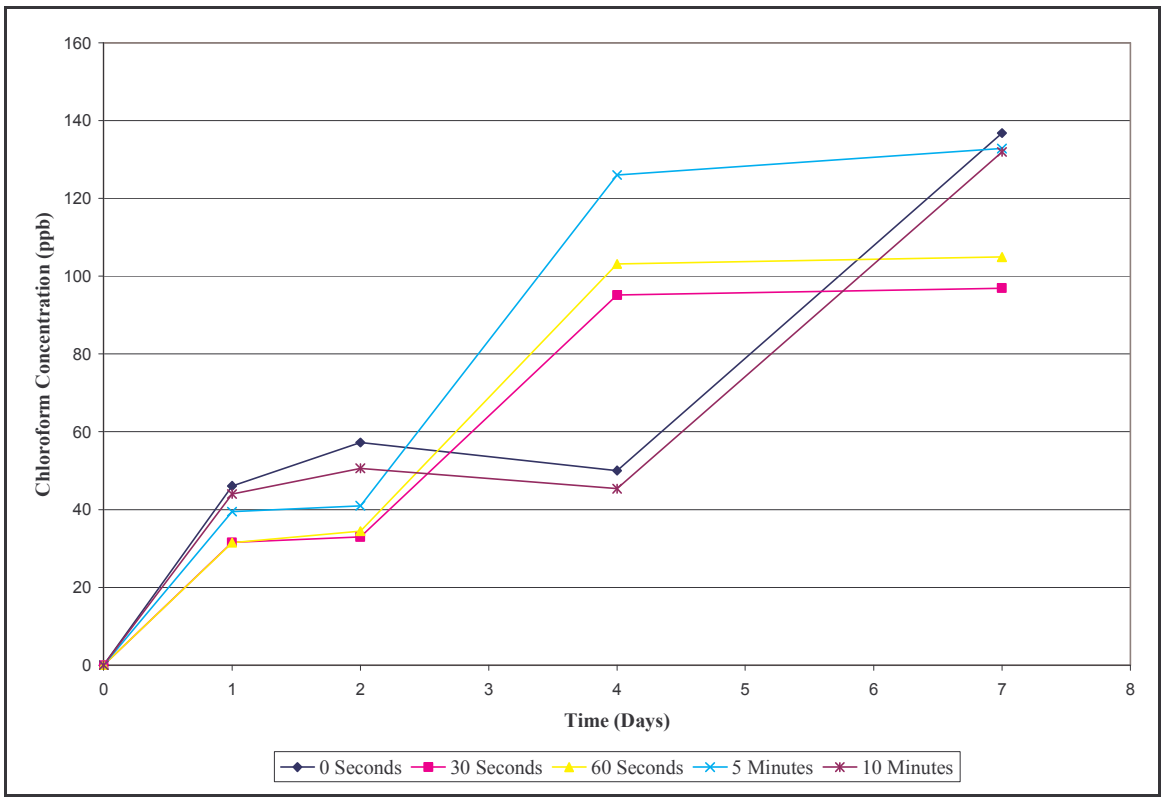
Set 2, 2.5 mg/L



Set 3, 1 mg/L



Set 3, 2.5 mg/L


160
140
120
100
80
60
40
20
0
Chloroform Concentration (ppb)
0
1
2
3
4
5
6
7
8
Time (Days)
0 Seconds
30 Seconds
60 Seconds
5 Minutes
10 Minutes

Appendix D: Statistical Analyses Raw Data

Raw Data for Averages and Standard Deviations Calculations

Average Concentration	Day 1		Day 2		Day 4		Day 7	
	1.0	**2.5**	**1.0**	**2.5**	**1.0**	**2.5**	**1.0**	**2.5**
0 Seconds	43.0	85.0	24.3	81.0	62.2	87.0	87.0	130.0
30 Seconds	33.0	57.0	37.5	65.7	51.9	92.4	53.0	101.0
60 Seconds	49.0	90.0	55.8	108.6	62.9	130.7	68.9	128.0
5 Minutes	25.0	62.0	28.7	68.6	45.0	105.0	48.0	105.0
10 Minutes	34.0	58.0	68.1	49.8	35.8	55.8	51.0	105.0
% Reduction								
10 minutes	20.9	31.8	**-180.5**	38.6	42.5	35.9	41.4	19.2
5 minutes	41.9	27.1	-18.2	15.3	27.6	-20.7	44.8	19.2
30 seconds	23.3	32.9	-54.5	18.9	16.6	-6.2	39.1	22.3

0 Seconds									
	Day 1		**Day 2**		**Day 4**		**Day 7**		
	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	
Average	43.0	85.4	24.3	81.0	62.2	87.0	87.0	130.0	
Standard Deviation	30.6	48.1	2.3	68.4	68.1	45.9	45.9	52.8	
Percent Error	71.1	111.7	9.6	84.4	109.5	52.8	52.8	40.6	66.6
30 Seconds									
	Day 1		**Day 2**		**Day 4**		**Day 7**		
	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	
Average	33.1	56.6	37.5	65.7	51.9	92.4	52.5	101.0	
Standard Deviation	12.8	25.0	14.3	29.1	9.8	4.7	11.1	13.5	
Percent Error	38.6	44.2	38.0	44.4	18.8	5.1	21.2	13.3	27.9

60 Seconds									
	Day 1		Day 2		Day 4		Day 7		
	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	
Average	49.2	89.6	55.8	108.6	62.9	130.7	68.9	128.9	
Standard Deviation	31.9	56.1	36.8	79.7	37.8	51.0	40.3	61.8	
Percent Error	64.9	62.6	66.0	73.3	60.2	39.0	58.5	47.9	59.1
5 Minutes									
	Day 1		Day 2		Day 4		Day 7		
	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	
Average	25.4	62.1	28.7	68.6	45.0	105.0	47.5	104.7	
Standard Deviation	6.3	23.8	9.3	28.6	11.6	25.5	16.5	32.1	
Percent Error	24.7	38.4	32.4	41.7	25.7	24.3	34.7	30.6	31.6
10 Minutes									
	Day 1		Day 2		Day 4		Day 7		
	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	1 mg/L	2.5 mg/L	
Average	34.2	57.7	68.1	49.8	35.8	55.8	50.6	104.9	
Standard Deviation	14.4	23.3	36.2	1.2	14.4	22.3	9.4	28.6	
Percent Error	41.9	40.3	53.1	2.3	40.2	40.0	18.6	27.3	33.0

ANOVA Raw Data

1 mg/L NOM						
Sonication Time	**0**	**30**	**60**	**5**	**10**	
Replicate 1	140	52	115	45	60	
Replicate 2	59.36	41	39	32.23	50	
Replicate 3	61.7	63.66	53.44	65	41.37	
2.5 mg/L NOM						
Sonication Time	**0**	**30**	**60**	**5**	**10**	
Replicate 1	178	116	199	112	108	
Replicate 2	112.98	90	83	69.74	75	
Replicate 3	136.82	96.94	104.91	132.78	131.92	
Anova: Single Factor						
1 mg/L NOM						
SUMMARY						
Groups	***Count***	***Sum***	***Average***	***Variance***		
0	3	261.06	87.02	2106.529		
30	3	156.66	52.22	128.4052		
60	3	207.44	69.14667	1629.025		
5	3	142.23	47.41	272.8243		
10	3	151.37	50.45667	86.92563		
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	3348.098	4	837.0246	0.990864	0.455734	3.47805
Within Groups	8447.418	10	844.7418			
Total	11795.52	14				
Anova: Single Factor						
2.5 mg/L NOM						
SUMMARY						
Groups	***Count***	***Sum***	***Average***	***Variance***		
0	3	427.8	142.6	1081.956		
30	3	302.94	100.98	181.2412		
60	3	386.91	128.97	3798.163		
5	3	314.52	104.84	1031.96		
10	3	314.92	104.9733	816.8421		
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	4039.192	4	1009.798	0.730662	0.591276	3.47805
Within Groups	13820.32	10	1382.032			
Total	17859.52	14				