Characterization of TOX Produced During Disinfection Processes

3rd Progress Report
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CONTENTS

3

LIST OF TABLES

LIST OF FIGURES

8

FOREWORD

The Awwa Research Foundation is a nonprofit corporation that is dedicated to the implementation of a research effort to help utilities respond to regulatory requirements and traditional high-priority concerns of the industry. The research agenda is developed through a process of consultation with subscribers and drinking water professionals. Under the umbrella of a Strategic Research Plan, the Research Advisory Council prioritizes the suggested projects based upon current and future needs, applicability, and past work; the recommendations are forwarded to the Board of Trustees for final selection. The foundation also sponsors research projects through the unsolicited proposal process; the Collaborative Research, Research Applications, and Tailored Collaboration programs; and various joint research efforts with organizations such as the U.S. Environmental Protection Agency, the U.S. Bureau of Reclamation, and the Association of California Water Agencies.

This publication is a result of one of these sponsored studies, and it is hoped that its findings will be applied in communities throughout the world. The following report serves not only as a means of communicating the results of the water industry's centralized research program but also as a tool to enlist the further support of the nonmember utilities and individuals.

Projects are managed closely from their inception to the final report by the foundation's staff and large cadre of volunteers who willingly contribute their time and expertise. The foundation serves a planning and management function and awards contracts to other institutions such as water utilities, universities, and engineering firms. The funding for this research effort comes primarily from the Subscription Program, through which water utilities subscribe to the research program and make an annual payment proportionate to the volume of water they deliver and consultants and manufacturers subscribe based on their annual billings. The program offers a cost-effective and fair method for funding research in the public interest.

A broad spectrum of water supply issues is addressed by the foundation's research agenda: resources, treatment and operations, distribution and storage, water quality and analysis, toxicology, economics, and management. The ultimate purpose of the coordinated effort is to assist water suppliers to provide the highest possible quality of water economically and reliably. The true benefits are realized when the results are implemented at the utility level. The

foundation's trustees are pleased to offer this publication as a contribution toward that end.

[Project specific paragraph.]

ACKNOWLEDGMENTS

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EXECUTIVE SUMMARY

During the first 12 months of the project, research efforts focused on TOX method testing and optimization, data analysis for utility selection, and refinement of methods for advanced TOX characterization. Over the following 6 months a series of bulk samples were collected and analyzed in the UMass and OSU laboratories

In the early stages of the project, several analytical methods to characterize and identify unknown TOX molecules were developed or refined. After much study and testing, a final set of conditions to be used in the CuO degradation studies was adopted. An extensive search of the literature and consultation with researchers applying these methods was instrumental in arriving at this hybrid method. A draft SOP is nearly complete, along with a full set of QC protocols.

In addition, a set of techniques was developed and tested on NOM and chlorination byproducts. The first was a technique to extract organic molecules from natural water samples with subsequent ESI-MS in mind. By employing a C_{18} disk SPE, over 60% of the DOM in acidified natural water can be isolated and desalted in the field. This material was found to retain its original functional group distribution. From the high resolution mass spectrum and elemental analysis of DOM, it was found that series of molecules with a mass difference equivalent to -CH_2, -H_2 and -O and a low content of nitrogen contribute to the observed odd mass dominant peak pattern. A second technique employed preparative capillary GC to acquire large amounts of highly-resolved chlorination products. This was done successfully with laboratory halogenated samples of NOM extracts.

An ultra-high resolution FT-ICR technique was applied to some extracted samples to produce highly resolved mass spectra. Elemental compositions of each peak observed in the mass spectra can be calculated. This demonstrated the feasibility of constructing elemental composition libraries from water samples before and after they are subjected to halogenation process. The obtained libraries of elemental compositions can be compared to identify unknown TOX molecules. Using van Krevelen analysis we will be able to investigate and visually present plausible reaction pathways of molecules displaying resolved peaks in an ultra-high resolution mass spectrum.

Analysis of TOX standards showed that recovery is complete for THMs, and polyhalogenated acetic acids, regardless of the GAC used. In contrast, the monohaloacetic acids

are partly washed out during sample preparation. This washout may not occur to the same extent with the coal-based GAC. An intermediate amount of nitrate solution (ca. 15 mL) should be used as a compromise value for future tests. There was no obvious difference between the two analyzers when used in standard (coulometric detection) mode. Detailed study of 2 alternative carbons failed to show any consistent advantage over the standard material. A completely new approach to TOCl, TOBr and TOI analysis involving peroxide-assisted UV oxidation followed by in-line analysis by IC was partially developed and validated.

Use of the front-end TOX adsorption and pyrolysis with off-line IC resulted in 100% recovery of TOCl and TOBr based on analysis of standards. Ion chromatographic analysis using commercial columns and the conventional detector (i.e., conductivity) required that two columns using two different eluents be used to achieve the required level of sensitivity and accuracy. Applying this halide-specific TOX analysis to the chlorinated raw waters showed the method to be quite accurate (<10% difference) as compared to conventional TOX.

Multi-oxidant laboratory treatment of two contrasting waters (from Winnipeg and Tulsa) was followed by extensive analytical work with an aim toward comparing TOX methodologies. Both readily formed brominated and iodinated byproducts in the presence of the bromide and iodide, respectively. Some important qualitative differences were noted between the two reactive systems. TOX analysis using the Euroglas analyzer with IC and the standard carbon was found to perform without detectable bias and with a high level of precision.

Finally, a preparative capillary GC protocol was developed and applied to chlorinated samples of natural aquatic organic matter. Also completed is the analysis by LC-TOF MS of extracts of the treated Winnipeg sample (Task 1b). These show some classic features of NOM (signs of homologous series' and various levels of unsaturation). Chlorination seemed to complicate the spectra.

FOREWORD TO THE THIRD PROGRESS REPORT

The purpose of this report is to transmit progress from the fifth and sixth quarterly project periods (September 15, 2003 to March 15, 2004) and to integrate this into work from preceding periods. Work during these two most recent periods covered tasks 1, 2, and 4.

This is prepared in the style of an AWWARF final report, and was created using the AWWARF MS Word template. As such it contains sections that deal with tasks not yet undertaken. This report also contains a final chapter that presents information on the state of progress and general project management. In addition, much of the results chapters are written in the "on-going" style of a progress report, rather than a final report. As portions of this project come to completion, the corresponding results chapters will be modified to read like a "retrospective" final report.

CHAPTER 1: INTRODUCTION

It has been known since the early 1970s that the application of disinfectants, especially chlorine, results in the formation of new chemical compounds known as disinfection byproducts (DBPs). Most of these are organic compounds that represent the end products of chemical oxidation of naturally occurring organic matter (NOM). A certain fraction of these compounds contain covalently bound chlorine, bromine or iodine. These are know as the halogenated DBPs, and they can be measured by a non-specific analytical method know as total organic halide (TOX).

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CHAPTER 2: BACKGROUND

Despite nearly 3 decades of research on the formation of halogenated disinfection byproducts in drinking water, there still remains a large fraction of material that has not been identified. We know that there are many unknown chlorinated and brominated byproducts, thanks to the development of the total organic halide (TOX) analyzer. This instrument and its associated methodology, is capable of measuring all or nearly all of the organically-bound chlorine, bromine and iodine in a disinfected water sample. By comparing the TOX values with the halides attributed to known identifiable byproducts (trihalomethanes, haloacetic acids, etc.) we can estimate the unknown TOX (abbreviated here as UTOX).

Researchers have been attempting to close the TOX gap for many years by identifying more and more of the UTOX. When using free chlorine, the trihalomethanes (THMs) and the haloacetic acids (HAAs) can together comprise as much as 50% of the TOX. Although large in number, other identified groups of halogenated byproducts account for very little of the remaining 50%. Efforts to identify more of these and to account for more of the TOX are ongoing. One of the most complete and recent compilations of DBPs can be found in the review article by Richardson (1998).

Although the earliest work on DBP and TOX centered on the use of free chlorine, more attention has recently been paid to the alternative disinfectants. These have gained favor largely because of the DBP issue. For example, chloramination is becoming more widely used in the US as utilities re-evaluate their operations in light of the new DBP/microbial cluster of regulations. A recent survey has shown that 29.4% of medium and large US utilities were using chloramines as of 1998, as compared to 20% in 1989 (Connell et al., 2000). Chloramines offer many potential advantages over chlorine, most notably lower THM and HAA levels (Bryant et al., 1992). Nevertheless, chloramination has been shown to produce substantial amounts of TOX, which increases from hours to days (Johnson & Jensen, 1986; Stevens et al., 1989). The amount of TOX produced has been shown to be greater at lower pHs. Stevens also showed that a similar trend exists for THM formation, in direct contrast with the behavior for free chlorination. Also, with certain types of activated aliphatic compounds, reaction with chloramines is nearly as fast as the analogous reaction with free chlorine (McKnight and Reckhow, 1992).

18

Symons and co-workers (1996) conducted a detailed study of chloramination and DBP formation under the sponsorship of AWWARF. Symons' data support the earlier findings of Jensen that an especially large fraction of the TOX formed by chloramines are not in the form of the common DBPs (i.e., THMs, HAAs). These authors found that only 10-35% of the TOX could be accounted for by these major byproducts.

One question that persists with chlorimination centers on the potential significance of the unidentified TOX. There are indications that chloramination produces mostly high molecular weight TOX (e.g., Johnson & Jensen, 1986). The higher MW material might not be toxicologically significant due to membrane transport issues (ILSI, 1998).

Another widely used alternative disinfectant is ozone. Due to its lack of stability, ozone is not used as a residual disinfectant in the US. However, it is becoming more common as a primary disinfectant, preceeding free chlorination or chloramination. Ozone, itself, does not produce chlorinated organic byproducts. However, it can oxidize ambient bromide or iodide and produced TOBr and TOI compounds. It will also modify the organic precursors so that upon subsequent chlorination or chloramination, the DBP yields are altered.

Preozonation has been known for many years to result in both increases and decreases in subsequent THM formation during free chlorination. This is a result of complex set of sequential reactions who's ultimate outcome depends on the pH's at various points, the ozone dose, the bicarbonate concentrations, the reaction time, and the nature of the NOM ((Riley at al., 1978; Reckhow and Singer, 1984). The case for TOX formation is similarly complex, but most observers have reported decreases as a result of preozonation. Symons and co-workers have presented some data that indicates similar effects of preozonation when chloramines are used instead of free chlorine.

It's clear that in this time of rapid changes in US disinfection practice, we need to acquire a better understanding of the importance of unidentified byproducts. The TOX measurement gives us a window on to these compounds. If we cannot identify them at a structural level, we must use the TOX measurement to characterize them in a way that can help engineers, toxicologists and regulators make intelligent decisions.

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CHAPTER 3: MATERIALS AND METHODS

RESEARCH OBJECTIVES

Objectives of this research were: (1) to determine the nature and chemical characteristics of the unknown fraction of the total organic halogen (UTOX) produced during chlorination and alternative disinfection processes (i.e., chloramination, chlorine dioxide, ozone disinfection), (2) to assess the impact of treatment on removal of UTOX precursors; (3) to assess the stability of UTOX in a model distribution system and (4) to determine the best TOX protocol for use with IC analysis for the purposes of discriminating between TOCl, TOBr and TOI.

GENERAL APPROACH

This work was conducted in several phases; and it built upon the latest fundamental advancements in NOM characterization. First, a series of TOX methodology studies (Task 1) were undertaken. This was needed to validate existing TOX methods before they could be reliably applied to the analysis of TOBr and TOI. Next, a broad survey of North American utilities was conducted (Task 2). This involved the collection of waters of diverse quality and geographic location for laboratory treatment with 5 basic disinfection scenarios (chlorination, chloramination, both with and without preozonation, and chlorine dioxide). Analysis of these samples for TOX species and known DBPs was undertaken to help the PIs better assess the full range of UTOX occurrence and the raw water characteristics that are associated with higher levels. In addition, distribution system samples were fractionated according to hydrophobicity and molecular size, and then analyzed for UTOX. This was intended to help in assessing the likelihood that UTOX compounds are biologically active. Task 3 focused on factors influencing UTOX concentrations, especially engineering factors. This task was designed to examine impacts of chemical conditions during disinfection on ultimate UTOX concentrations. The final phase (4) was directed to the application of advanced chemical techniques (borrowed from the humics researchers) to the characterization of UTOX. This included analysis of bulk disinfected waters (Task 4a), and analysis of carefully fractioned samples (Task 4b). A set of three innovative and complementary techniques were used: TMAH thermochemolysis GC/MS,

electrospray ionization high resolution MS, and CuO oxidation GC/MS & LC/MS.

Synopsis of Project Tasks

General Comments

Standard IC analysis of furnace pyrolysates were used for TOCl, TOBr and TOI analysis. All IC analyses employed the chemical suppression method. In general, all disinfected samples were analyzed for the full suite of specific halogenated byproducts, and residual disinfectant species. This includes the neutral extractables (including all 10 THMs, the haloacetonitriles, haloketones, etc.) and all 19 haloacetic acids. All samples were further analyzed for TOX, and its halogen-specific fractions, TOCl, TOBr and TOI. The halide-based difference between the specific compound analysis and the bulk OX analysis was then used to calculate unknown TOX (UTOX). This can be further resolved into unknown TOCl, unknown TOBr, and unknown TOI.

Task 1: Preliminary Assessment of TOX Method Performance

This first portion of task 1 involved the analysis of known solutions of chlorine, bromine and iodine containing HAAs, THMs and other compounds. Each was analyzed for TOX at varying concentrations using the Euroglass instrument and the standard activated carbon. Solutions of halogenated compounds were also run on the Dohrmann instrument. In other tests, halogenated standard solutions were run using alternative activated carbons. Final determination was by IC (to get TOCl, TOBr, and TOI) as well as microcoulometric detection (standard TOX).

The comparison between these two analyzers is quite important, because they represent the two different approaches that have been used in commercial instruments. One uses oxygen with carbon dioxide as an auxiliary gas (Dohrmann). The other uses only oxygen (Euroglass). This distinction is important for two reasons. First the oxidative environments in the two systems are different, so pyrolysis reactions may proceed in different ways. It is important to know if this impacts recovery of TOCl, TOBr or TOI. Second, the use of carbon dioxide results in potential interference in IC analysis of the halides. Minear and coworkers were forced to purge much of the dissolved CO_2, thereby creating new opportunities for loss of HX, or sample

contamination.

The second group of Task 1 experiments made use of two contrasting groups of precursors for production of unknown TOX that can be used to test the methodologies. Our approach was to pick a water that has NOM with a substantial autochthonous content and another dominated by allochthonous or pedogenic material. Both had a substantial TOC, so that a high yield of TOX was obtained. It's also important that neither had a high bromide level. This was important in permitting us to evaluate the impacts of added bromide. The waters selected for this task are raw waters from Tulsa's Jewell plant and from the city of Winnipeg. The former is largely allochthonous and the latter is heavily autochthonous as evidenced by their SUVA values. These two waters represent extremes when considering the range of values noted for the ICR plants.

The waters used in Task 1b were treated with chlorine after being dosed with varying levels of bromide and iodide ion. The purpose was to form a range of unknown brominated and iodinated byproducts (contrasting with the known ones from Task 1a), which could be tested for relative recovery by the various TOX protocols. Additional experiments were run where the halide ions were added after quenching the chlorine. The purpose here was to see if bromide or iodide ions would interfere with TOX measurements using these protocols.

All samples were analyzed for the full suite of specific halogenated byproducts, and residual disinfecant species. This includes the neutral extractables (including all 10 THMs, the haloacetonitriles, haloketones, etc.) and all 19 haloacetic acids. All samples were further analyzed for TOX, and its halogen-specific fractions, TOCl, TOBr and TOI.

Task 2: Survey of unknown TOX formation in disinfected waters

Task 2 was intended to generate data on the range of UTOX values that may be observed in waters across North America. The first step was to identify about two dozen waters of differing quality (considering various combinations of TOC, SUVA, bromide/iodide, alkalinity/hardness, and region) for study. This was done using available data (ICR and other sources) and in consultation with the AWWARF project officer and the PAC. Once selected, raw waters and finished waters were collected from each site at different points throughout the project period. These were shipped to UMass for treatment with disinfectants and chemical

analysis. At UMass each was treated with the five disinfection scenarios (chlorine, chloramine, both with an without preozonation, and chlorine dioxide). A standard set of protocols was used for all samples (see Table 2). All samples were then be quenched and analyzed for the full suite of DBPs (THM, HAAs, TOX, TOCl, TOBr and TOI).

Table 1: Task 2 Test Conditions

	Standard conditions
Bromide/Iodide	Ambient
PH	Ambient
Pre-O_3 dose	1 mg-O_3/mg-C
Free Cl_2 target residual	1.5 mg/L
Chloramine target residual	2.5 mg/L
Cl_2/N ratio	4.5 g/g
ClO_2 dose	1.5 mg/L
Free Cl_2 Contact Time	12 hr
Disinfectant Contact Time	48 hr
Temp	20°C

At the same time, a characteristic distribution water sample was collected from each of the Task 2 plants, quenched and shipped to UMass. This was analyzed for the full suite of DBPs. In addition, a portion of this sample was fractionated based on molecular size (ultrafiltration) and hydrophobicity (hydrophobic resin adsorption). The resulting fractions were analyzed for the full set of DBPs as well. The intention was to develop a database on the general character (e.g., hydrophobicity and apparent molecular weight) of UTOX in North American waters.

Task 3: Conditions affecting UTOX formation and destruction

The purpose of task 3 was to determine the impact of a variety of treatment conditions (disinfection conditions) on UTOX concentration. In Task 3, a smaller set of water samples was selected from the task 2 plants. Selection criteria was based on raw water characteristics and UTOX yields and characteristics. An attempt was made to include a set of waters that

24

adequately captures the full range of behavior as observed in task 2. These waters were treated with the same combinations of disinfectants as used in Task 2, but some additional experimental variations were used. These included variations in pH, bromide level and iodide level.

Task 4: Advanced characterization of unknown TOX

The purpose of task 4 was to borrow some of the most promising advanced techniques from the field of NOM characterization, and to apply these to the problem of UTOX. The selected methods included TMAH thermochemolysis, ESI/MS, and CuO oxidation with GC/MS & LC/MS. The first two are relatively new techniques that have been pioneered by one of the PIs (Hatcher). These have been employed quite successfully in the last few years for the characterization of NOM in drinking water. The last one is an older technique with some new elements added. It has traditionally been one of the most useful approaches to the characterization of the lignin content in humic substances. The three represent a complementary group. One is largely a reductive technique (TMAH), another (CuO method) is oxidative, and the third (ESI) is relatively non-degradative. None of these techniques had been previously applied to the focused study of halogenated NOM as proposed here. In task 4a these techniques were applied directly to the bulk disinfected waters. Task 4b carried this further by means of preparative-scale extraction and fractionation protocols prior to advanced chemical analysis.

In task 4a we collected a subset of waters from Task 2, and treated these in the laboratory using the 5 major disinfection scenarios (chlorine, chloramines, both with and without preozonation, and chlorine dioxide). Each was extracted or lyophilized as needed and analyzed by the selected advanced methods.

Task 4b incorporated preparative-scale fractionation into the experimental design for task 4a. Because of the labor-intensive nature of this fractionation, only the two most common disinfection scenarios could be examined. We treated a water selected from the task 2 studies with chlorine and another aliquot of the same water with chloramines. Laboratory disinfection was done in a large bulk sample (about 300L) which was then subject to preparative scale resin extraction followed by UF fractionation of each of the resin extracts. This resulted in about 24 separate fractions based on combinations of size, charge and hydrophobicity. All were analyzed by standard OC (TOC, UV-Vis absorbance) and DBP analysis (TOX, TOCl, TOBr, TOI, THM,

HAA). Some of the fractions will had a large abundance of organic carbon, and those fractions were analyzed by the advanced techniques. Special attention was paid to those fractions that are considered to be likely candidates for passive transport through biological membranes.

LABORATORY TREATMENTS

Chlorination/chloramination procedures

Chloramination and chlorination were conducted by widely used methodologies. Generally, reagents were added in the form of concentrated solutions under conditions of high-speed mixing. Symons and co-workers (1996) have concluded that the exact nature of this mixing is not of primary importance in simulating full-scale chloramination with bench-scale experiments. Nevertheless, to avoid any possible complication of this type, we used pre-formed chloramines. These are produced by careful mixing of concentrated solutions of sodium hypochlorite and ammonium chloride. This is done at low temperatures, and at a controlled pH. Experience with this approach at UMass has shown that relatively stable and pure solutions of monochloramine can be produced in this way. Additional details may be found in the UMass SOP for Chlorination (in the project QAPP).

Ozonation Procedures

As required for task 2,3&4 studies, samples were ozonated in a semi-batch system. Ozone was generated from pure oxygen by means of a laboratory corona discharge generator. The ozone/oxygen product gas was introduced into a 2-L glass reaction vessel containing the water to be treated. Flow was controlled with an electronic flow controller, and the ozone content was monitored by direct UV absorbance spectrophometry. The gas was mixed with the sample by a porous quartz frit. Off-gas was re-directed through a spectrophotomer for determination of ozone content. A membrane ozone electrode (Orbisphere) was fitted into the side of the glass reactor so aqueous ozone concentration could be continuously monitored. Ozone transferred was determined from the flow rates and the differences in ozone content in the applied gas versus the off-gas. Additional details may be found in the UMass SOP for Ozonation (in the project QAPP).

Chlorine Dioxide Treatment

Where required, samples were preoxidized with chlorine dioxide in a batch reactor. The reaction was conducted at darkness in BOD bottles in absence of air in order to avoid the possible loss of oxidant or volatile by-products produced during the course of the reaction (flasks will be filled up).

Chlorine dioxide was freshly generated as needed. Aqueous solutions were prepared from the gaseous chlorine dioxide generated from the acidification (i.e. sulfuric acid) of a solution of sodium chlorite. In order to avoid the presence of trace chlorine in the chloride dioxide stock solution, chlorine was removed from the gas stream by a $NaClO_2$ scrubber. Concentration in chlorine dioxide of solutions prepared using this protocol were found to range from 3 to 4 g/L of ClO_2. The concentration of the chlorine dioxide stock solution was checked before each use using the LSB method as developed by Bubnis and others.

Ultrafiltration

Ultrafiltration was used for assessing apparent molecular size of TOX compounds. Samples were treated using a stirred 300-mL Amicon pressure cells under a nitrogen atmosphere. We used membranes rated at 1K and 10K Daltons. These were applied in a parallel configuration. The smaller UF membrane were used to determine those TOX molecules that are most likely to pass through biological membranes. It has been proposed that the low MW TOX contains the toxicologically important compounds. The 10K UF membrane were used to help determine which TOX molecules are of sufficient size as to be considered macromolecular for the purposes of physical and chemical treatment processes (e.g., coagulation, adsorption).

CHEMICAL ANALYSIS: VALIDATED METHODS

Total Organic Carbon

Total organic carbon (TOC) was measured on nearly all samples in this research. It was be measured by the high-temperature combustion method (APHA et al., 1999). At UMass a Shimadzu 5000 was used for these measurements. Additional details may be found in the

UMass SOP for TOC Analysis (in the project QAPP).

UV Absorbance

The full UV-Visible absorbance spectrum was measured for all waters prior to treatment with disinfectants. UV Spectroscopy has been extensively used in studying humic substances. Specific UV absorbance at 254 nm is widely used to assess the humic content of NOM. Though their UV spectra are often featureless, the ratio of absorbance at 465 nm to 665 nm (i.e., E_4/E_6 ratio) has been successfully used as an indictor for the degree of humification and aromaticity of NOM (Stevenson, 1995; Chen at al., 1977). The E_4/E_6 ratio decreases with increasing molecular weight and condensation of aromatic constituents. Molar absorptivity at 280 nm of NOM is also indicative of humification and molecular size (Chin et al., 1994; Chin et al., 1997).

Korshin and co-workers have shown that there are certain wavelengths (ca. 272 nm) that present especially strong correlations between absorbance and formation of TOX following chlorination (Korshin et al., 1996). We measured UV absorbance (full range of wavelengths) before and after disinfection on all samples. All absorbance measurements were made at UMass a Hewlett-Packard diode array spectrophotometer.

Residual Chlorine (Free and Combined)

Residual chlorine was measured by titrimetric DPD methodology (4500-Cl, D and F: APHA et al., 1999). We measured residual chlorine species on all samples collected for DBP analysis.

THMs and other Neutral Extractables

Trihalomethanes and other neutral extractables (haloacetonitriles, haloketones, chloropicrin, etc.) were measured on all disinfected samples and controls. We used the standard micro-extraction method with GC and electron capture detection (ECD) (APHA et al., 1999). This method was expanded to include the 6 iodinated THMs, and as many iodinated neutral extractables as possible given availability of standards. Additional details may be found in the UMass SOP for THMs (in the project QAPP).

Haloacetic Acids

The full suite of haloacetic acids were measured along with the THMs whenever samples are disinfected. Haloacetic acids were measured by the micro-extraction method with methylation and separation/detection by GC with ECD. More specifically, we used the acidic methanol derivatization (US EPA method 552.2) which avoids the use of highly-toxic reagents as required for the diazomethane method. Acidic methanol has proven to give better and more reliable recoveries of all HAA9 species, especially the brominated forms (Pat Fair, personal communication, 2000). The existing method was expanded to include the 6 iodinated trihaloacetic acids, the 3 iodinated dihaloacetic acids and monoiodoacetic acid. This resulted in a total of 19 HAAs. Additional details may be found in the UMass SOP for HAA Analysis (in the project QAPP).

Conventional Total Organic Halide (with microcoulometric detection)

Total organic halide (TOX) was measured on nearly all of the samples in this study. Task 1 analyses (at UMass) employed a Euroglass instrument as well as a Dohrmann DX-20 unit. Subsequent tasks used only the Euroglass instrument. Both instruments operate under the standard GAC adsorption, pyrolysis and coulometric detection scheme. However, one (Dohrmann) uses a carbon dioxide auxiliary gas, and the other (Euroglass) doesn't. Methodology generally followed that established in Standard Methods (APHA et al., 1999). Additional details may be found in the UMass SOP for TOX Analysis (in the project QAPP).

CHEMICAL ANALYSIS: NON-STANDARD METHODS

Total Organic Halide with IC Detection

In this study we developed methodologies for measuring TOCl, TOBr and TOI as separate fractions of the TOX. This was done by trapping the HX vapor in the pyrolysis tube gases, and subjecting these to inorganic halide analysis by ion chromatography. This approach has been used by a small number of researchers over the past 20 years. However, Minear is one of the few to actually publish a specific methodology (e.g., see: Echigo et al., 2000). They used

a heated transfer line, which was also flushed after each sample. We took the same general approach. However, unlike Minear and co-workers, we used the Euroglass TOX analyzer which does not use a CO_2 auxiliary gas.

Inorganic halide analysis were conducted with a dedicated ion chromatograph. The instrument for this used chemical suppression technology, and was equipped a data system.

Hydrophilic/Hydrophobic Content

The analysis of hydrophobic and hydrophilic content was performed on all treated drinking water samples collected in Task 2. Non-ionic resin fractionation by XAD resin adsorption chromatography was used to determine the DOC distribution of operationally defined hydrophobic, transphilic and hydrophilic DOC fractions. The methodology was scaled down from the design employed by Aiken et al. (1992). Two sequential columns containing DAX-8 and DAX-4 resins[1] were used to adsorb (the column distribution coefficient, $k'_{0.5r}$, is set equal to 50 for both XAD-8 and XAD-4 resins, $V_{0.5r} = 2V_0 (1+k'_{0.5r})$ with V_0 .: Void volume) hydrophobic and transphilic DOC, respectively. The XAD-8 resin is an acrylic ester polymer and the XAD-4 resin is a styrene divinylbenzene copolymer. Phosphoric acid was used to acidify samples to pH ~ 2 prior to application to the columns. Acidified samples were first passed through a column containing XAD-8 resin at an approximate flow rate of 2 mL/min, and then subsequently passed through an additional column containing XAD-4 resin at the same flow rate. TOC measurements of influents and effluents of columns were used to perform a carbon mass balance, which yielded hydrophobic, transphilic and hydrophilic TOC fractions. Hydrophobic TOC are compounds that adsorb onto XAD-8 resin, transphilic TOC are compounds that adsorb onto XAD-4 resin, and hydrophilic TOC are compounds that pass through both columns.

Preparative-scale fractionation based on hydrophobicity and charge

Samples used for Task 4b were subject to preparative-scale fractionation. The proposed scheme used resin extraction to produce 8 major fractions based on hydrophobic behavior and organic charge. The organic extraction system consisted of three resin columns connected in

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[1] These are equivalent to the older XAD-8 and XAD-4 resins.

30

series in accordance with the method of Leenheer and Noyes[2]. The first column was filled with DAX-8 resin[3], a nonionic acrylic ester resin (Figure 2). The second column was filled with a cation exchange resin, MSC-IH, and the third column with Duolite A-7, an anion exchange resin. All resin columns were cleaned according to methods developed by Leenheer and co-workers. Two-liter glass liquid chromatography (LC) columns (Spectrum Chromatography Products, Dallas, TX) with Teflon end plates were used.

A total volume of about 300 liters of water was be pumped through the extraction system at a flow rate of 150 mL/min. The water was pumped through two cartridge filter units (Balston Co., Haverhill, MA) with glass fiber filters rated at 25 μm and 0.3 μm pore size and then through the column and fractionation system. The effluent from the columns was collected for subsequent recovery of the unretained hydrophilic neutral fraction.

The three resin columns were separately desorbed to recover the organic fractions after completion of the adsorption run. Weak hydrophobic acids were desorbed from the DAX- 8 column with a 0.1 N NaOH solution, followed by a deionized water rinse in the upflow direction. The eluant (1.5 liters) was immediately neutralized to pH 7 with H_2SO_4 to prevent alkaline oxidation and hydrolysis. Hydrophobic bases (5 liters) were desorbed from the DAX-8 column with a 0.1 N HCl solution. Hydrophobic neutrals were then recovered from the DAX-8 column by Soxhlet extraction of dried DAX-8 resin after desorption of hydrophobic bases and weak hydrophobic acids.

Hydrophilic bases were desorbed from the MSC-1H column with a 1.0 N NaOH solution and deionized water rinse. As before, the eluate (7 liters) was neutralized to pH 7 with H_2SO_4 to prevent alkaline oxidation and hydrolysis. Strong hydrophobic acids and hydrophilic acids were desorbed from the anion exchange column, Duolite A-7, by recycling a mixture of 10 N NaOH and deionized water through the column. Recycling was stopped when the pH of the eluant reached 11.5, after which the column was rinsed with deionized water.

Leenheer, Jerry A. and Noyes, T. I. A Filtration and Column-Adsorption System for Onsite concentration and Fractionation of Organic Substances from Large Volumes of Water. Washington, D.C.: U.S. Government Printing Office; 1984(U.S. Geological Survey Water Supply Paper; 2230).
[3] This is equivalent to the older XAD-8 resin.

31
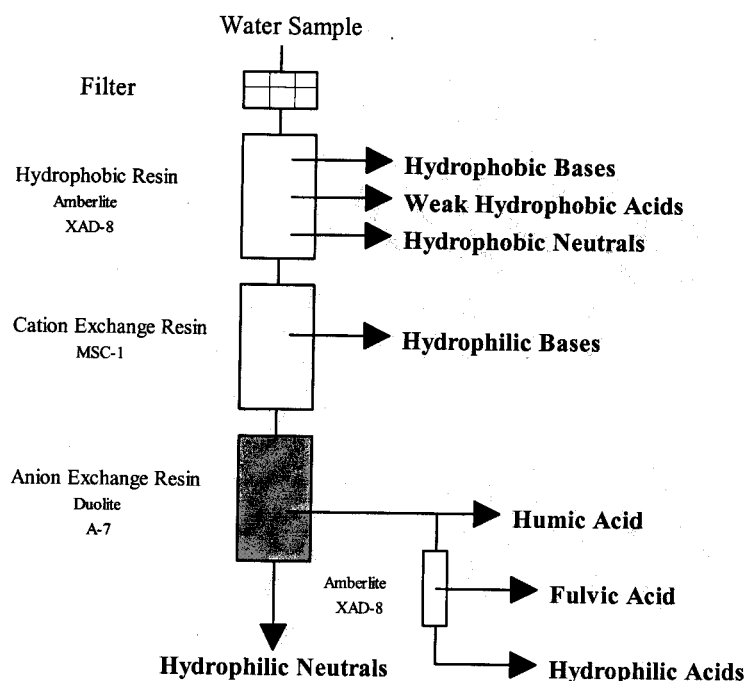
Figure 1. Preparative-scale resin fractionation scheme

TMAH thermochemolysis for characterization of chlorinated DOM

One technique for investigating chlorinated DOM molecular composition is the tetramethylammonium hydroxide (TMAH) thermochemolysis GC-MS procedure, developed by Challinor (1989, 1995). This method has been useful for investigating the molecular composition of organic matter in several recent studies of humic substances (HS) (Chefetz et al., 2000; del Rio et al., 1998; Hatcher & Clifford, 1994; Hatcher et al., 1996; Martin et al., 1995; McKinney et al., 1996; McKinney & Hatcher, 1996; Zang et al., 2000) and DOM (del Rio *et al.*, 1998; Mannino & Harvey, 2000; van Heemst *et al.*, 2000; Wetzel *et al.*, 1995). The TMAH reaction serves both as a degradative technique as well as a derivatization technique. Labile C-O bonds such as esters, amide bonds, some ether bonds with α-hydroxy groups (β-O-4 bonds in lignin), and to some extent glycosidic bonds, are cleaved resulting in fragments. This degradation occurs mainly through a base-catalyzed hydrolysis reaction. Acidic protons, such as those found on carboxylic acids and phenols, are methylated whereas esters are transesterified into the corresponding methyl esters (Filley et al., 1999). The results are products of increased volatility that can be separated and analyzed using GC-MS.

32

The TMAH thermochemolysis GC-MS procedure was used here as a complementary technique to other non-standard methods proposed for this research. For example, CuO oxidation has been found to be particularly useful to study lignin-derived material in DOM. However, to the authors' knowledge other biogenic contributions to DOM have not been represented by this approach aside from short chain fatty acids (<6 carbon units) (Ertel et al., 1984; Hautala et al., 1997; Hautala et al., 1998; Hyotylainen et al., 1997; Louchouarn et al., 2000). Pyrolysis GC-MS has also been useful for structural studies of DOM (Bruchet *et al.*, 1990; Schulten, 1999; van Heemst *et al.*, 1996; van Heemst *et al.*, 1999). However, substantial amounts of CO and CO_2 are produced during pyrolysis, which result from the polar functionalities that are important structural features of DOM (Saiz-Jimenez, 1994). These may be retained with the TMAH GC-MS technique since sub-pyrolysis temperatures are used (250°C) and since methylation deactivates polarity and the tendency to undergo thermal transformations.

A drawback to the TMAH procedure is that natural methoxy groups, such as those found in lignin, cannot be distinguished from those introduced during the TMAH reaction and that the strong base can remove Cl atoms from structural entities in chlorinated DOM by simple substitution reactions. However, these drawbacks can be overcome by using the new ^{13}C-labeled TMAH thermochemolysis GC-MS procedure. [13]C TMAH thermochemolysis maintains the same degradative and derivatization characteristics as that of unlabeled TMAH (Filley et al., 1999). However, ^{13}C TMAH thermochemolysis relies on ^{13}C labeled methyl groups in TMAH as the methylating agent so that naturally occurring methoxy groups can be distinguished from those produced during the TMAH thermochemolysis procedure. The position of the labeled methoxy group (or natural phenolic or hydroxyl precursor position) can often be determined by analysis of the mass spectral fragmentation patterns. Filley *et al.* (1999) demonstrated that there are minimal exchange reactions (<4%) with preexisting methoxy groups on TMAH products. This procedure yields vastly more information than that provided by other wet chemical degradation techniques for two reasons. Not only are chemically and thermally labile functionalities stabilized by methylation and thus the products more closely resemble their precursors (functionalities often not seen using other degradative techniques), but by using the ^{13}C TMAH procedure, one can more accurately identify the structure of the precursor prior to derivatization. We have recently employed this approach to evaluate the transformation of DOM into biodegradable DOM on plug-flow bioreactors (Frazier et al., 2001). By the combined use of

TMAH and ^{13}C-TMAH thermochemolysis we determined that the indigenous bacteria preferentially degrade and demethylate lignin. This is contrary to present belief that bacteria cannot demethylate lignin on time scales of a few hours.

In the case of chlorinated DOM for this project, we employed a dual methylation procedure, the first using diazomethane to methylate the hydroxyl functional groups with natural abundance methyls and the second using the ^{13}C-labeled TMAH to remove Cl and replace it with a labeled methyl. Mass spectrometry of resulting products allowed us to define the positions of Cl atoms in fragments of the molecular structure. This approach had never previously been attempted.

Although the thermochemolysis methodology describe above is non-standard, and contains some elements that had not previously been used, the basic approach followed an established protocol that had been developed in Dr. Hatcher's laboratory. This "standard operating procedure" is presented below.

A. TMAH thermochemolysis procedure

1. Prepare sample filter in advance by placing a plug of silica wool inside a short-necked Pasteur pippette, being careful not to break the plug up. Place in an evaporating dish and heat in furnace at 550 °C for 30 minutes. One filter is necessary per sample.

2. Obtain glass TMAH ampoules for as many samples as desired. Check the diameter of the ampoule with a long-necked Pasteur pipette; the pipette should be able to fit easily through the hourglass shaped ampoule. If not, discard it.

3. Weigh an appropriate (approximately 0.5 – 1 mg of organic matter) amount of sample on microbalance.

4. Place sample in ampoule, taking care not to leave any excess sample on the side of the tube.

5. Rinse tip of 200 μL pipette with 3 200 μL portions of methanol.

6. Add an appropriate amount of TMAH to sample ampoule. (Typically, for every milligram of organic material, add 200 μL of TMAH).

7. When finished adding TMAH to sample, purge TMAH bottle under house nitrogen for approximately 2 minutes, close cap tightly, and seal with Teflon tape.

8. Gently blow samples to dryness using higher purity nitrogen from tank. Make sure to not let sample splatter on sides of tube.

9. While sample is drying, prepare cold trap on vacuum line, close off all line valves except the main manifold valve, start pump and let purge for roughly 10 minutes.

10. Once sample is dry (after approximately 20 minutes), insert into connection on vacuum line, open valve slowly to keep sample at the bottom of the tube, and let sample ampoule purge for several minutes. When done, close off sample to vacuum.

11. Flame seal ampoule. To do this, open both oxygen tank and house gas line, Open red (gas) torch knob about ¼ of a turn and ignite. Adjust gas flow to get approximately a 6" flame. Slowly open oxygen (green) knob. Add just a small amount to give direction to the flame. Heat the vial gently at first using this oxygen-lean flame at a 45o angle focused on the center of the hourglass and the areas immediately above and below it. Be sure to not hold the flame on a specific point; rotate it round the tube as much as possible. Heat for about 2 minutes.

12. At this point, add additional oxygen to give a bright blue cone and heat quickly. Touch the tip of the cone at the center of the hourglass at roughly the same angle. Hold for several seconds at a specific point. Repeat this several times at points all around the circumference of the ampoule. Take care not to melt a hole in the vial and open up to air.

13. Once the glass starts to melt, pull gently down on the vial and cut off the bottom portion of the ampoule.

14. Heat tip of the tube rapidly for several seconds more, rotating tube in hand.

15. Slowly decrease oxygen flow and continue to rotate tube while heating.

16. Turn off oxygen flow completely and heat gently for several more minutes. When finished the tip of the ampoule should be charred black.

17. Let tube cool.

18. Wrap ampoule in aluminum foil, covering both ends completely.

19. Place in oven basket and heat in TMAH oven for 30 minutes at 250 °C.

20. Set up filtration apparatus with filter placed above a GC vial.

21. After baking, score sample ampoule and place in a beaker of liquid nitrogen to freeze sample and break at score mark.

22. Wash 200 μL pipette with 3 portions of dichloromethane and add 50 μL eicosane (internal standard to calculate the recovery rate) to ampoule.

23. Add about 600 μL ethyl-acetate to sample ampoule and about 200 μL to the broken tip.

24. Pull extract out with a long-necked Pasteur pipette and filter into GC vial. Repeat extraction of ampoule until the GC vial is nearly full with extract.

25. Evaporate extract under nitrogen from tank (as in step 8) until the volume reaches about 50 μL.

B. Quantitative analysis procedure by LECO GC TOF (time of flight) MS

1. Dilute and make a series of each PGS and FAME standard solutions from stock solutions (These are compounds that are readily available as standards and represent the types of compounds to be expected in DOM).

2. Transfer 190 μL of each PGS (lignin) and FAME (fatty acid methyl ester) standard solutions to GC ampoules.

3. Add 10 μL of GC internal standard (2-chloro-5-nitro-benzophenone) solution to each ampoule in GC vials.

4. Transfer 19 μL of samples to ampoules in GC vials and add 1 μL of GC internal standard (2-chloro-5-nitro-benzophenone) solution.

5. Load vials on the GC-MS instrument and start analysis.

6. After GC runs, assign peaks in PGS and FAME standard GC chromatograms.

7. Add the assigned standard peaks into standard graph program in Pegasus software and type the concentrations of each standard solution.

8. Make sure that 2-chloro-5-nitro-benzophenone is checked as an internal standard and eicosane checked as a surrogate standard in the software.

9. Start to calculate the standard graph.

10. After calculation, process the chromatograms from each TMAH treated samples.

Electrospray Ionization Mass Spectrometry for characterization of chlorinated DOM

Electrospray ionization (ESI) mass spectrometry is a novel technique that has been

applied recently to the characterization of humic substances (McIntyre et al., 1997; Fievre et al., 1997; Brown and Rice, 1999; Solouki et al., 1999; Leenheer et al., 2001; Plancque et al., 2001; Kujawinski et al., 2001). ESI is a "soft" ionization technique in which ionizable compounds such as proteins, polar molecules, and humics become charged by the action of a volatilizing nebulizer spray. This process has been shown not to fragment the components of similar molecules, such as proteins (Gaskell, 1997). Intuitively, it is thought that humic substances will remain intact as well. This assumption is crucial considering the debate on whether humic substances are high molecular weight macromolecules or aggregates of noncovalently linked molecules (Piccolo and Conte, 2000) such as sugars, carbohydrates, and fatty acids.

In this research, we have applied ESI ionization coupled to a quadrupole time of flight mass analyzer to identify and describe chlorinated DOM structures. The ESI-QqTOF method is capable of achieving resolving powers in excess of 10,000, which is sufficient to resolve many of the peaks in the spectrum of DOM. The molecular weight distribution from this spectrum is consistent with reported spectra of fulvic acid from natural water (Plancque et al., 2001). This fact is very encouraging because fulvic acid should have a lot of common structures with DOM prepared in this protocol. From earlier work, we conclude that there are many series of molecules with differences of 2H, O, CH_2 and H_2O, which could be an explanation for observed peak patterns (Brown and Rice, 1999).

Much higher resolving power can be attained for humic substances with other techniques such as Fourier transform ion cyclotron resonance (FT ICR) mass spectrometry (Brown and Rice, 1999; Kujawinski et al., 2001). The QqTOF analyzer was chosen because of its robust and sensitive nature and ability to show little mass discrimination over a relatively wide range of masses. Several types of adducts are possible, such as H^+, Na^+, K^+, and NH_4^+, but only H^+ and Na^+ are expected in these samples as demonstrated previously (Kujawinski et al., 2001). The sodium ion would be expected as a result of extraction in sodium hydroxide. However, it appears that the peaks in these samples are expected to consist mostly of hydrogen adducts. In our protocol, DOM was isolated from water by solid phase extraction. In this way, we were able to reduce or eliminate sodium adduct peaks, with the resulting spectrum characterized by peaks reflecting primarily H^+ adducts. This is very crucial to identifying chlorinated DOM.

One particular feature of high resolution mass spectrometry is the ability to separate compounds having relatively large mass defects, especially chlorine-containing compounds that

have two isotopes each having a large negative mass defect. This property allowed us to clearly identify a DOM component containing chlorine. Without interfering ions, carbon (12.0000 amu), nitrogen (14.0031 amu), hydrogen (1.0078 amu) and oxygen (15.9949 amu) is the main elemental composition of DOM, and their exact mass numbers in any sort of added proportions are close to their nominal mass numbers (maximum difference is 0.0078). Compared to these elements, chlorine (34.9689), bromine (78.9183) and iodine (126.9045) have much larger mass defect (minimum difference is 0.0311). Substitution of any of main elements with halogen will change the mass defect of DOM molecules. By comparing the mass defect patterns in the spectra of natural and chlorinated DOM, we were able to determine the contribution of halogenated molecules. From the high-resolution data, we were able to identify the elemental composition of individual halogenated molecules. These identified molecules then can be subjected to MS/MS analysis for structural elucidation (Plancque et al., 2001).

The ESI-MS methodology described above is non-standard, and contains some elements that had not previously been used. As with the thermochemolysis techniques, there was an established protocol for ESI-MS investigation of NOM that has been developed in Dr. Hatcher's laboratory. This "standard operating procedure" is presented below.

1. Prepare sample by dissolving natural organic matter into methanol and water mixture (typically 50:50). The typical concentration of Humic substance for mass spectrometric analysis is about 1mg/ml.
2. Select a standard material (e.g. poly-ethylene-glycol (PEG) solution) that matches the molecular weight range of your sample.
3. Run the selected PEG solution and optimize instrumental conditions (such as capillary temperature).
4. Run NaI solution to calibrate mass to charge ratio at given condition.
5. Flush a transfer-line and syringe with MeOH to remove the residual NaI.
6. Analyze the sample by direct infusion method.

CuO Oxidation and Product Analysis by GC/MS and LC/MS

Oxidative degradation methods have been used along with GC/MS for the characterization of NOM since the early 70s. While many different oxidants have proven successful in preserving structural features in degraded NOM, CuO oxidation has probably been the most useful (Christman et al., 1983; Ertel et al., 1984; Hautala et al., 1997; Hautala et al., 1998; Hyotylainen et al., 1997; Liao et al., 1983; Louchouarn et al., 2000). Using this technique, researchers from both Ertel's laboratory and Christman's laboratory have clearly identified a range of lignin-based structures in aquatic NOM. Cupric oxide methods are mild and have been reported to preserve 25-75% of such lignin structures in environmental samples.

As part of this work a specific alkaline CuO protocol was selected, refined, tested, and applied to the halogenated water samples that were subject to detailed characterization. This methodology was based on the classical method (Hedges & Ertel, 1982) with modifications selected from subsequent studies. We also used a variation of this method with LC/MS analysis.

CHAPTER 4: LABORATORY ASSESSMENT OF METHOD PERFORMANCE

During the 3rd and 4th quarterly project periods, research at UMass focused on the following areas within Task 1 (Preliminary Assessment of TOX Methods):

> Exploration of recent advancements made by Dionex in combining TOX with IC
> Exploration of potential PACs for testing
> Testing the Euroglass instrument for TOX compound recovery
> Testing IC recovery
> Combining Euroglass adsortion/pyrolysis with IC
> GC method development for iodinated DBPs

In the 5th and 6th project periods this work was largely completed. A small amount of additional model compound testing will continue.

PROPOSED NEW INSTRUMENT DEVELOPMENT

During the first year of the project, we entered into discussions with a potential commercial partner (Dionex Corporation) on the feasibility of developing a sensitive and robust instrument for measuring TOCl, TOBr and TOI in drinking waters. This discussion included a meeting with Tekmar-Dohrmann (T-D) at their Ohio facilities in February 2003.

Subsequent to this, one of the PIs (Reckhow) and a graduate research assistant (Hua) traveled to the Sunnyvale CA laboratories of Dionex for evaluation of a prototype oxidation unit. This was a new German product that consisted of an ultra-high intensity UV lamp with proprietary cooling and circulating systems. This was examined in connection with off-line IC analysis and pretreatment with a variety of Dionex ion exchange materials. After nearly three days of laboratory testing by Reckhow, Hua and two Dionex applications specialists, a workable instrument design emerged. In a simple form, the stages are as follows:

> Sample withdrawal from an autosampler tray
> Sample pre-treatment through a battery of IC traps
> > o Including two Ag cartridges in series
> Introduction of pre-treated sample into UV reactor
> Introduction of UV reactor effluent to IC injector loop
> Analysis by IC

o   Chloride, bromide, iodide, nitrate, bicarbonate, sulfate

One of the objectives of this work was to develop a completely aqueous system that did not require solids handling. This would avoid some of the problems that have rendered existing TOX methods cumbersome, prone to bias, imprecise and expensive. Of course, the other prime objective was to develop a method and instrument that could differentiate between TOCl, TOBr and TOI. For this to work as conceived, we would need a means of retaining inorganic halide prior to analysis of the organic matter.

Extensive studies were conducted with model TOX compounds and chlorinated waters both with and without added inorganic halide. The use of two Ag cartridges (cation exchangers pre-loaded with silver ions) in series was completely successful in dropping the inorganic halide levels below a few micrograms per liter. Whatever halide background that might remain (if any) could be subtracted from the final oxidized level (e.g. the TOX) by on-line IC analysis of an unoxidized sample. UV based oxidation with added hydrogen peroxide was found to achieve 95-100% mineralization efficiency (forming inorganic halide and bicarbonate). The resulting anions were easily measured by off-line IC, and detection limits were estimated to be in the microgram per liter level (i.e. as low or lower than those for existing TOX instruments).

EXPLORATION OF POTENTIAL PACS FOR TESTING

Currently there are only two types of commercially available TOX carbons. Both are coconut based. Although we've been unable to find any comparative data on their performance, the manufacturer reports that one of the carbons has a higher TOX blank than the other. Our testing supports this. For the early stages of this work, we proposed to look at both of the commercial products. In addition, Calgon has a relatively new activated carbon with an extremely low inorganic halide content. This is the Filtrasorb 600, and its chloride level is substantially lower than F400, which was the TOX standard for many years (partly for reasons of its low blank halide level). We acquired the necessary equipment and developed protocols for hand-grinding, sieving and packing cartridges with this material as a third carbon type.

TESTING FOR TOX COMPOUND RECOVERY

TOX recovery with microcoulometric detection: Phase 1 tests

The first portion of Task 1 involves the analysis of known solutions of chlorine, bromine and iodine containing HAAs, THMs and others (e.g. halogenated nitrogenous compounds). Table 1 summarizes work done on the first extensive set of TOX recovery experiments. These used the Euroglas analyzer and the standard carbon columns supplied by CPI (carbon #1). For these tests sample volumes of 50 mL were used, and columns were rinsed with 30 mL of the Euroglas nitrate washing solution.

TOX as determined from the first column and the sum of the two columns for each compound are summarized in Figure 2 through Figure 9. From Table 2 and Figure 2 through Figure 9, the recoveries of TOX from the THMs are nearly complete:

- 84-87% for chloroform,
- 85-92% for dibromochloromethane and
- 95-100% for bromoform.

In contrast, the recoveries of the HAAs are species specific. The monohaloacetic acids were poorly recovered:

- 41-60% for chloroacetic acid and,
- 60-76% for bromoacetic acid,

whereas the dihalo and trihaloacetic acids showed recoveries that were similar to the THMs:

- 78-87% for dichloroacetic acid,
- 90-100% for dibromoacetic acid and
- 86-96% for trichloroacetic acid.

The TOX recovery data for model compounds shows a general trend of increasing recovery with decreasing concentration, but the degree of increase varies with different compounds. Another phenomenon that we observed from the TOX recovery tests is that some HAAs, especially monochloroacetic acid, monobromoacetic acid and dichloroacetic acid, are partly washed out by nitrate washing step. This can be seen by comparing the TOX of the first and second columns (Figure 5 - Figure 9).

42

Table 2: TOX standards tests using the Euroglas analyzer

Name	Molecular Formula	Standard (µg Cl/L)	TOX Concentration (µg Cl/L)			
			1st Column	2nd Column	Total	% Recovery
Trihalomethanes (THMs)						
Chloroform	CHCl$_3$	349.7	272.4	27.1	299.5	85.6%
		235.3	188.2	8.3	196.5	83.5%
		88.4	72.7	3.8	76.5	86.5%
Dibromochloromethane	CHClBr$_2$	500	414.2	9.5	423.7	84.7%
		300	272.7	4.1	276.8	92.3%
		100	84.7	4.9	89.6	89.6%
Bromoform	CHBr$_3$	500	465.8	7.1	472.9	94.6%
		300	283.1	3.0	286.1	95.4%
		100	98.3	2.3	100.6	100.6%
Monohaloacetic Acids (MHAA)						
Monnochloroacetic Acid	CH$_2$ClCOOH	500	72.2	132.3	204.5	40.9%
		300	46.6	95.8	142.4	47.5%
		100	26.0	34.2	60.2	60.2%
Monobromoacetic Acid	CH$_2$BrCOOH	500	179.6	130.7	310.3	62.1%
		300	91.0	134.7	225.7	75.2%
		100	42.2	33.4	75.6	75.6%
Dihaloacetic Acids (DHAA)						
Dichloroacetic Acid	CHCl$_2$COOH	500	195.4	202.7	398.1	79.6%
		300	109.3	123.6	232.9	77.6%
		100	48.4	38.2	86.6	86.6%
Dibromoacetic Acid	CHBr$_2$COOH	500	423.7	44.9	468.6	93.7%
		300	251.4	19.8	271.2	90.4%
		100	100.2	3.3	103.5	103.5%
Trihaloacetic Acids (THAA)						
Trichloroacetic Acid	CCl$_3$COOH	500	386.5	43.3	429.8	86.0%
		300	266.8	20.7	287.5	95.8%
		100	86.6	5.1	91.8	91.8%

43
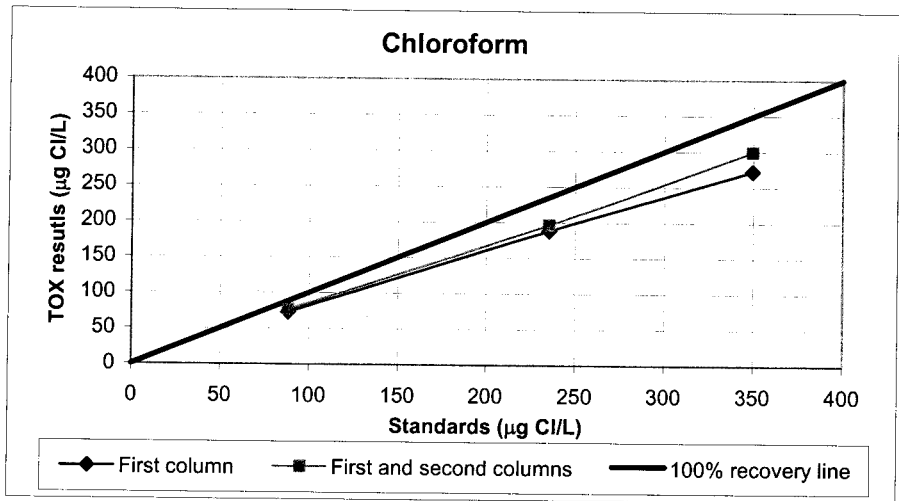
Figure 2: Recovery of Chloroform standards by TOX
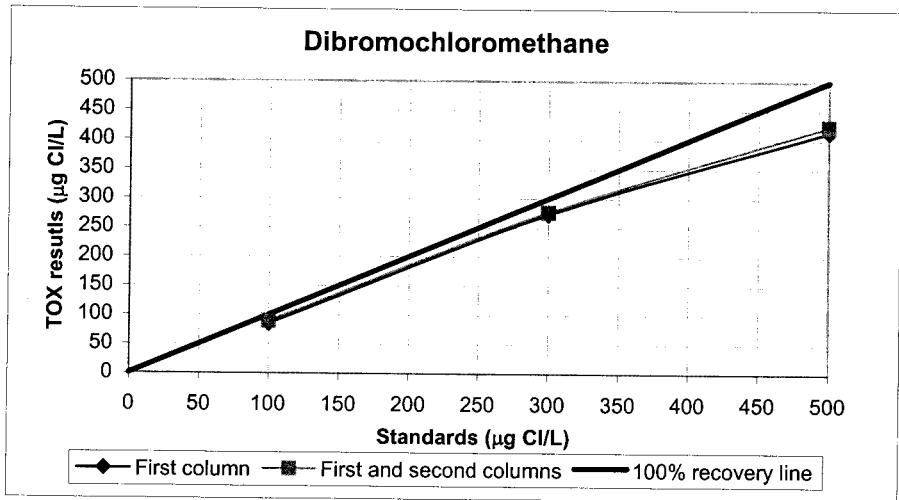
Figure 3: Recovery of Dibromochloromethane standards by TOX
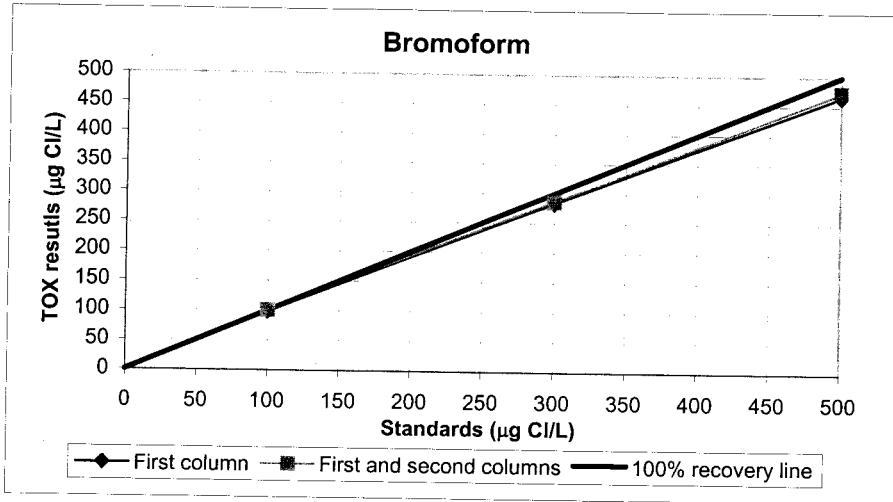
Figure 4: Recovery of Bromoform standards by TOX
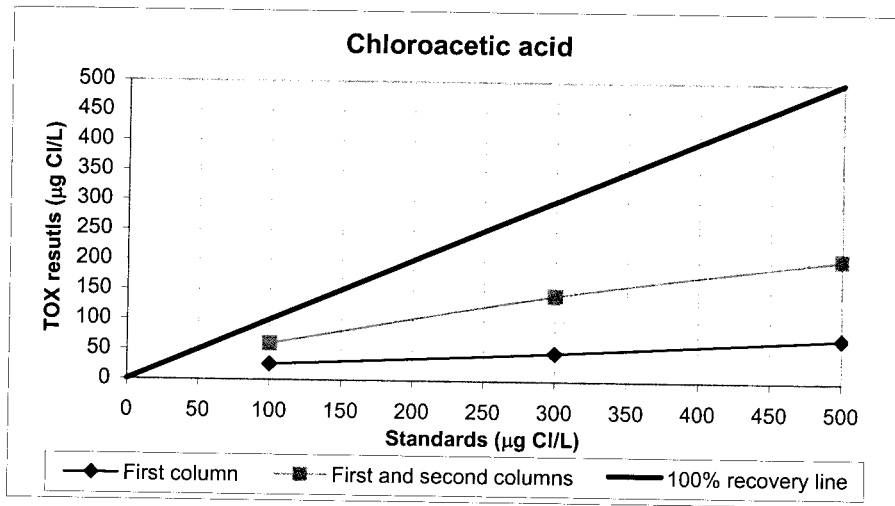
Figure 5: Recovery of Monochloroacetic acid standards by TOX
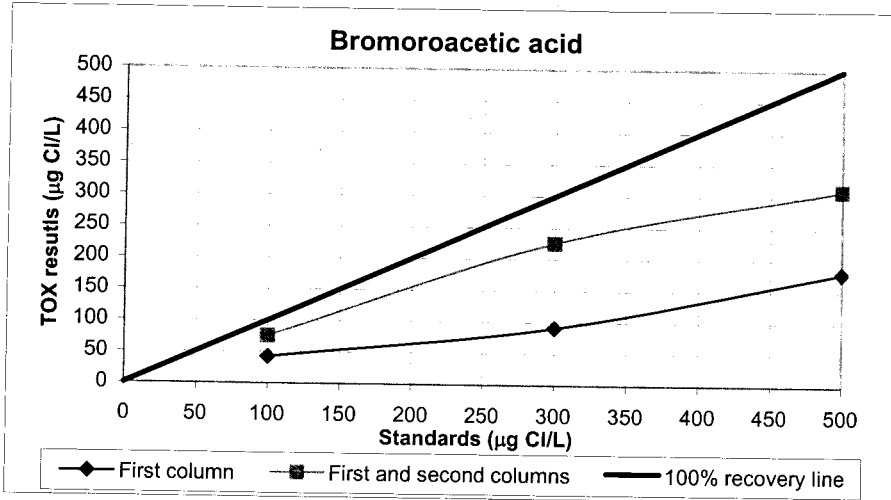
Figure 6: Recovery of Monobromoacetic acid standards by TOX
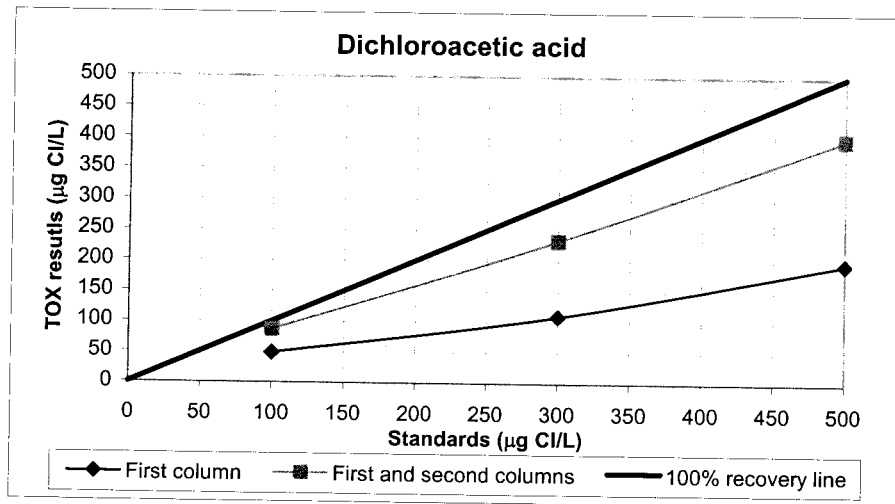
Figure 7: Recovery of Dichloroacetic acid standards by TOX

46
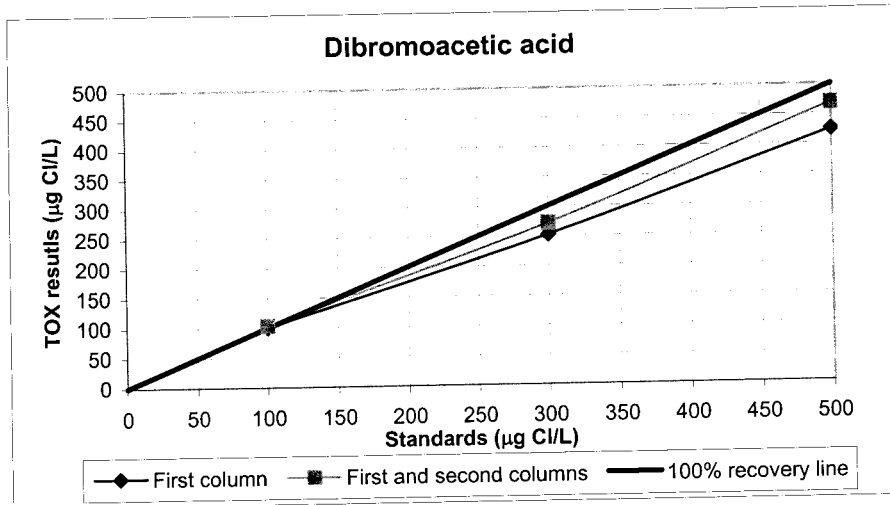
Figure 8: Recovery of Dibromoacetic acid standards by TOX
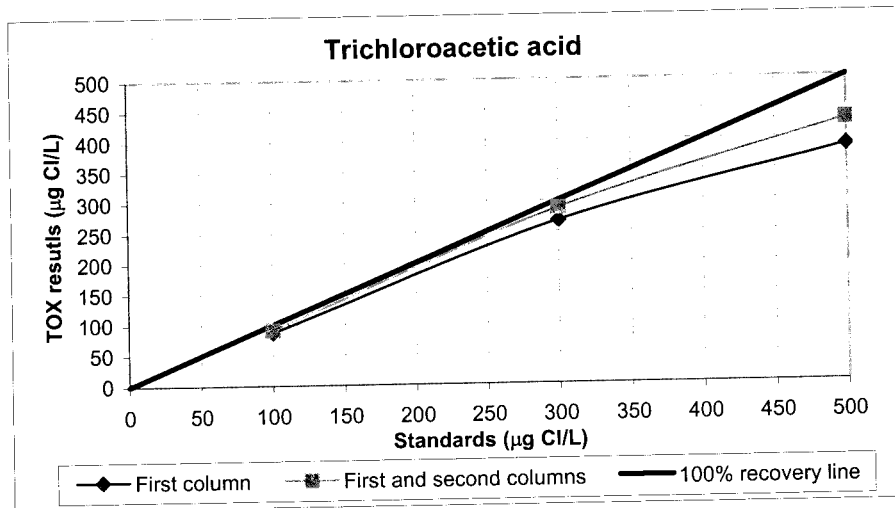
Figure 9: Recovery of Trichloroacetic acid standards by TOX

Impact of nitrate rinse volume and Cl⁻ concentrations on TOX measurement

From the TOX recovery testing of model compounds, it was observed that some HAAs were partly washed out during the nitrate rinse step. Further tests were conducted to evaluate the possibility of improving the recovery of TOX by reducing the nitrate rinse volume. The impact of the inorganic chloride on TOX measurement was also studied.

47

First, it is instructive to review the "standard" protocols as recommended by the two equipment manufacturers and by the generic standard. Nitrate rinsing methods for Euroglas and Dohrmann and Standard Methods are summarized below. It can be seen that the three methods are quite different as far as the concentration and volume of nitrate washing solution is concerned.

Euroglas
(1) Stock Nitrate Solution: Weigh out 17 g of $NaNO_3$, Transfer it to a 1000 ml measuring flask and add 1.4 ml nitric acid (HNO_3) 65%, top up the solution to 1000 ml.
(2) Nitrate Washing Solution: Pour 100 ml of the stock nitrate solution into a 1000 ml measuring flask and fill to 1000 ml.
(3) Wash microcolumns with 25 ml nitrate washing solution at a rate of 3 ml/min for 100 ml sample. This equals to 31.0 mg NO_3^-/sample.

Dohrmann
(1) A 5000 ppm nitrate solution is prepared by dissolving 8.2 gm of reagent grade KNO_3 in 1 liter of reagent water.
(2) Washing microcolumns with 2 ml nitrate washing solution at a rate of 0.5 ml/min for 100 ml sample. This equals to 10 mg NO_3^-/sample.

Standard Methods
(1) Dilute 8.2 g KNO_3 to 1000 ml with reagent water. Adjust to pH 2 with HNO_3. 1L = 5000 mg NO_3^-.
(2) Pass 2 to 5 mL NO_3^- solution through columns at a rate of approximately 1 mL/min. This equals to 10 mg to 25 mg NO_3^-/sample.

Recovery tests using dichloroacetic acid standards were conducted with different nitrate rinse volumes (Euroglass-based concentrations). Results are shown in Table 3. From Table 3, the recovery of DCAA increases form 78% to 95% when reducing the nitrate rinse volume from 30 mL to 13 mL. By comparing the TOX of the first and second columns, it becomes clear that the problem of analyte wash-out is greatly improved by reducing the nitrate rinse volume.

Table 3: Test on DCAA recovery with different Euroglas nitrate rinse volumes

Standards	Rinse Volume	Nitrate loading	First Column (μg Cl/L)	Second Column (μg Cl/L)	Overall Concentration (μg Cl/L)	Recovery
DCAA 300μg Cl/L	30 mL	37.3 mg NO_3^- /sample	107.5	125.4	232.9	77.6%
DCAA 300μg Cl/L	25 mL	31.0 mg NO_3^- /sample	141.4	112.2	253.6	84.5 %
DCAA 300μg Cl/L	13 mL	16.1 mg NO_3^- /sample	207.6	76.9	284.5	94.8%

The purpose of the nitrate wash is to remove the inorganic chloride from the carbon columns, thus removing the interference of inorganic chloride on TOX measurement. From the data presented above, reducing the nitrate rinse volume can improve the DCAA recovery. But the reduced nitrate rinse volume must also ensure adequate removal of inorganic chloride to guarantee unbiased TOX measurement. Tests were conducted to evaluate the impact of varying chloride concentrations and nitrate volumes on TOX measurement.

Protocol: Make chloride solutions with different concentrations, pass 100 ml of each solution through 2 carbon columns, then wash the columns with different nitrate washing volumes, measure TOX of columns by Euroglas analyzer.

Solutions:

(1) Nitrate washing solution: Dissolve 1.63g KNO_3 into 1000 ml deionized water, adjust pH to 2 by HNO_3 acid. 1L = 1000 mg NO_3.
(2) Chloride solutions: 0.5, 1.0 and 2.0 g Cl/L, adjust pH to 2 by HNO_3 acid

Table 4 presents a summary of the results of the test on the impact of varying chloride concentrations and nitrate rinse volumes on TOX. For DI water spiked with 0.5 g Cl/L, the use of either 10 or 15 mL of nitrate rinse solution gave measured TOX values that were nearly equal to the blank value. However, if the DI water is spiked to a 1.0 g Cl/L level, the measured TOX increases by 2.4 μg Cl/L compared to blank value if a 10 mL volume of nitrate rinse is used. In

contrast, the TOX can be brought back to the blank value, if 15 or 20 mL of nitrate rinse is used. For a DI solution containing 2.0 g Cl/L, measured TOX increases by 3 μg Cl/L and 6.2 μg Cl/L for nitrate rinse volumes of 20 mL and 15 mL, respectively. From this test, it is concluded that 15 mL of the 1000 mg NO_3^-/L rinse solution can achieve adequate removal of inorganic chloride when the influent level is 1 g Cl/L. Larger amounts of rinse would not be necessary, and could result in unwanted washout of weakly adsorbed organic halides. Therefore, it was decided that 15 mL of the 1000 mg NO_3^-/L rinse solution should be used for future TOX analyses. In cases of ambient chloride levels in excess of 1 g Cl/L, modifications in the protocol should be examined, such as use of larger rinse volumes or sample dilution. We don't anticipate that such high chloride levels will be encountered in this research.

Table 4: Impact of varying chloride concentrations and nitrate volumes on TOX

Cl⁻ (g/L)	Nitrate washing volume (ml)	First column (μg Cl/L)	Second column (μg Cl/L)	Total (μg Cl/L)
blank	15	5.3	5.7	11.0
0.5	10	6.0	4.8	10.8
0.5	15	5.4	5.6	11.0
1.0	10	7.6	5.8	13.4
1.0	15	6.4	5.3	11.7
1.0	20	6.7	4.6	11.3
2.0	15	8.9	8.3	17.2
2.0	20	6.1	8.0	14.0
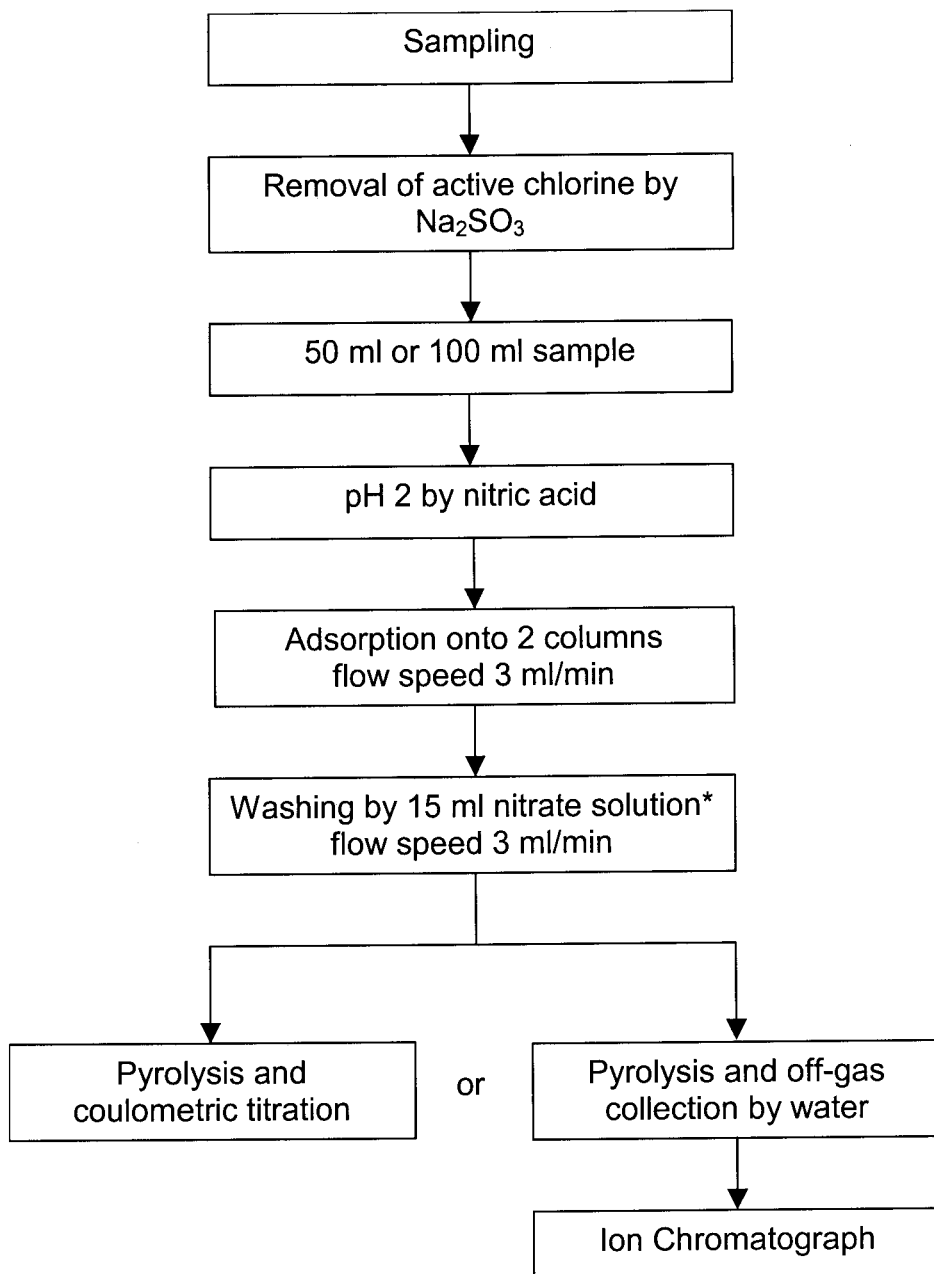
Figure 10: Summary of Refined TOX Analytical Procedure

* Nitrate rinse solution: Dissolve 1.63g KNO_3 into 1000 ml deionized water, adjust pH to 2 by HNO_3 acid. 1L = 1000 mg NO_3.

Testing the 3 GACs with Euroglass adsortion/pyrolysis

The three GAC materials chosen for this work include both commercial products offered by CPI Corporation. In addition, we selected a relatively new product (Filtrasorb-600) sold by

51

Calgon Corporation (Table 5). The latter carbon is not marketed for use with TOX analyzers; however, it is an especially high-purity, highly adsorptive product. This carbon is produced from coal, much like the Filtrasorb-400 that was commonly used in TOX analysis prior to the widespread use of CPI carbons. For this reason, F-600 was selected for further study. Use of this carbon required that it be hand ground in a mortal and pestle, followed by repeated sieving to achieve a relatively uniform 100/200 mesh.

Table 5: Three Activated Carbons Selected for TOX Analysis

| Characteristics[4] | Carbon Number | | |
	1 (standard)	2	3
Supplier	CPI	CPI	CALGON
P/N	475-002	475-001	F-600
Source	Coconut	Coconut	Coal
Particle Size	100-200 mesh	100-200 mesh	Granular[5]
Background	0.4 µgCl/40mg	≤1.0 µgCl/40mg	unknown

Blanks of the three carbons were measured using the Euroglas analyzer (Table 6). The results showed that all three achieved acceptably low values, quite close to the value advertised by the manufacturer (Table 5). As expected, carbon #1 had a slightly lower blank than #2. However, the freshly ground and sieved carbon #3 had a lower blank value than even #1 (0.24µg/40 mg). Furthermore, preliminary testing with a problematic standard (monochloroacetic acid) showed that whereas the two CPI carbons exhibited incomplete recovery (40-60%), the F-600 gave nearly complete recovery. Subsequent testing with monobromoacetic acid, another problematic compound, (Table 7) showed that F-600 and CPI-002 gave nearly complete recovery showed, whereas CPI-001 carbon exhibited incomplete recovery (79-89%). However, by comparing the TOX of the first and second columns (Figures 2-4), it is clear that much less bromoacetic acid was washed to second column of F-600 than CPI carbons. Similar results were also observed for monochloroacetic acid.

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[4] Information provided by manufacturer
[5] Requires grinding with mortar and pestle followed by extensive sieving.

Table 6: Blanks of three carbons

Carbon	Carbon Type	First column (µg Cl/L for 50 ml)	Second column (µg CL/L for 50 ml)	Average Concentration (µg Cl/L for 50 ml)	Background
1	CPI-002	9.7	9.6	9.7	0.49µg/column
2	CPI-001	11.3	11.4	11.4	0.57µg/column
3	F-600	5.5	4.1	4.8	0.24µg/column

TOX recovery with microcoulometric detection: Phase 2 tests

Table 7 and Table 8 summarize work done on TOX recovery from the second phase of model compound tests. This part of Task 1 was done using the following refined TOX analytical procedure (Figure 10).

Based on the data in Table 7, the recovery of bromoform is nearly complete using Euroglas analyzer, regardless of the particular carbon used:

• 96%-100% for Carbon CPI-002

• 96%-99% for Carbon CPI-001

In contrast, the recoveries of monobromoacetic acid are quite dependent on carbon types:

• 99%-100% for Carbon CPI-002

• 79%-89% for Carbon CPI-001

• 100% for Carbon F-600

Based on the data in Table 8, the recoveries of trichloroacetic acid and tribromoacetic acid are nearly complete when using Dohrmann analyzer and standard carbon (CPI-002).

- ~100% for trichloroacetic acid

- 94%-100% for tribromoacetic acid

Table 7: TOX standard tests using the Euroglas analyzer

Name	Molecular Formula	Carbon Type	Conc. (μg Cl/L)	TOX (μg Cl/L)			
				1st Column	2nd Column	Total	% Recovery
Bromoform	CHBr3	CPI-002	500	480.3	5.6	485.9	97.2%
			300	288.9	1.6	290.5	96.8%
			100	100.4	0.8	101.2	101.2%
		CPI-001	500	487.5	2.7	490.2	98.0%
			300	295.3	2.3	297.6	99.2%
			100	96.7	0	96.7	96.7%
Mono-bromoacetic Acid	CH2BrCOOH	CPI-002	500	343.5	160.5	504.0	100.8%
			300	227.2	71.5	298.7	99.6%
			100	88.0	23.0	111.0	111.0%
		CPI-001	500	179.6	215.9	395.5	79.1%
			300	117.8	119.5	237.3	79.1%
			100	51.1	38.3	89.4	89.4%
		F-600	500	450.9	67.1	518.0	103.6%
			300	278.6	41.4	320.0	106.7%
			100	98.1	10.2	108.3	108.3%

Table 8: TOX standard tests using the Dohrmann analyzer

Name	Molecular Formula	Carbon Type	Conc. (μg Cl/L)	TOX (μg Cl/L)			
				1st Column	2nd Column	Total	% Recovery
Trichloroacetic Acid	CCl3COOH	CPI-002	300	298.8	13.0	311.8	103.9%
			300	302.0	7.0	309.0	103%
			300	301.2	17.6	318.8	106.2%
Tribromoacetic Acid	CBr3COOH	CPI-002	300	287.0	0	287.0	95.7%
			300	283.4	0	283.4	94.5%
			300	302.2	2.0	304.2	100.5%

Testing the 3 GACs with Euroglas adsorption/pyrolysis
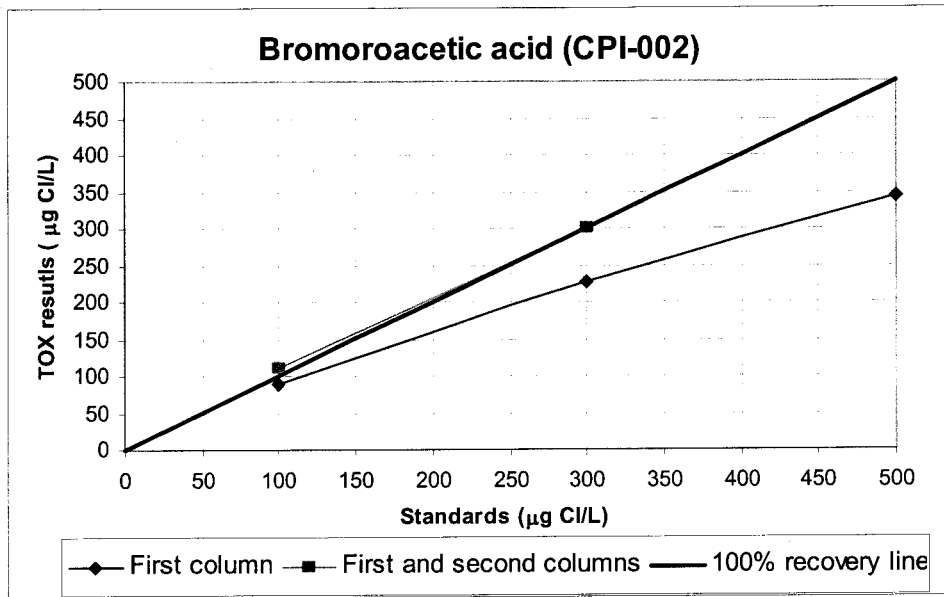
Figure 11: Recovery of Bromoacetic acid standards by Carbon CPI-002 and Euroglas Analyzer
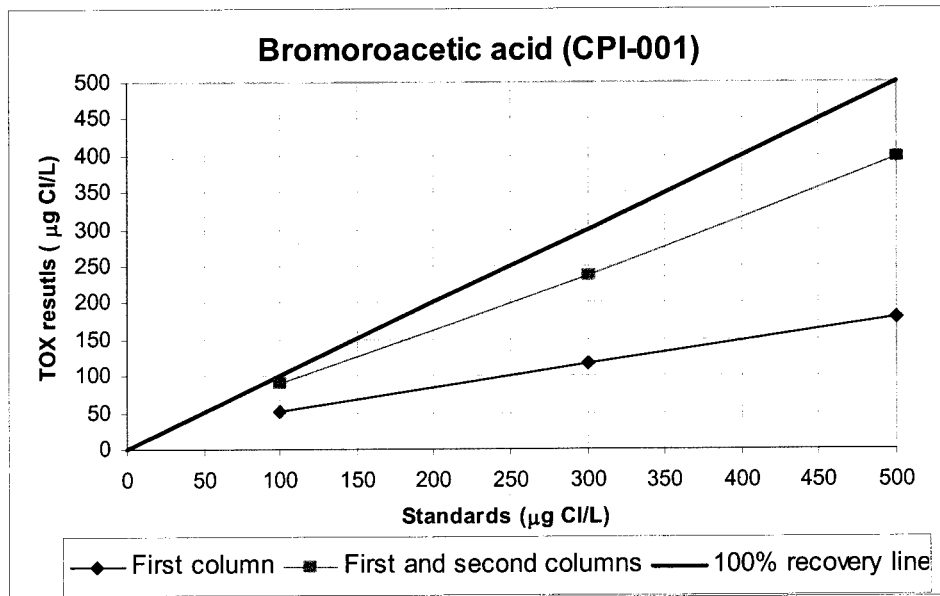
Figure 12: Recovery of Bromoacetic acid standards by Carbon CPI-001 and Euroglas Analyzer
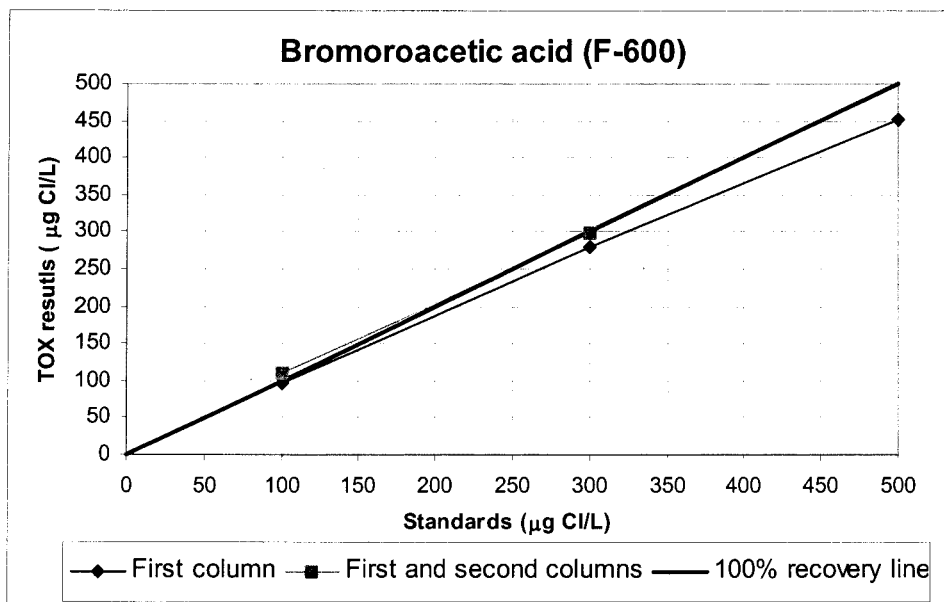
Figure 13: Recovery of Bromoacetic acid standards by Carbon F-600 and Euroglas Analyzer

As expected, those compounds that exhibited poor recovery also showed a substantial amount of carry-over into the 2nd column. Figure 14 shows this is a simple graphical form. Most of the data are clustered within a recovery area of 0.85 to 1.05 and within 0 to 0.1 for fraction in 2nd column. Those falling outside this are the two monohaloacetic acids and a dichloroacetic acid set.
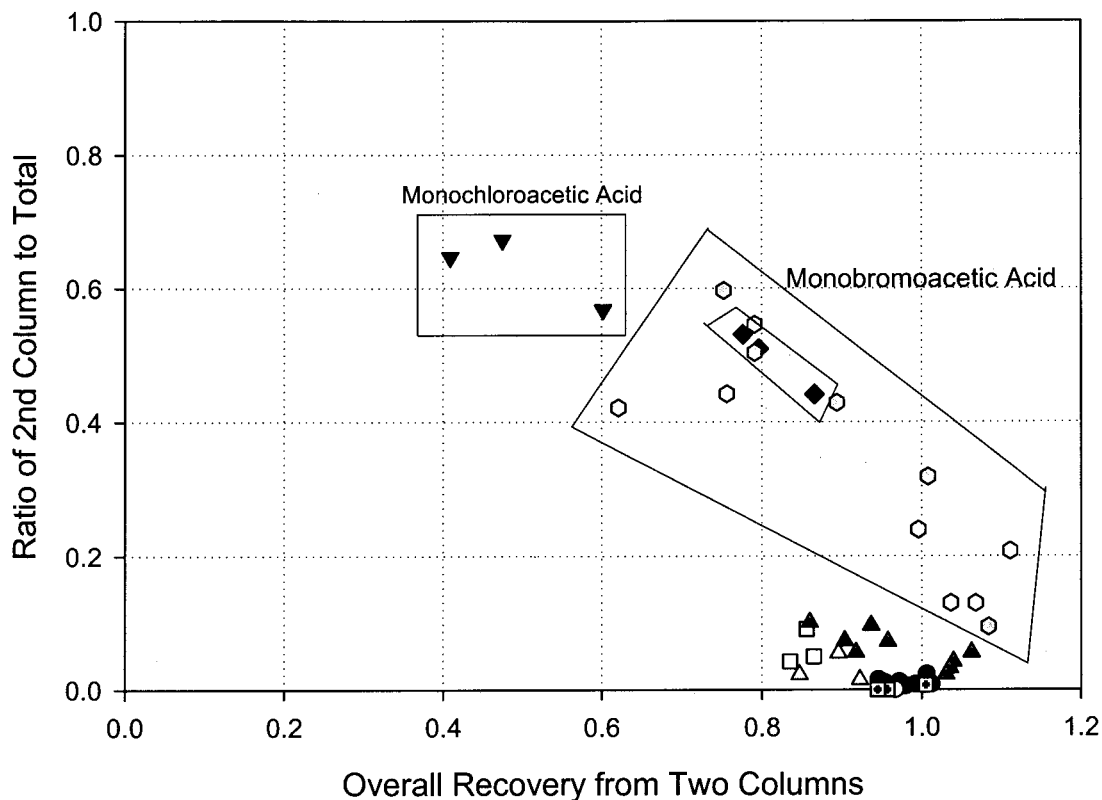
Figure 14. Relationship between Recovery and Carry-over to 2nd Column

ION CHROMATOGRAPHY OF THE HALIDES: TESTING AND REFINEMENT

The objective of this work was to identify an IC method (column phase and program, flow rates) that allows analysis of all three halides of interest (chloride, bromide and iodide) without substantial interference from other ions. Classical IC methods for chloride and bromide use conductivity detection, whereas iodide is most commonly associated with electrochemical detection. While iodide can be quantified by conductivity detection, it places certain constraints on the chromatography system. Experimentation at UMass and consultation with Dionex applications chemists led us to the conclusion that analysis of all three halides may not be feasible in a single IC run. The most common IC columns (e.g., AS-9, AS-11, AS-14) do not produce chromatograms with clear and quantifiable iodide peaks. Another phase (AS-16) does allow well-behaved iodide elution as appropriate to low level detection with the conductivity

detector (Figure 15). However, this phase does not resolve bromide from nitrate (compare Figure 15 with Figure 16). Subsequent testing at UMass showed that small amounts of nitrate from the nitrate rinse step are carried over into the pyrolysate trap. It may be possible to find alternatives to nitrate for removing inorganic halide from activated carbon. However, this sort of investigation was considered outside of the scope of the current project. Dionex applications chemists are aware of our dilemma, and have also concluded that commercial phases do not exist at present that would fully meet our needs. We will continue to keep our concerns in the minds of our Dionex contacts, as they work toward developing new columns. Until an ideal solution presents itself, we will use two separate columns for IC analysis, AS-14A for chloride and bromide, and AS-16 for iodide. The former uses 8 mM $NaCO_3$ as an eluent , and the latter uses 35 mM NaOH. Both will use a 1 mL/min flow rate and conductivity detection with chemical suppression.
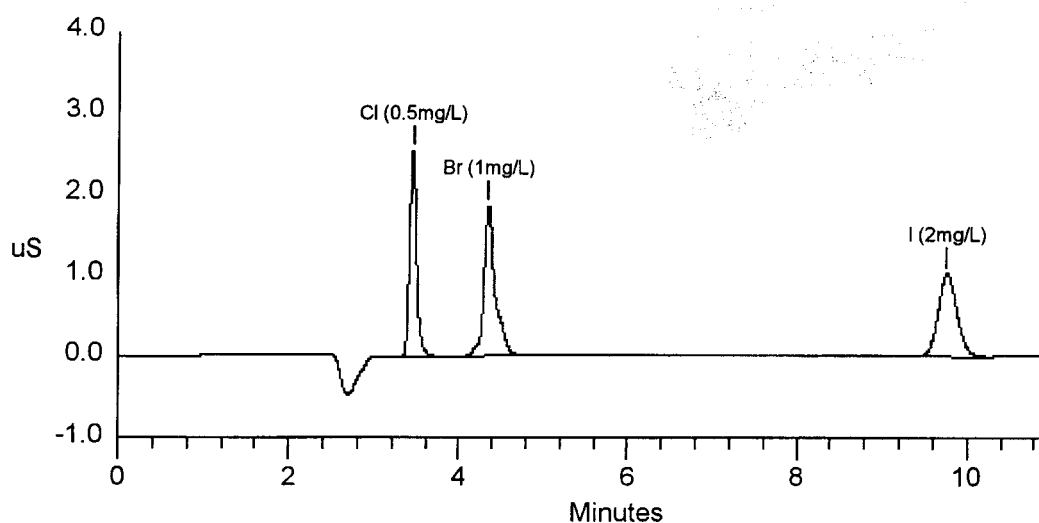
Figure 15: Ion Chromatogram of Three Halide Standards (AS-16 column)
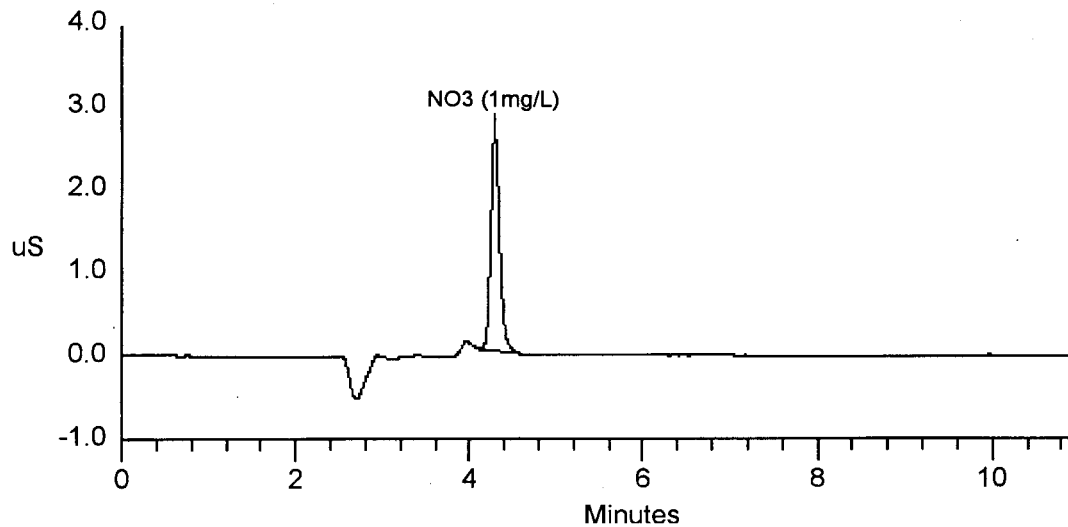
Figure 16: Ion Chromatogram of a Nitrate Standard (AS-16 column)

A DX-500 ion chromatography system with a conductivity detector was used for the halides analysis. IonPac® AS14A column (Dionex) was used for chloride and bromide analysis. IonPac® AS16 column (Dionex) was used for iodide analysis. A 100μl volume injection was used for all samples. Figures 7 and 8 show typical chloride and bromide standard curves from the UMass laboratory.
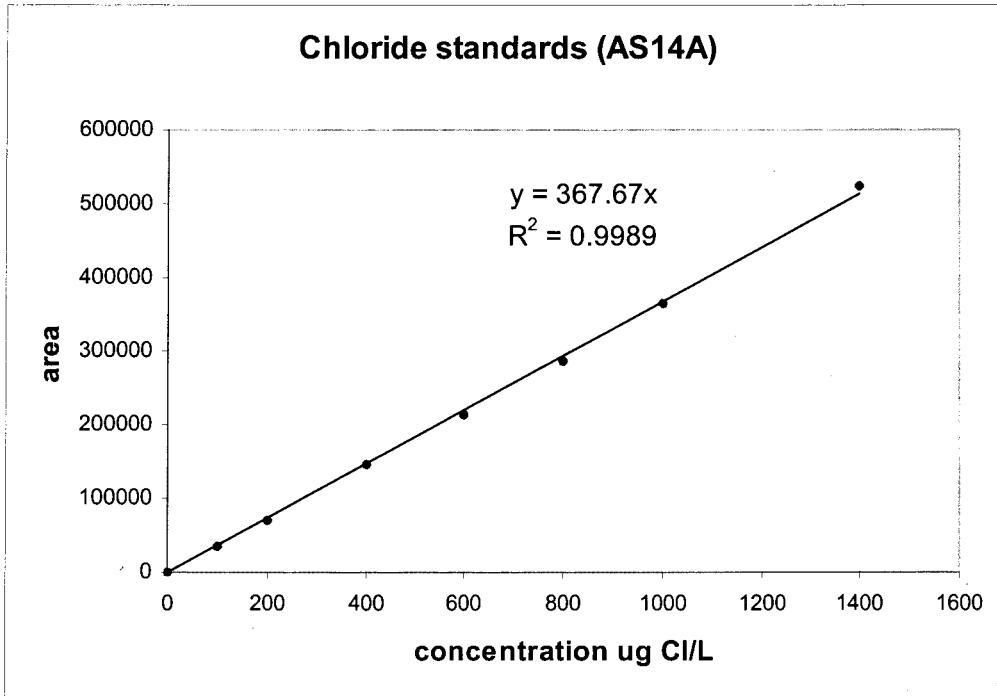
Figure 17. Chloride standard curve using the AS14A column
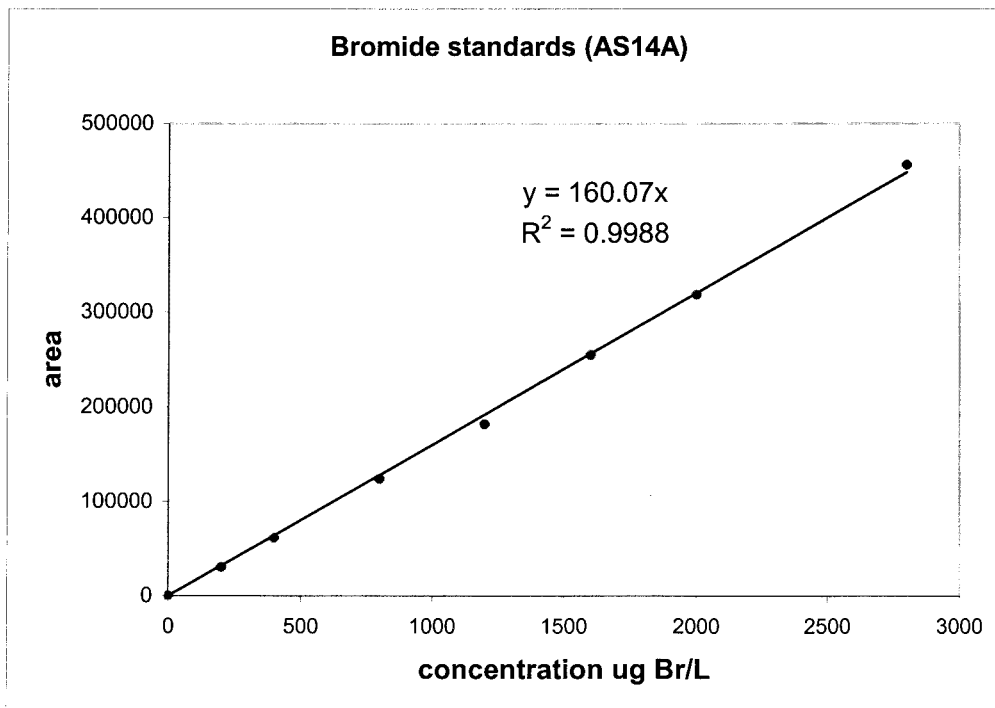
Figure 18. Bromide standard curve using the AS14A column

60

Combining Euroglass adsortion/pyrolysis with IC

Interference from Carbon Dioxide with the Dohrmann Analyzer

Preliminary experiments with the Dohrmann analyzer in connection with IC showed that an apparent bicarbonate peak (note that this analyzer uses carbon dioxide as an auxiliary gas) interferes with the chloride ion peak (Figure 19). The interference by this peak can be removed by sparging the sample with nitrogen gas for 5 minutes (Figure 20). No such interference was found when analyzing Euroglas analyzer pyrolysate with IC. Certainly there is some carbon dioxide that enters the trap solution, however a substantial amount of this is probably purged out during the trapping phase. Without the steady inflow of CO_2 from a carrier gas, the ending trap concentration is apparently quite low.
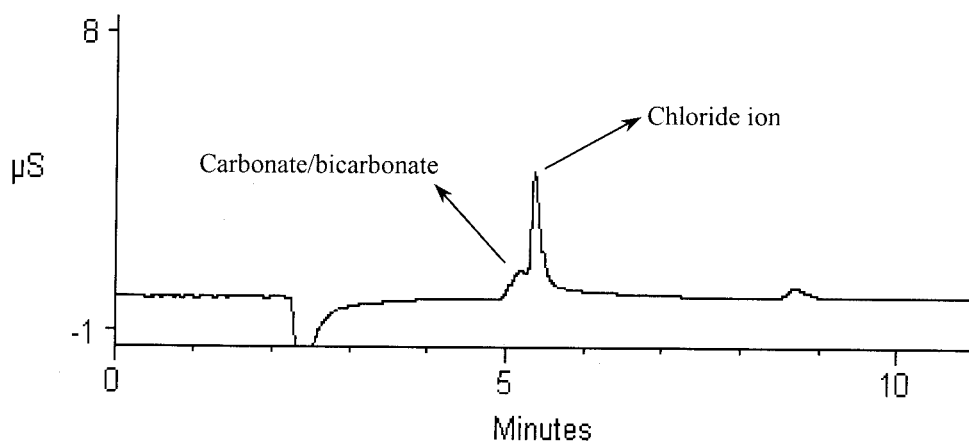
Figure 19 Ion Chromatogram of Unsparged Dorhmann Pyrolysate

61

Figure 20. Ion chromatogram of Sparged Dohrmann Pyrolysate

TOX recovery by combining adsorption/pyrolysis with IC

TOX standards were prepared in the same fashion as with the microcoulometric test. A 50 mL volume of each standard was passed through 2 carbon columns. After adsorption, the carbon columns were placed into the combustion tube of a TOX analyzer. Off-gas was collected into DI water. After combustion and absorption, the gas transfer tube was flushed with DI water to remove condensed halides in the tube. The total volume of water in the collecting tube was adjusted to exactly 20 mL before ion-chromatographic analysis. A 5-minute sparge step was applied to samples processed with the Dorhmann analyzer before ion-chromatographic analysis.

Preliminary testing with IC analysis was done by collecting off-gas from the Euroglass combustion tube. A 50 mL volume of a 300 µgCl/L bromoform standard was passed through 2 carbon columns and placed into the combustion tube of the Euroglas analyzer. Off-gas was collected by bubbling the furnace exit flow into a beaker with 50 mL water. The sample was analyzed by IC after a 10 min collection period. The result was a TOBr measurement of 303.5 µgCl/L, a 101% recovery.

This was repeated using different sparger designs, including fine bubblers and glass-fritted diffusers (Figure 21). Better mixing was evident with the fritted sparger. In addition, there were indications that recoveries were more reliable with this design. Accordingly, we

62

decided to adopt the fritted sparger design for future testing. The complete trap design incorporated a small volume (about 20 mL) of trapping liquid in a cylinder (Figure 22). The cylinder geometry was selected to achieve good mixing, and gas transfer without excessive bubbling and foaming. The trap base design for the two instruments was different so that it could accommodate the particular geometry of each analyzer. Similar design considerations had to be made with the inlet structure. Each trap was entirely made of borosilicate glass.
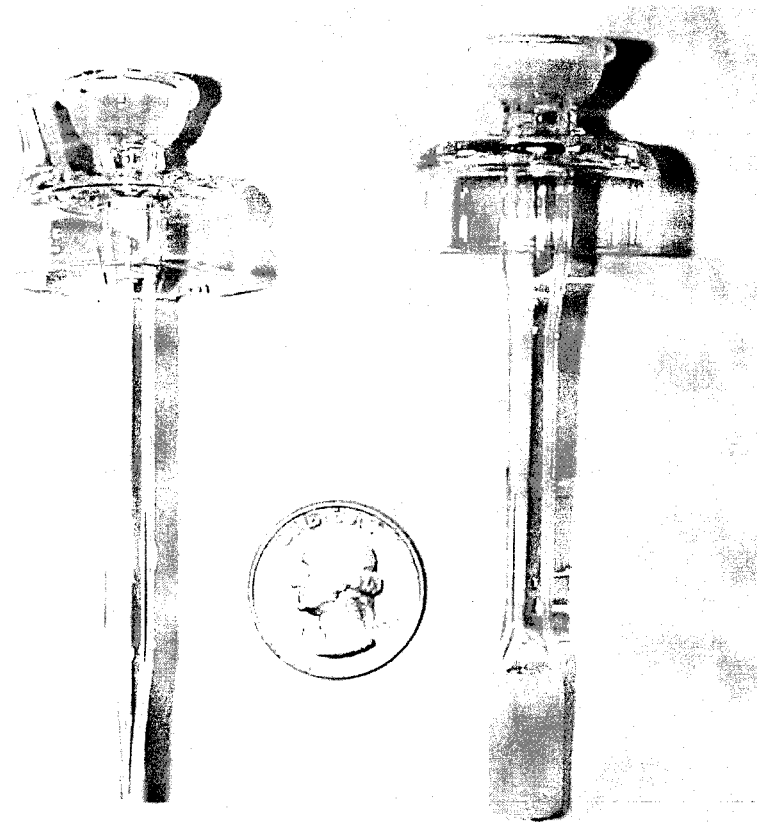
Figure 21: Fine Bubble and Glass Frit Spargers Tested
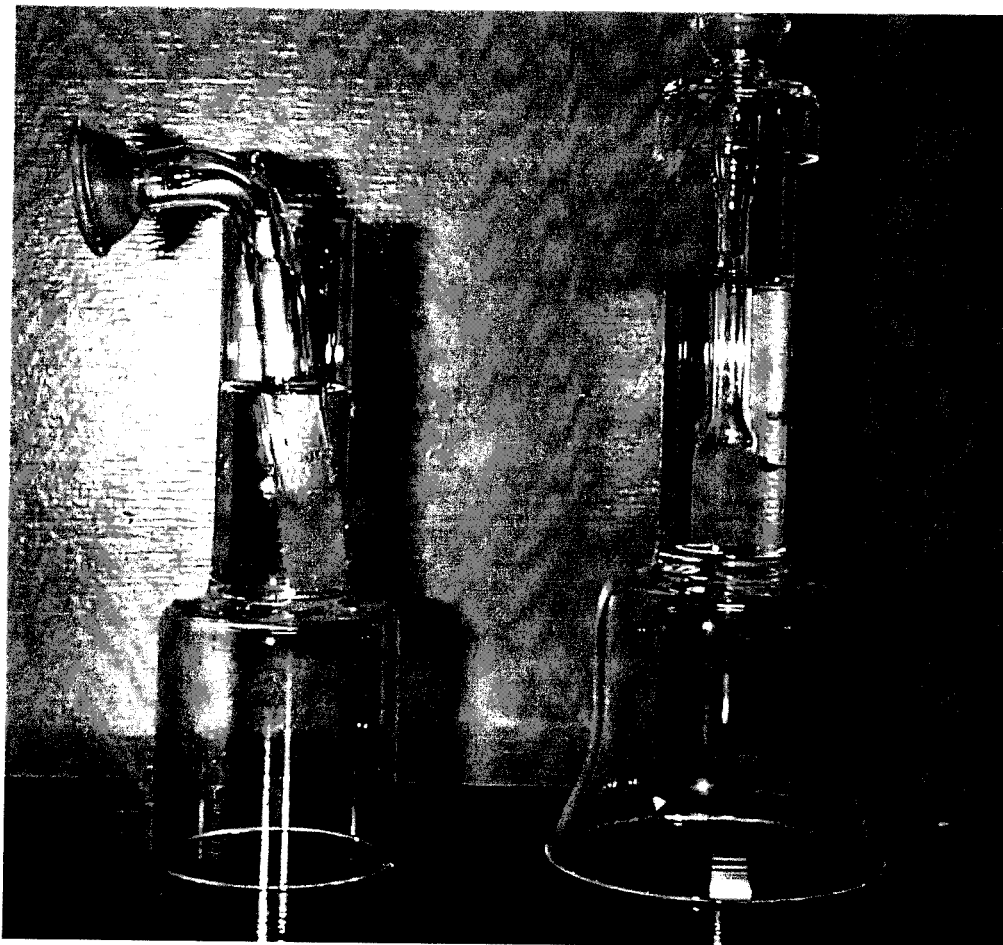
Figure 22. Final Design for the Halide Traps for the Dohrmann (left) and Euroglas (right) Instruments

Table 9 summarizes the TOX recoveries by combing adsorption/pyrolysis and IC. Standard carbon (CPI-002) was used in these tests. The results these early tests showed that the recoveries of trichloroacetic acid and tribromoacetic acid are nearly complete by both analyzers, although the Euroglas analyzer showed slightly higher recoveries than the Dohrmann analzer, the difference is probably not significant.

Table 9: TOX standard tests by combining adsorption/pyrolysis and IC.

Name	Molecular Formula	Carbon Type	Analyzer	Conc. (μg Cl/L)	TOX (μg Cl/L)			
					1st Column	2nd Column	Total	% Recovery
Trichloroacetic Acid	CCl₃COOH	CPI-002	Euroglas	300	296.8	15.3	312.1	104.0%
			Dohrmann	300	289.5	9.5	299.0	99.7%
Tribromoacetic Acid	CBr₃COOH	CPI-002	Euroglas	300	294.4	7.0	301.4	100.5%
			Dohrmann	300	275.5	7.5	283.0	94.3%

GC METHOD DEVELOPMENT FOR IODINATED DBPS

It was our intention that we would acquire or synthesize as many of the iodinated THMs and HAAs as feasible within the budget. We would use these for the purpose of refining the existing methods so that the full suite of compounds (i.e., including those containing iodine) could be analyzed. Analysis would be fully quantitative where authentic standards of known purity were available. If this was not possible, we would present a semi-quantitative estimate based on analogous sensitivities and recoveries.

From the beginning of the project, the synthesis of iodinated DBPs was pursued. Aside from iodoform and monoiodoacetic acid, none of the common DBPs are generally available as iodinated analogues. With the assistance of Susan Richardson and Stewart Krasner, we found a synthetic chemist who had previously made the iodinated THMs. For a fee, we were able to retain his services for the production of about 200 mg of each of the six iodinated THMs. These were used to prepare aqueous solutions that were subsequently extracted in pentane and analyzed by GC/MS (Varian ion trap). In each case, multiple peaks were observed, indicating the presence of substantial levels of contaminants. In at least one case, we were unable to find any peaks with mass spectra indicative of an iodinated methane.

We corresponded with the supplier and their analytical chemist regarding this purity issue. There was some acknowledgment by the supplier that purity had not been verified. There was also some uncertainty on our end as to whether the compounds could have partly degraded upon transit, storage or preparation of standard solutions. As a gesture of good will, the supplier offered to send us more samples for testing. However, at that point our Varian GC/MS had begun to fail, and we were looking forward to using the new Waters GC/MS once it arrived.

During the first 4 months of the project, Dr. Onu attempted to synthesize several iodinated acetic acids. We now have three of these in a crystalline form, but GC analysis of two of these also showed them to be of low purity. The third has yet to be subject to GC/MS analysis. We expect to look at this one with the new instrument.

In September 2003, the GC-TOF arrived at UMass, and it was installed later in the fall. Since that point, we have been able to verify the purity of our iodinated THM standards and do some quantitative work with them. The installation of the GC-TOF has been plagued by problems, and as of this writing the field engineers are still trying to verify proper operation.

CHAPTER 5: FIELD TESTING OF TOX METHODOLOGIES

This chapter presents the data on extensive testing of two contrasting waters in accordance with Task 1b.

INTRODUCTION

Task 1b experiments are intended to make use of two contrasting groups of precursors for production of a "natural spectrum" of TOX compounds that can be used to test the various methodologies. The waters selected for this task are raw waters from Tulsa's Jewell plant and from the city of Winnipeg. The former is largely allochthonous and the latter is heavily autochthonous. The waters used in Task 1b were to be treated with chlorine after being dosed with varying levels of bromide and iodide ion (Figure 23). The purpose is to form a range of unknown brominated and iodinated byproducts that can be tested for relative recovery by the various TOX protocols. Addition experiments have also been run where the halide ions are added after quenching the chlorine. The purpose here is to see if bromide or iodide ions will interfere with TOX measurements using these protocols.
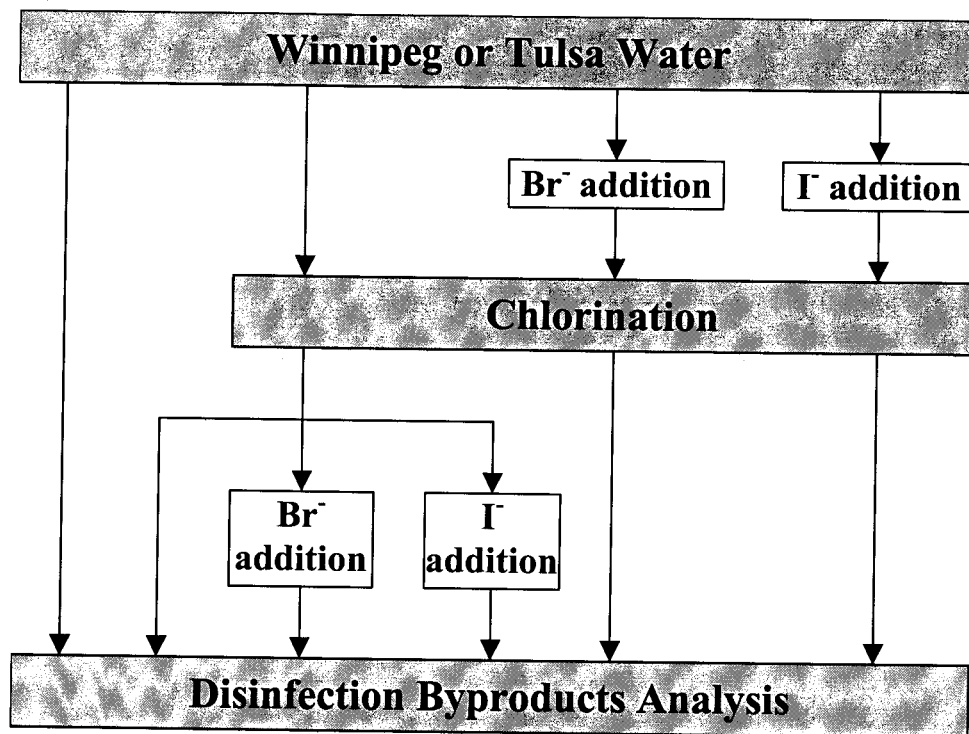
Figure 23. Raw water chlorination test schematic for Task 1b

RAW WATER SAMPLES

Bulk samples were collected by utility personnel in late 2003 and shipped in refrigerated containers to UMass by overnight carrier. The water quality of the two raw water samples was typical of the historic values for the two plants (Table 10). This Winnipeg water has an exceptionally low SUVA, considering its high TOC. Much of this organic matter is expected to be from algal activities. The Tulsa water had a higher SUVA, but not quite as high as is typically observed in the summer.

Table 10. Water Quality of Samples Collected for Task 1b

Sample Location	Date of collection	TOC (mg/L)	DOC (mg/L)	UV_{254} (cm-1)	SUVA (L/mg/m)	Br^- (μg/L)	pH
Winnipeg	08/21/03	8.50	8.47	0.131	1.55	9	7.5
Tulsa	12/11/03	5.33	5.13	0.160	3.12	63	7.6

WINNIPEG TESTS

Preliminary Chlorination Demand Test

The first experiment that was run with the raw water was a chlorine demand study. A dose/residual curve has been developed so that we could estimate the appropriate dose to achieve the desired residual (0.5 mg Cl_2/L) at the end of the contact time (48 hours) at the desired temperature of 20°C. The pH of water samples was adjusted to 7 with a phosphate buffer prior to chlorination.
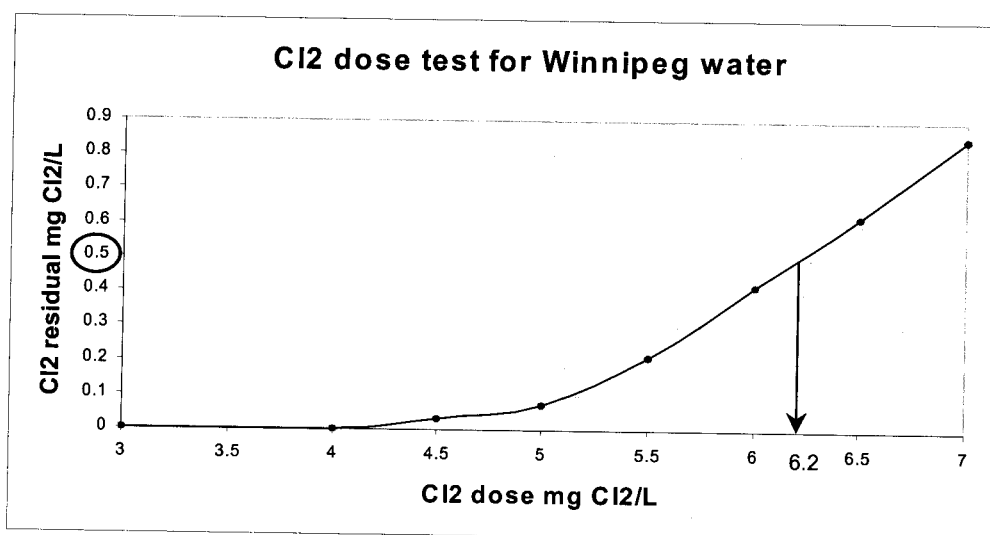
Figure 24. Chlorine demand test on Winnipeg water

The dose that was adopted for bench-scale chlorination tests was based on this demand curve. The value chosen was 6.2 mg Cl_2/L.

Treated Water Chlorine Residuals

Three levels of Br^- and I^- ions addition were chosen for this test, 2, 10 and 30 µmole/L.

Chlorine residuals were measured using the DPD method for all treated samples after 48 hours incubation time. The key numbers (#1 to #14) represented in Figure 25 correspond to the different points for which chlorine residuals were measured.
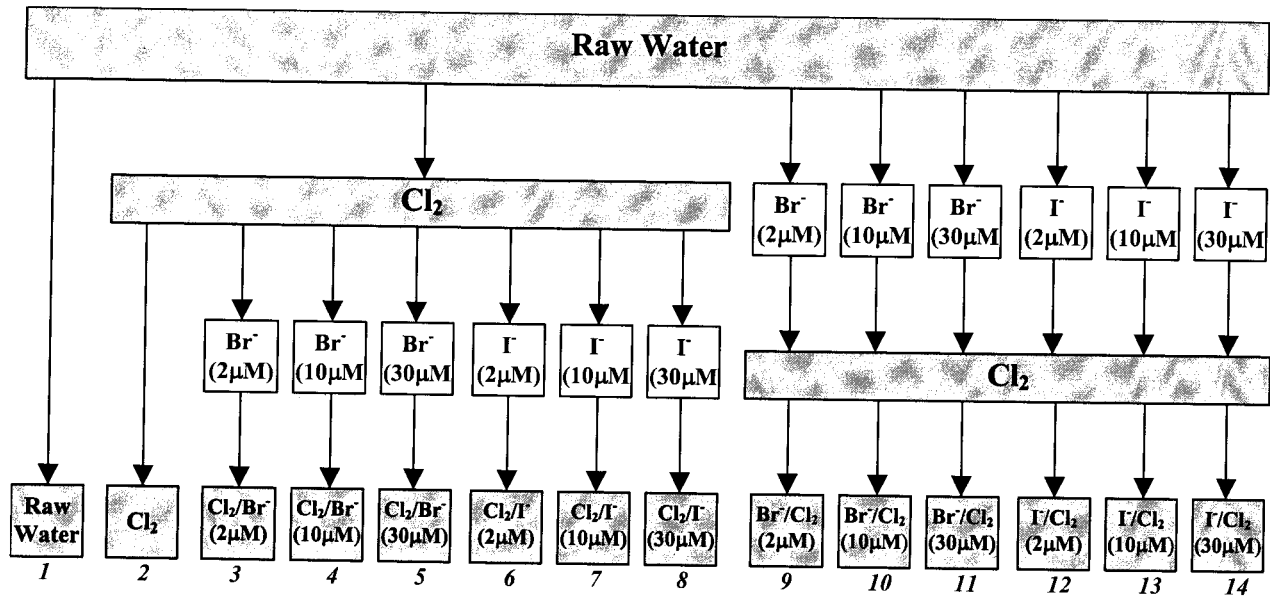
Figure 25. Sample Numbering Key for Task 1b Chlorination Test

Table 11 shows chlorine residual and pH of the treated water samples. The chlorinated water samples without bromide and iodide addition before chlorination (#2-#8) show a chlorine residual range of 0.46-0.5 mg Cl_2/L, which is very close to the target value of 0.5 mg Cl_2/L. Chlorination following bromide and iodide addition shows a higher chlorine demand (samples #9-#14 vs samples #2-#8). The chlorine residuals of bromide and iodide addition at level of 2 μmole/L are 0.28 and 0.26 mg/L respectively. The chlorine residual was essentially depleted at the end of 48 hour contacting when increasing the bromide and iodide concentrations to 10 and 30 μmole/L (samples #10, #11, #13 and #14). These data indicate that added bromide and iodide ions were involved in the chlorination, and produced higher chlorine demand. Brominated and iodinated products are expected in these samples.

Table 11: Chlorine residuals and pH of the treated Winnipeg water samples

Number	Sample	Chlorine Residual after 48 hr incubation (mg/L)	pH after 48 hr incubation
1	Raw water		
2	Cl_2	0.46	7.28
3	Cl_2 /Br$^-$ (2μmole/L)	0.50	7.29
4	Cl_2/Br$^-$ (10μmole/L)	0.50	7.28
5	Cl_2/Br$^-$ (30μmole/L)	0.48	7.31
6	Cl_2/I$^-$ (2μmole/L)	0.47	7.30
7	Cl_2/I$^-$ (10μmole/L)	0.47	7.33
8	Cl_2/I$^-$ (30μmole/L)	0.47	7.34
9	Br$^-$/Cl_2 (2μmole/L)	0.28	7.27
10	Br$^-$/Cl_2 (10μmole/L)	0.00	7.42
11	Br$^-$/Cl_2 (30μmole/L)	0.00	7.40
12	I$^-$/Cl_2 (2μmole/L)	0.26	7.42
13	I$^-$/Cl_2 (10μmole/L)	0.00	7.38
14	I$^-$/Cl_2 (30μmole/L)	0.00	7.30

Specific DBPs

THMs, HAAs and TOX were analyzed for all of the treated water samples. As expected, increasing bromide levels resulted in a shift in the THM and HAA speciation to the more brominated forms (Figure 26 to Figure 29). It is interesting to note that the TTHM concentration on a molar basis increases substantially with bromide (Figure 30). In contrast, the HAA9 only increases slightly (Figure 31). Not surprisingly, the HAA5, which is heavily weighted toward the chlorinated forms, decreases with increasing bromide.
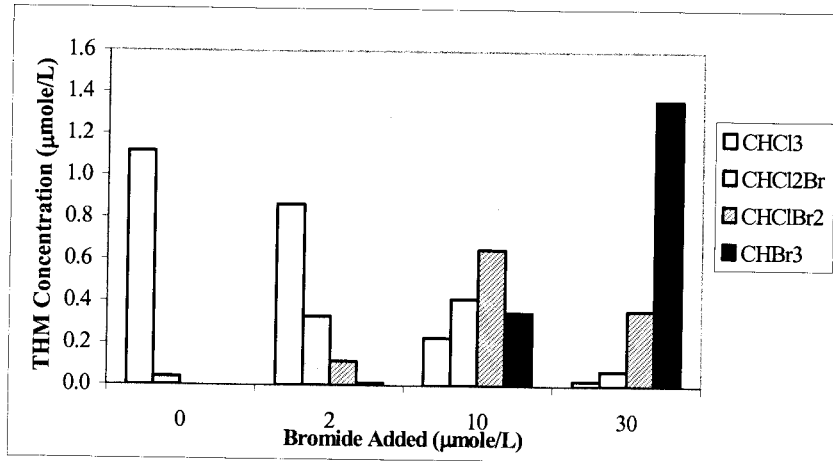
Figure 26. Winnipeg Water: THM Concentrations versus Added Bromide.
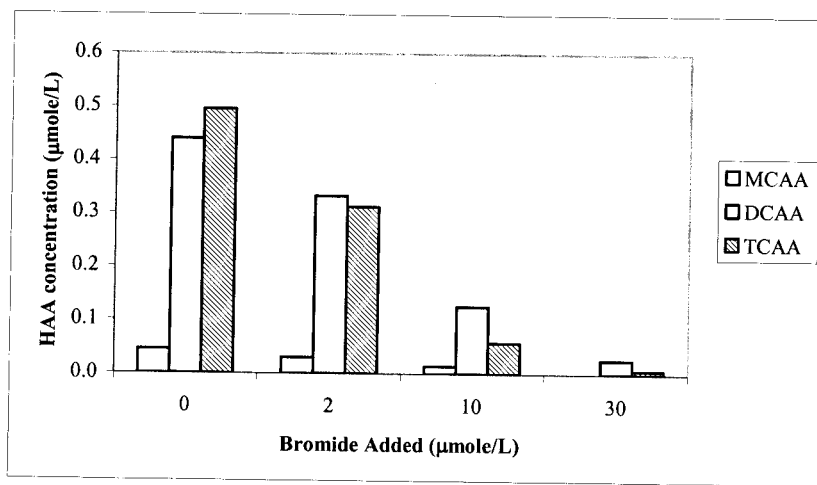
Figure 27. Winnipeg Water: Chlorinated HAA Concentrations versus Added Bromide.

72
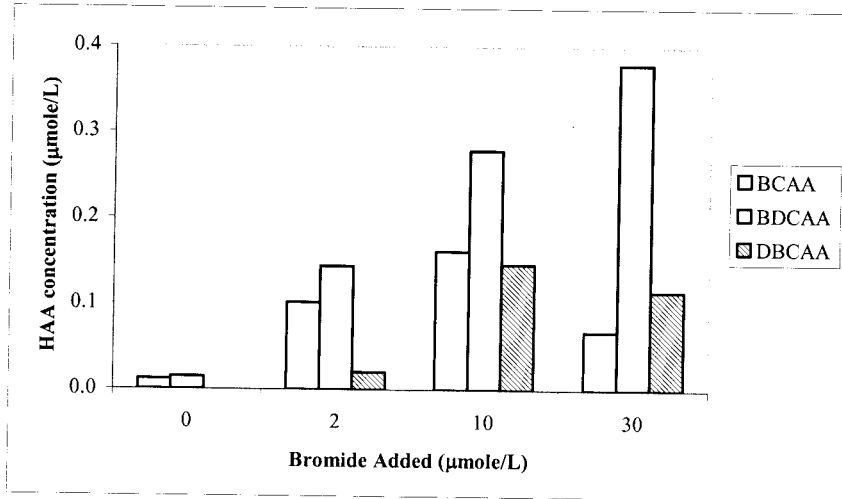
Figure 28. Winnipeg Water: Mixed HAA Concentrations versus Added Bromide.
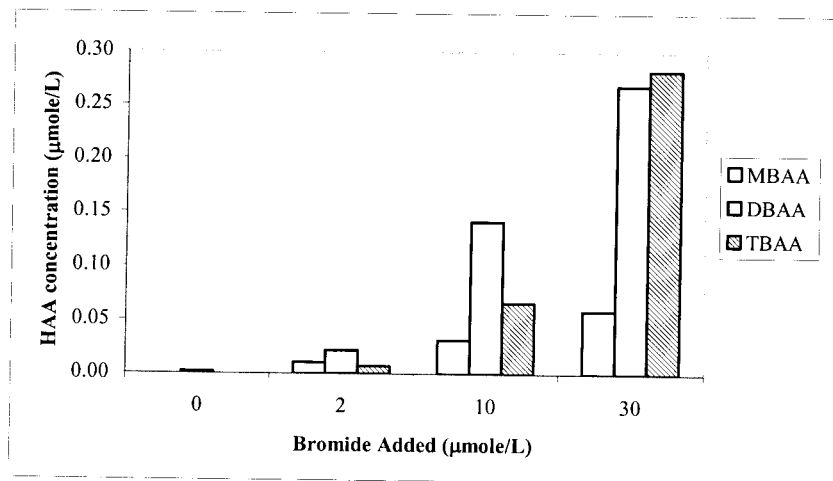
Figure 29. Winnipeg Water: Brominated HAA Concentrations versus Added Bromide.
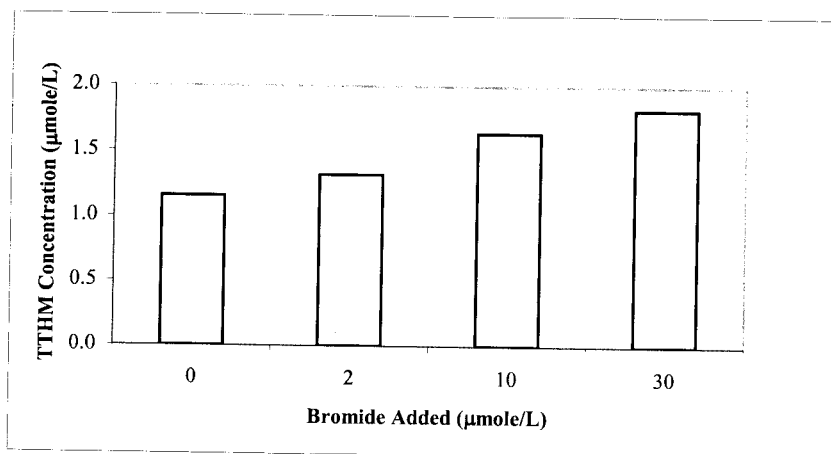
Figure 30. Winnipeg Water: Molar TTHM versus Added Bromide.
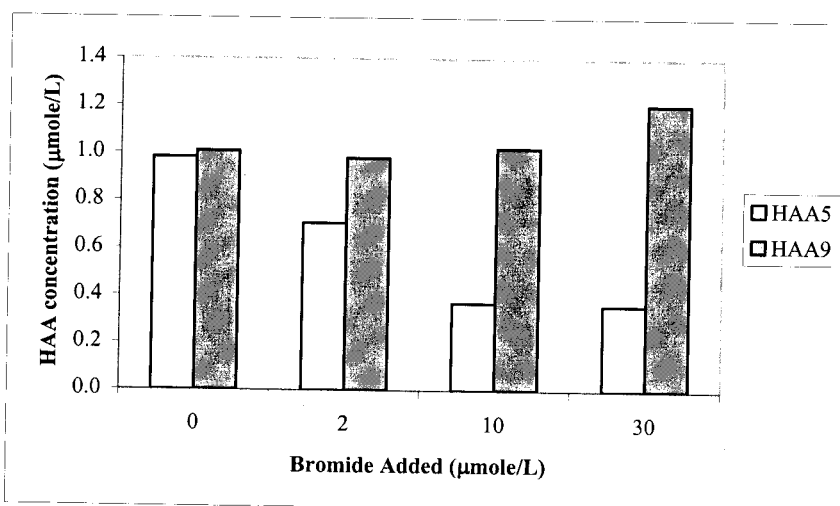
Figure 31. Winnipeg Water: Molar HAA5 and HAA9 versus Added Bromide.

Calculation of the bromine incorporation ratio (or the analogous chlorine incorporation ratio) is sometimes a clearer way of seeing the impacts of bromide on speciation. Figure 32 and Figure 33 show these values for the THMs and HAAs, respectively. Because the HAAs comprise 3 distinct classes of byproducts (mono, di and tri halogenated acetic acids), it's more appropriate to separate these out when considering chemical phenomena. Figure 34 shows the bromine incorporation factors for these three HAA groups as well as the THMs. The reader should note that the trihaloacetic acids generally show less bromine incorporation than the THMs. This is a phenomenon that we have noted with the ICR data as well.

74

Comparison across species groups with different numbers of halogens is best done with a molar fraction metric. Whereas the bromine incorporation factor ranges from zero to 1, 2 or 3 depending on the species; the bromine/halogen molar fraction will always range from 0 to 1 regardless of the total number of halogens. Figure 35 shows how the bromine/halogen molar fraction changes for different levels of bromide and for different classes of DBPs. These data suggest that the DHANs are most readily populated with bromine atoms and the THAAs are least likely to be brominated.
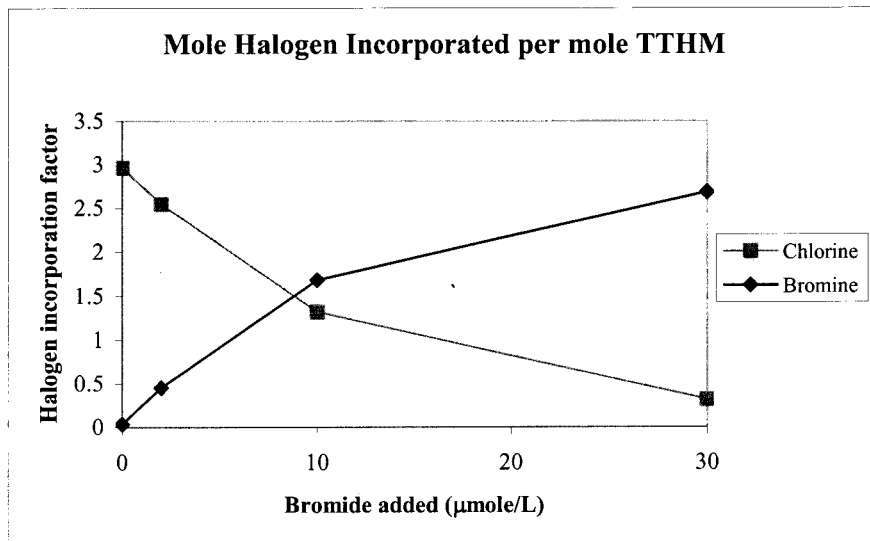
Figure 32. Winnipeg Water: Halide Incorporation in THMs versus Added Bromide.
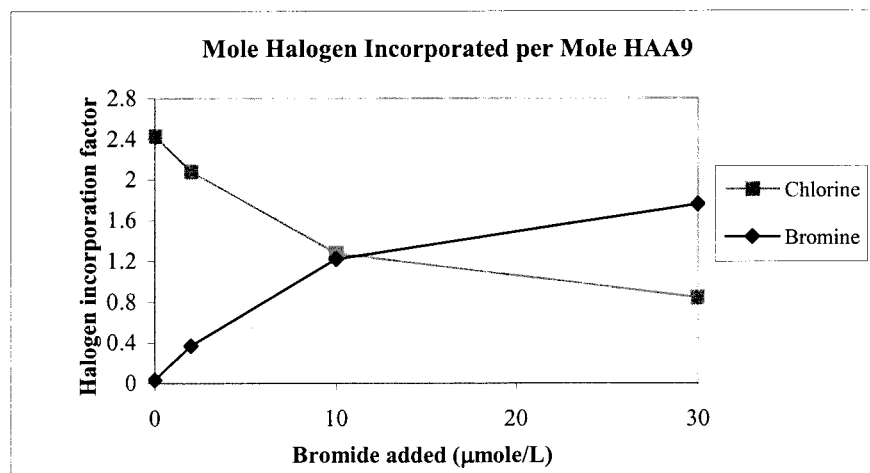
Figure 33. Winnipeg Water: Halide Incorporation in HAAs versus Added Bromide.
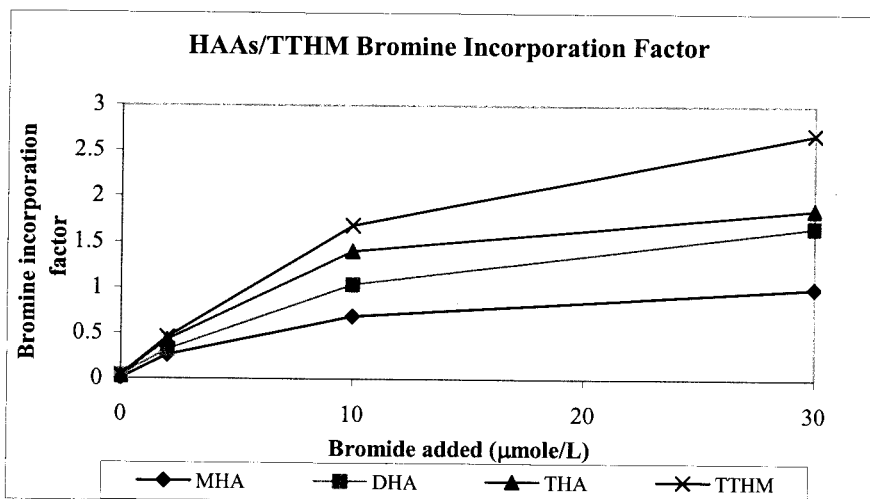
Figure 34. Winnipeg Water: Halide Incorporation in DBP Families versus Added Bromide.
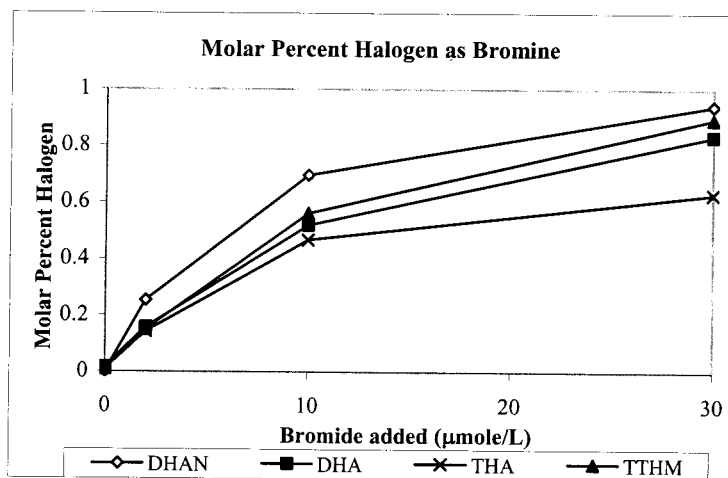
Figure 35. Winnipeg Water: Bromine/halogen Molar Fraction versus Added Bromide

Addition of iodide produces iodinated byproducts much like bromine leads to brominated compounds. There is a substantial speciation shift in the THMs as iodide is added with chloroform declining and the iodinated forms becoming more prominent (Figure 36). With small ambient levels of bromide, there is a persistent presence of bromodichloromethane, however no other brominated forms were detected in these experiments. It is interesting to note that the species profile at the highest iodide level appears anomalous and bimodal. This behavior is not

76

generally seen with analogous bromide spiking studies. Careful review of the data failed to indicate any systematic error. There may be some kinetic reasons for the relatively low level of chlorodiiodomethane as compared to the iodoform and Dichloroiodomethane.
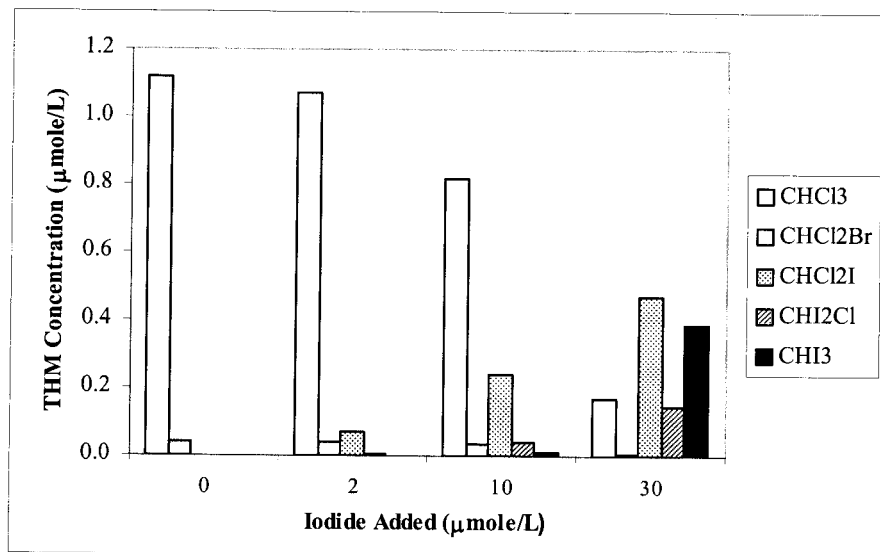
Figure 36. Winnipeg Water: THM Concentrations versus Added Iodide.

Data from the Winnipeg tests indicates that iodide is somewhat less reactive than bromide as measured by tendency to form THMs. First, there is almost no change in molar TTHM level with increasing iodide (Figure 37). This is in contrast to the bromide tests. Second, the chlorine incorporation factor requires higher levels of iodide (molar basis) before it starts to decline as compared to the case with bromide (Figure 38). This may be a kinetic effect, a steric effect, or a manifestation of the instability of some iodinated intermediates.
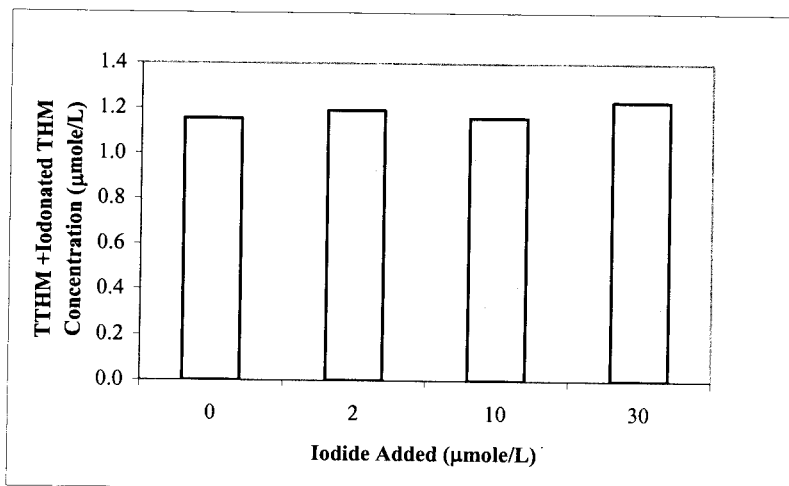
Figure 37. Winnipeg Water: Molar TTHM versus Added Iodide
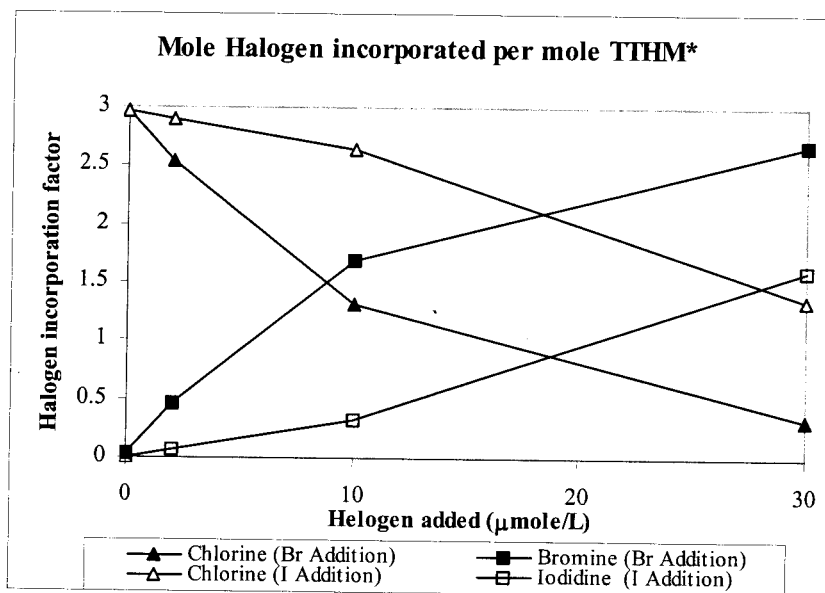
Figure 38. Winnipeg Water: THM Halogen Incorporation versus Added Halide

At present we do not have authentic standards for the iodinated HAAs, so we can only reliably quantify the chloro-bromo species. Neverthess, the HAA show a clear decline in TCAA and DCAA with increasing iodide levels (Figure 39 and Figure 40). This is analogous to the decline in chloroform in Figure 36. Note that TCAA drops more abruptly than DCAA, as expected based on the larger number of possible iodinated trihaloacetic acids.
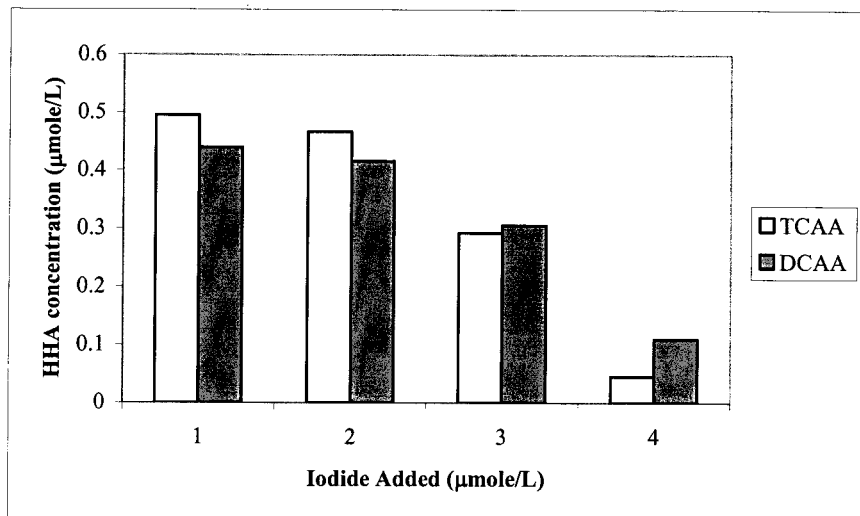
Figure 39. Winnipeg Water: TCAA and DCSS Concentrations versus Added Iodide.
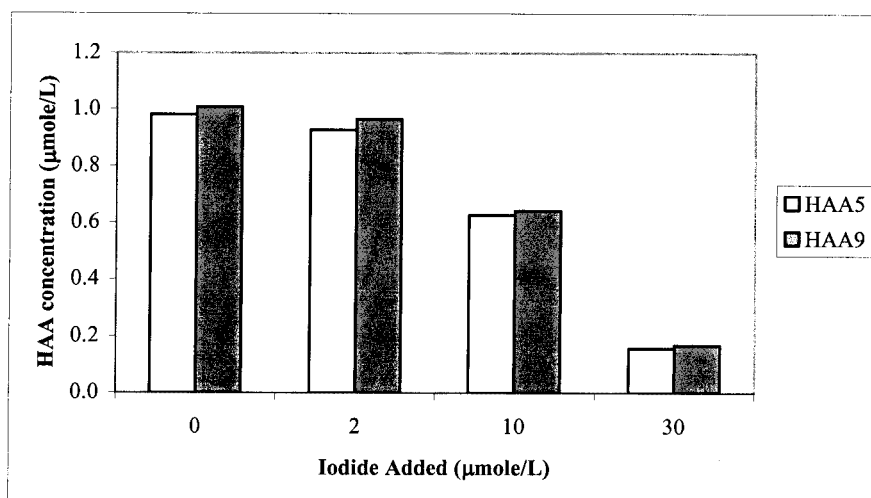
Figure 40. Winnipeg Water: HAA5 and HAA9 Concentrations versus Added Iodide.

Total Organic Halides

Table 12 presents the classical TOX data from this experiment. While there are differences among the three carbons in their degree of breakthrough (to the second column), all seem to show a similar trends. The brominated compounds tend to show less breakthrough than the chlorinated ones (compare 2nd GAC columns for raw and 30 uM bromide).. It also appears that the iodinated compounds are even better retained on the GAC than the brominated ones.

Table 12. TOX results for Task 1b Winnipeg water

Samples	Carbon Type	Analyzer	TOX (μg Cl/L)		
			1st Column	2nd Column	Total
Raw water/Cl₂	CPI-002	Euroglas	481	102	582
	F-600	Euroglas	381	164	545
	CPI-002	Dohrmann	499	73	572
Br/Cl₂ 2μM	CPI-002	Euroglas	456	105	561
	F-600	Euroglas	356	146	502
	CPI-002	Dohrmann	494	75	569
Br/Cl₂ 10 μM	CPI-002	Euroglas	461	64	525
	F-600	Euroglas	361	110	471
	CPI-002	Dohrmann	488	23	511
Br/Cl₂ 30 μM	CPI-002	Euroglas	565	44	609
	F-600	Euroglas	443	106	549
	CPI-002	Dohrmann	566	22	588
I/Cl₂ 2μM	CPI-002	Euroglas	490	97	587
	F-600	Euroglas	399	169	567
	CPI-002	Dohrmann	566	40	606
I/Cl₂ 10 μM	CPI-002	Euroglas	457	77	533
	F-600	Euroglas	374	145	519
	CPI-002	Dohrmann	506	30	536
I/Cl₂ 30 μM	CPI-002	Euroglas	367	36	403
	CPI-002	Dohrmann	398	21	419

In addition to the classical TOX measurements, halide specific measurements were made on these samples. This was done using both the Eurglass and Dorhmann analyzer and by trapping the off gas as described previously and analyzing by ion chromatography. Data show that as bromide dose increases the TOCl gives way to TOBr (Figure 41). The same phenomenon is apparent in the experiments where iodide was added (Figure 42).

The overall TOX data are summarized in Figure 43. While addition of bromide has mixed or subtle effects on the TOX, addition of iodide appears to result in a net decrease in the molar concentration of organic-bound halogen. This reinforces the observations based on THM

data that iodide is slightly less reactive than bromide in forming DBPs. The TOX speciation in Figure 41 and Figure 42 also support earlier observations on the need for higher levels of iodide as compared to bromide to achieve the same level of incorporation.
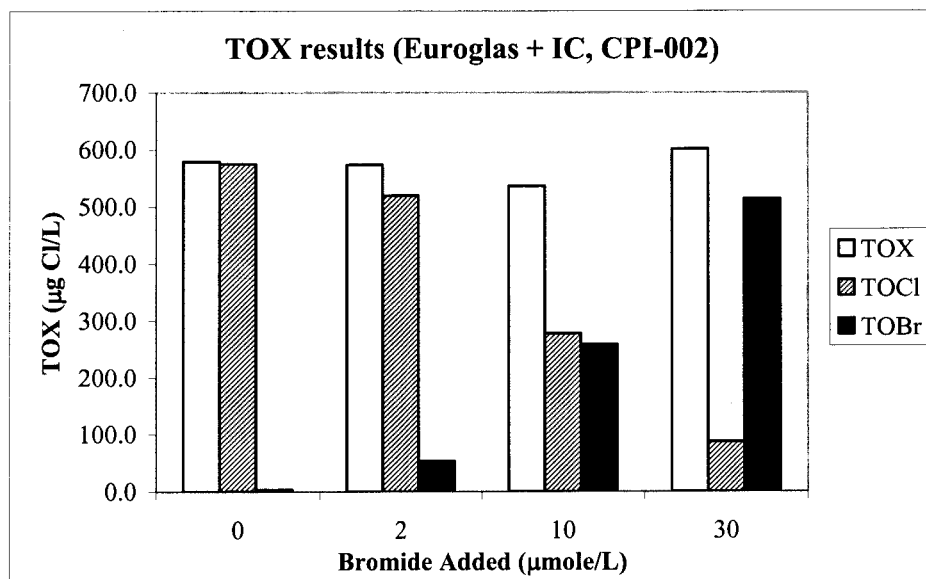
Figure 41. Winnipeg Water: TOX , TOCl and TOBr concentrations versus Added Bromide
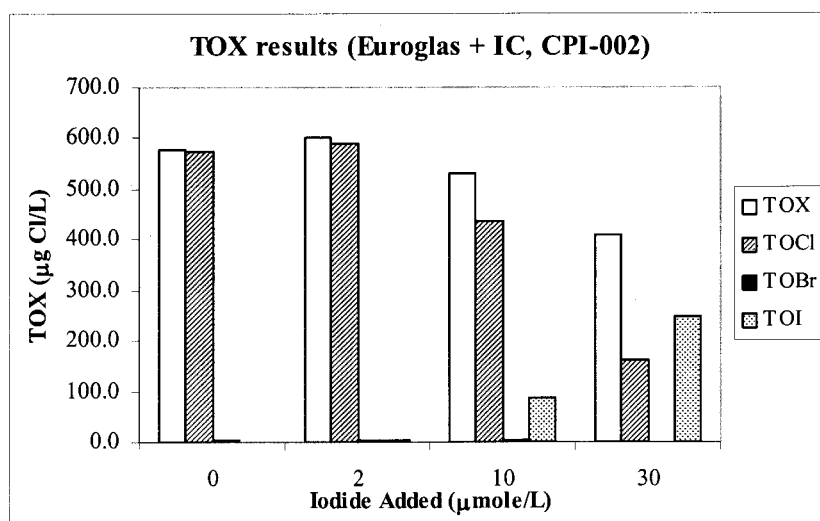
Figure 42. Winnipeg Water: TOX , TOCl and TOBr concentrations versus Added Bromide

81
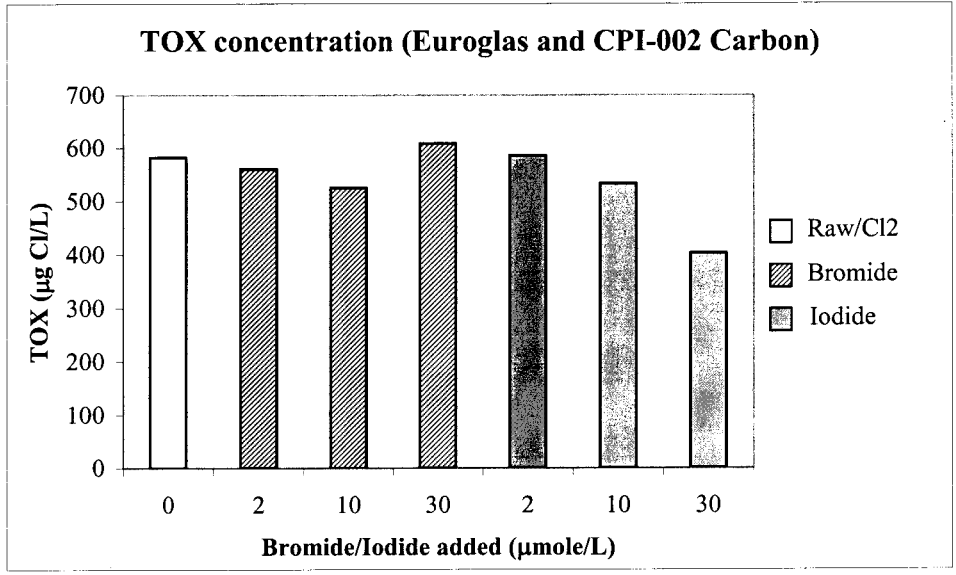
Figure 43. Winnipeg Water: TOX Concentrations versus Added Bromide and Iodide.

Table 13 Shows the results of calculations concerning the total amount of analytically identified DBPs, expressed as TOX, and designated as KnTOX. These are calculations based on the THM and HAA data. From this, a measure designated as the unknown TOX or UTOX is determined as the difference between the measured TOX and the calculated KnTOX. This shows that the ratio of known to unknown TOX increases as either the bromide or iodide level increase. The implications are that bromide and iodide produce smaller amounts of non-regulated DBPs. Nevertheless, there is still a substantial amount of these compounds present, even at the highest bromide and iodide levels.

Table 13. Known and Unknown TOX Results Winnipeg water (Euroglas+CPI-002)[‡]

Sample	KnTOCl	KnTOBr	KnTOI	KnTOX	KnTOCl/ UTOCl	KnTOBr/ UTOBr	KnTOX/ UTOX
Raw/Cl2	217.1	2.5		219.5	0.61	2.49	0.61
Br/Cl2 (2mM)	198.7	36.0		234.6	0.62	2.03	0.69
Br/Cl2 (10mM)	127.0	149.6		276.6	0.84	1.37	1.06
Br/Cl2 (30mM)	55.7	259.2		314.9	1.77	1.02	1.10
I/Cl2 (2mM)	214.8	2.9	3.2 [*]	220.9 [*]	0.57		0.58 [*]
I/Cl2 (10mM)	169.3	2.0	12.8 [*]	184.3 [*]	0.63		0.53 [*]
I/Cl2 (30mM)	72.5	0.7	68.4 [*]	142.2 [*]	0.82		0.53 [*]

[‡] Kn prefix signifies "Known" and is a reference to the total amount of identified DBPs (THMs, HAAs) in TOX units; the U prefix signifies "Unknown" and is a reference to the difference between the analytical TOX and the KnTOX.
[*] May be underestimated due to lack of quantitative iodinated HAA data

Comparative Performance of Different TOX Protocols

The standard carbon (CPI-002) tends to result in higher levels of TOX than the alternative carbon (CPI-001; Figure 44). Furthermore, for all but the highest iodide level, Filtrasorb 600 gives slightly lower levels than CPI-002.
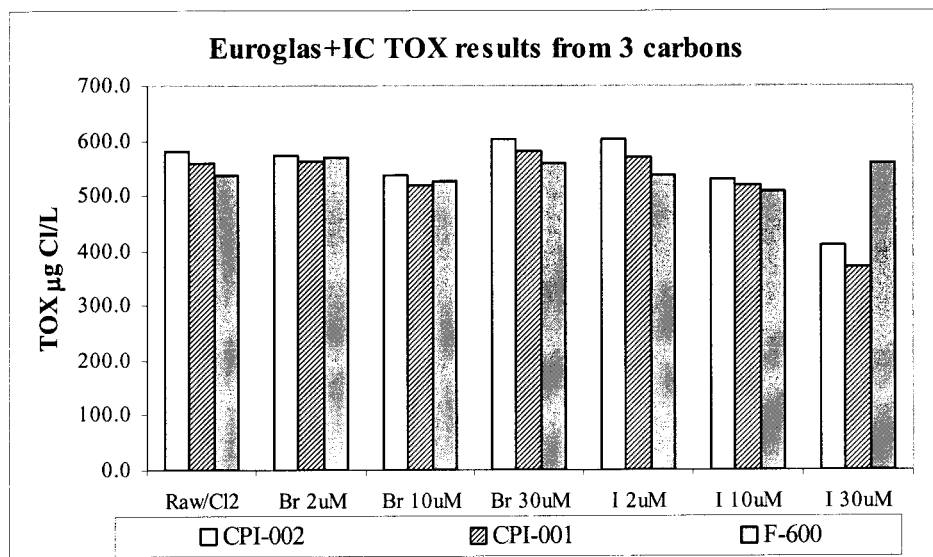
Figure 44. Winnipeg Water: Effect of Carbon Type on TOX Value

Based on the Winnipeg samples, the Euroglas instrument gave nearly identical results when comparing standard mode microcoulometric detection) with IC mode (Figure 45). This was not the case for the Dohrmann instrument (Figure 46). We suspect that the transfer and trapping of inorganic halide was not as efficient when applied to the Dohrmann architecture than with Euroglas. This is supported by the lack of any obvious bias in the comparison of the two instruments by standard microcoulometry (Figure 47). In contrast the analogous comparison of IC based measurements does show a negative bias on the part of the Dorhmann setup (Figure 48).
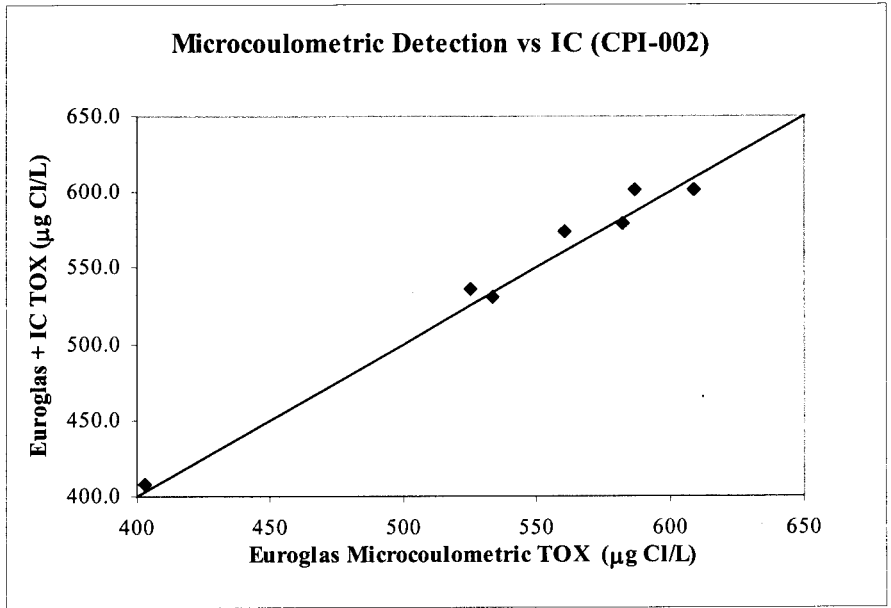
Figure 45. Winnipeg Water: Microcoulometric Detection versus IC Detection: Euroglas Instrument
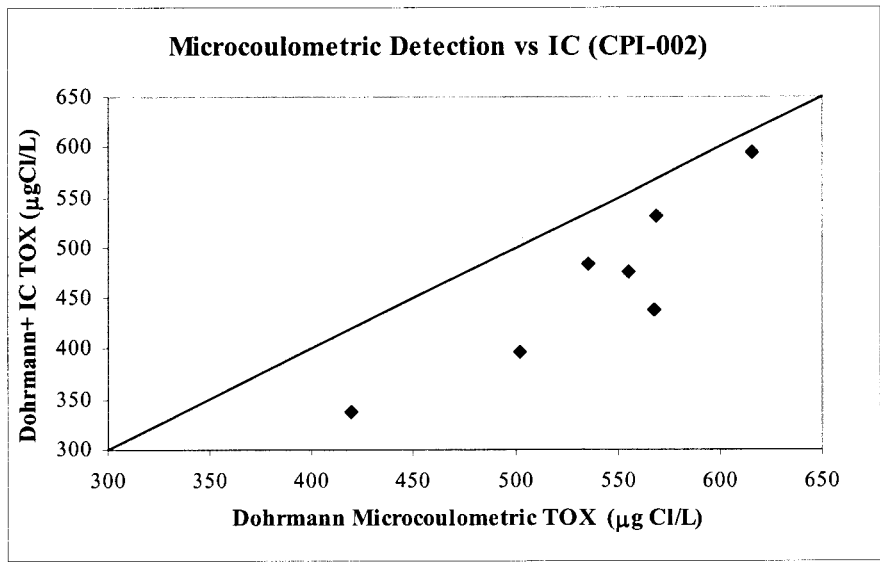
Figure 46. Winnipeg Water: Microcoulometric Detection versus IC Detection: Dohrmann Instrument

TOX by Microcoulometric Detection
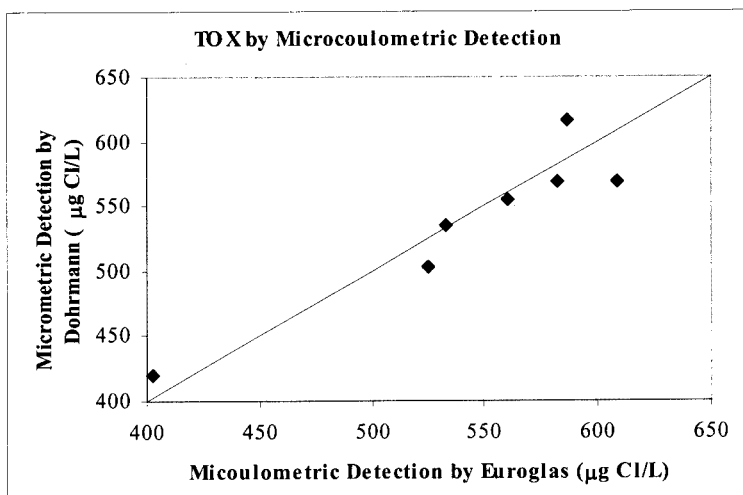
Figure 47. Winnipeg Water: Euroglas Microcoulometric Detection versus Dohrmann Microcoulometric Detection
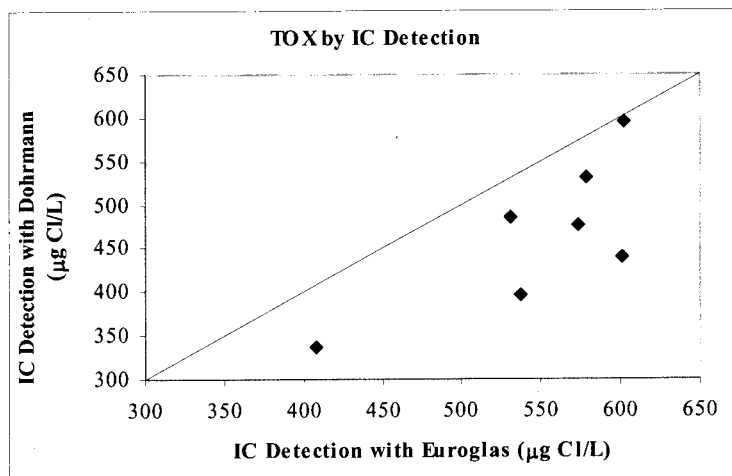
TOX by IC Detection

Figure 48. Winnipeg Water: Euroglas IC Detection versus Dohrmann IC Detection

Advanced Characterization of Unknown TOX

Four treated samples of Winnipeg water were selected for advanced characterization of unknown TOX by C_{18} solid phase disk extraction and high resolution electrospray ionization mass spectrometry. These samples are listed in Table 14.

87

Table 14. Samples selected for advanced characterization

Number	Sample Identification	Sample Description
1	Raw water	Raw water collected from Winnipeg, Canada
2	Chlorinated water	Chlorinated water at dose of 6.2 mg Cl_2/L, to reach a residual of 0.5 mg Cl_2/L after 48 hr incubation at 20 °C
11	Chlorinated water with bromide	Chlorinated water with the addition of bromide at the concentration of 30 μmole/L
14	Chlorinated water with iodide	Chlorinated water with the addition of iodide at the concentration of 30 μmole/L

C_{18} solid phase disk extraction of each sample was done at UMass after the 48-hour contact time. Following extraction, C_{18} disks were stored in cleaned glass bottles and shipped to Ohio State University by overnight delivery for further testing.

Sample preparation procedure at UMass:

(1) Chlorinated waters were quenched with sodium sulfite (~5mg/L).
(2) All water samples were filtered through GF/F glass filters.
(3) The pH of samples were adjusted to between 2 and 2.5 with hydrochloric acid.
(4) Aliquot of 800 ml of each sample was loaded onto the C_{18} extraction disk. Before extraction, disks were conditioned by soaking with 10 ml MeOH.

TULSA TESTS

Preliminary Chlorination Demand Test

As with the Winnipeg water, the first experiment that was run with the Tulsa raw water was a chlorine demand study. A dose/residual curve has been developed so that we could estimate the appropriate dose to achieve the desired residual (0.5 mg Cl_2/L) at the end of the contact time (48 hours) at the desired temperature of 20°C. The pH of water samples was adjusted to 7 with a phosphate buffer prior to chlorination.
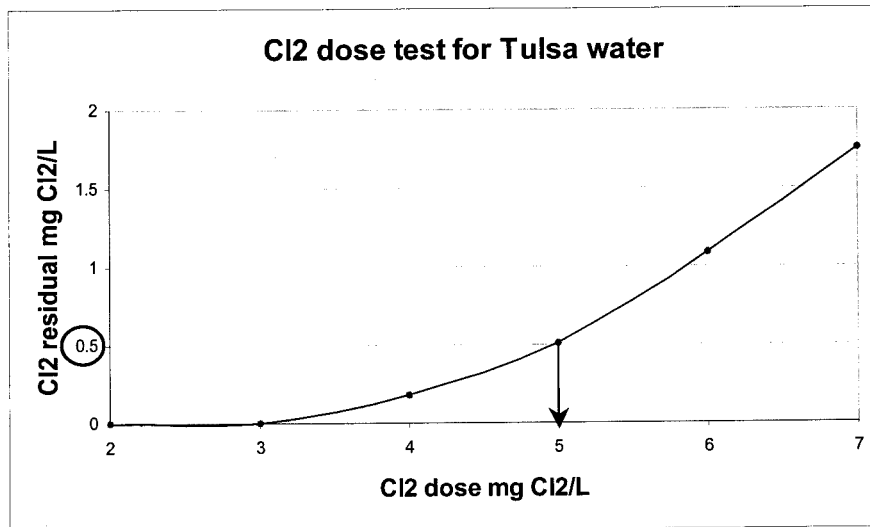
Figure 49. Chlorine demand test on Tulsa water

The dose that was adopted for bench-scale chlorination tests was based on this demand curve. The value chosen was 5.0 mg Cl_2/L.

Treated Water Chlorine Residuals

Three levels of Br^- and I^- ions addition were chosen for this test, 2, 10 and 30 μmole/L. Chlorine residuals were measured using the DPD method for all treated samples after 48 hours incubation time. The key numbers (#1 and 9 to #14) represented in Figure 25 correspond to the different points for which chlorine residuals were measured.
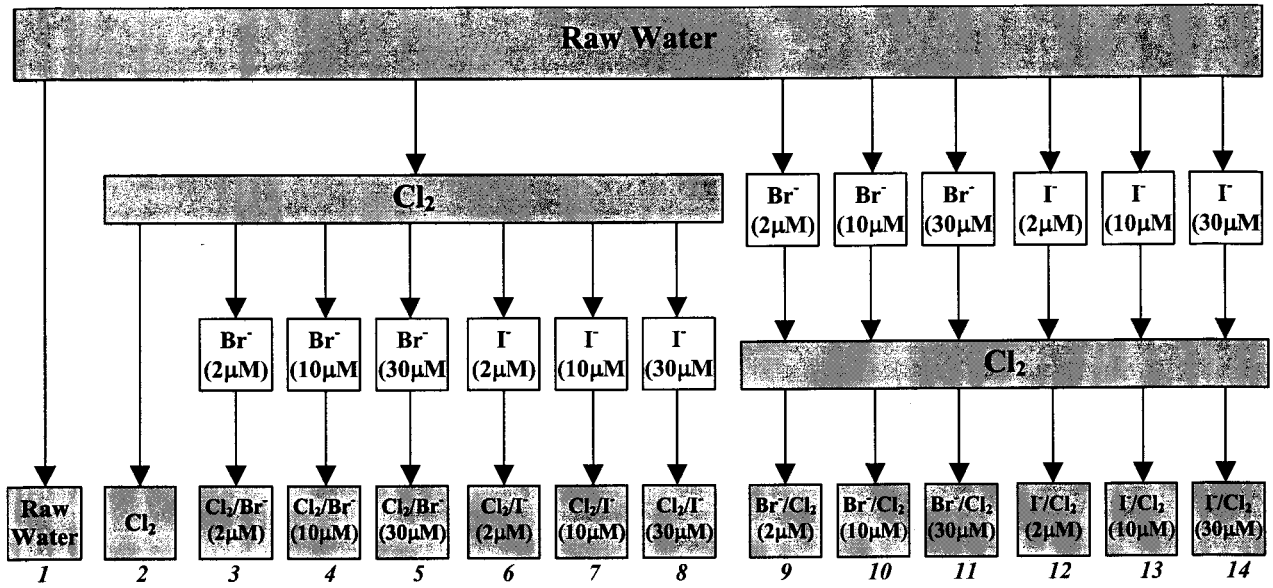
Figure 50. Sample Numbering Key for Task 1b Chlorination Test

Table 15 shows chlorine residual and pH of the treated water samples. The chlorinated water sample without bromide and iodide addition before chlorination (#1) shows a chlorine residual range of 0.51 mg Cl_2/L, which is very close to the target value of 0.5 mg Cl_2/L. Chlorination following bromide and iodide addition shows a higher chlorine demand (samples #9-#14 vs samples #1). The chlorine residuals of bromide and iodide addition at level of 2 μmole/L are 0.38 and 0.22 mg/L respectively. The chlorine residual was essentially depleted at the end of 48 hour contacting when increasing the bromide and iodide concentrations to 10 and 30 μmole/L (samples #10, #11, #13 and #14). These data indicate that added bromide and iodide ions were involved in the chlorination, and produced higher chlorine demand. Brominated and iodinated products are expected in these samples. These results are quantitative similar to those for the Winnipeg water

Table 15: Chlorine residuals and pH of the treated Tulsa water samples

Number	Sample	Chlorine Residual after 48 hr incubation (mg/L)	pH after 48 hr incubation
1	Raw water/Cl_2	0.51	7.3
9	Br^-/Cl_2 (2μmole/L)	0.38	7.34
10	Br^-/Cl_2 (10μmole/L)	0	7.28
11	Br^-/Cl_2 (30μmole/L)	0	7.26
12	I^-/Cl_2 (2μmole/L)	0.22	7.31
13	I^-/Cl_2 (10μmole/L)	0	7.25
14	I^-/Cl_2 (30μmole/L)	0	7.28

Specific DBPs

THMs, HAAs and TOX were analyzed for all of the treated water samples. As expected, increasing bromide levels resulted in a shift in the THM and HAA speciation to the more brominated forms (Figure 51 to Figure 54). It is interesting to note that the TTHM concentration on a molar basis increases substantially with bromide (Figure 55). In comparison, the HAA9 only increases slightly less (Figure 56). Not surprisingly, the HAA5, which is heavily weighted toward the chlorinated forms, decreases with increasing bromide.
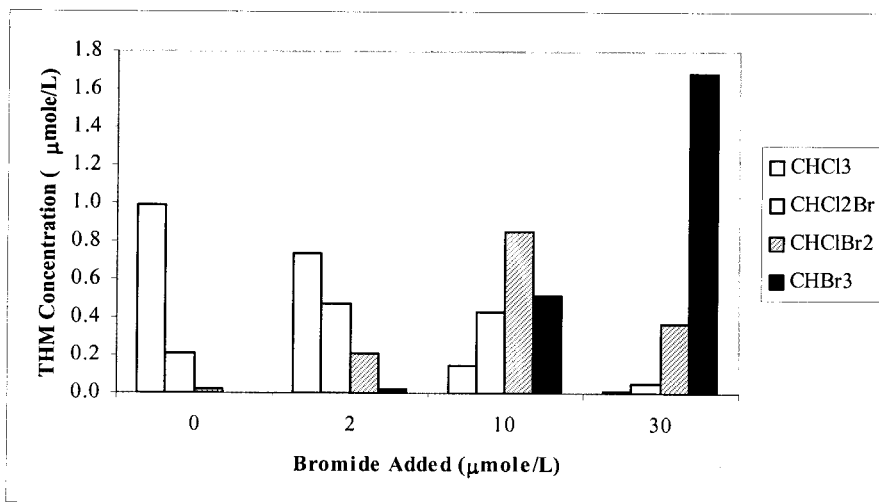
Figure 51. Tulsa Water: THM Concentrations versus Added Bromide.

92
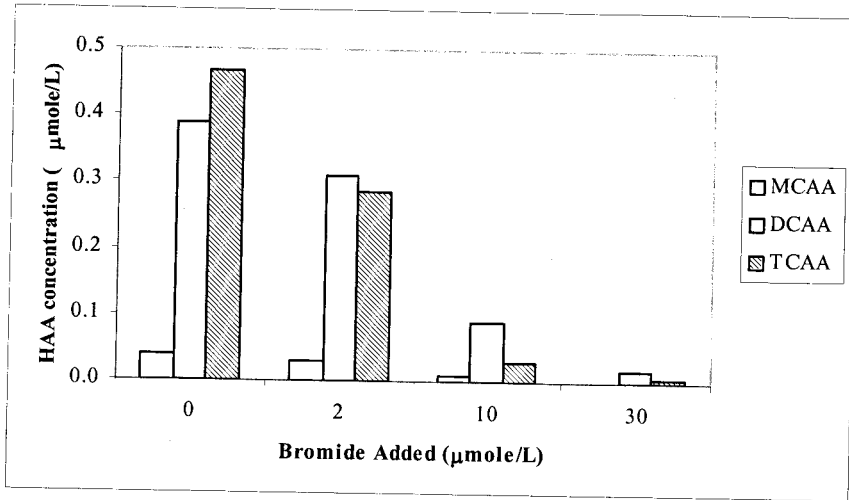
Figure 52. Tulsa Water: Chlorinated HAA Concentrations versus Added Bromide.
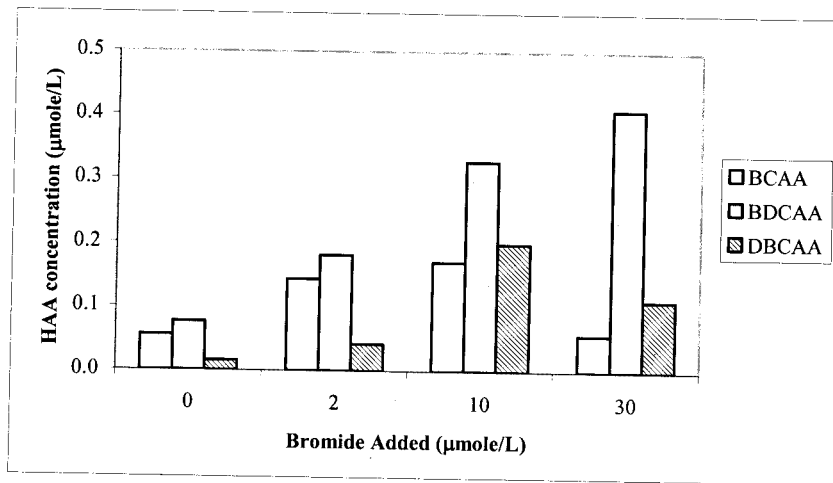
Figure 53. Tulsa Water: Mixed HAA Concentrations versus Added Bromide.
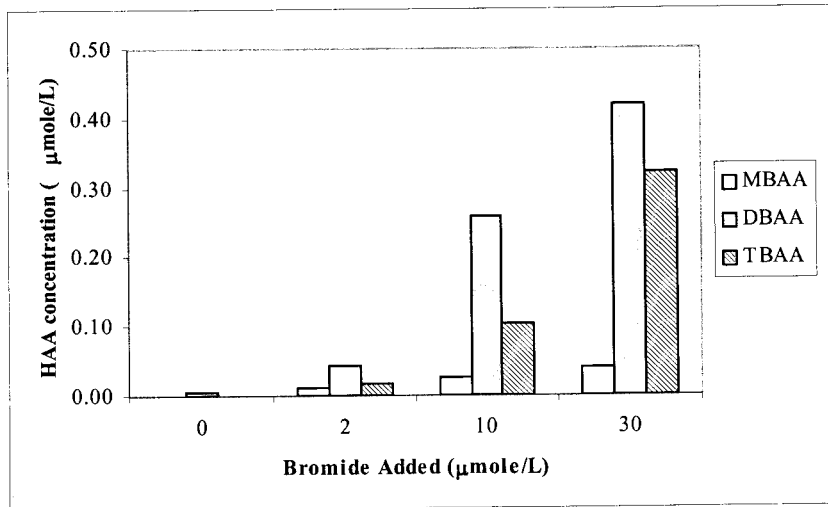
Figure 54. Tulsa Water: Brominated HAA Concentrations versus Added Bromide.
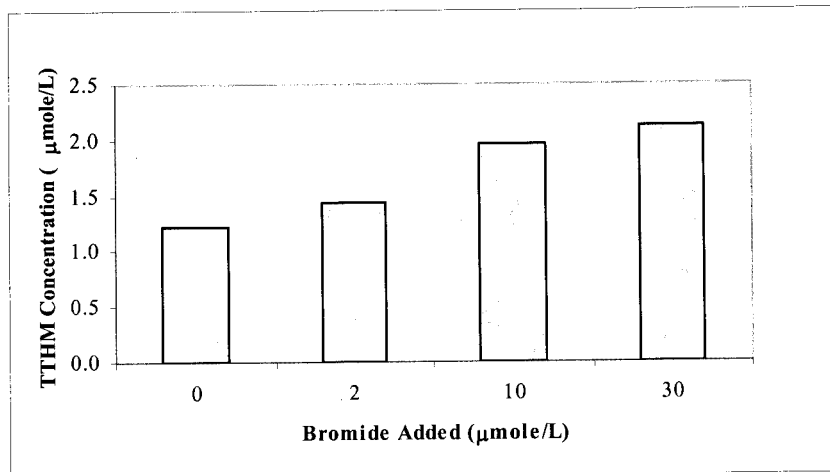
Figure 55. Tulsa Water: Molar TTHM versus Added Bromide.
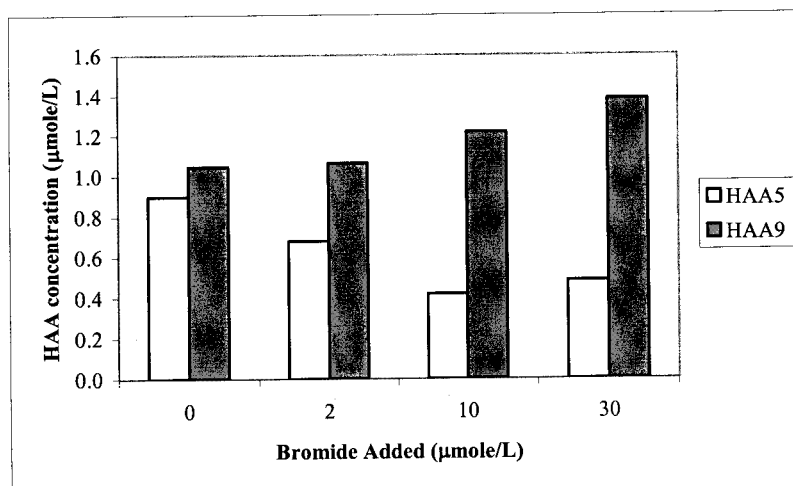
Figure 56. Tulsa Water: Molar HAA5 and HAA9 versus Added Bromide.

Calculation of the bromine incorporation ratio (or the analogous chlorine incorporation ratio) is sometimes a clearer way of seeing the impacts of bromide on speciation. Figure 57 and Figure 58 show these values for the THMs and HAAs, respectively. Because the HAAs comprise 3 distinct classes of byproducts (mono, di and tri halogenated acetic acids), it's more appropriate to separate these out when considering chemical phenomena. Figure 59 shows the bromine incorporation factors for these three HAA groups as well as the THMs. Once again the trihaloacetic acids generally show less bromine incorporation than the THMs. This is a phenomenon that we have noted with the ICR data as well.

Comparison across species groups with different numbers of halogens is best done with a molar fraction metric. Whereas the bromine incorporation factor ranges from zero to 1, 2 or 3 depending on the species; the bromine/halogen molar fraction will always range from 0 to 1 regardless of the total number of halogens. Figure 60 shows how the bromine/halogen molar fraction changes for different levels of bromide and for different classes of DBPs. These data suggest that the DHANs are most readily populated with bromine atoms and the THAAs are least likely to be brominated.
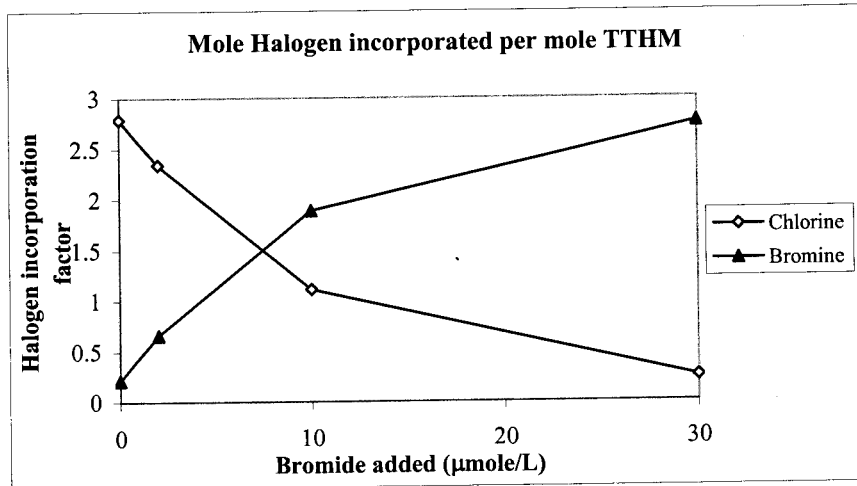
Figure 57. Tulsa Water: Halide Incorporation in THMs versus Added Bromide.
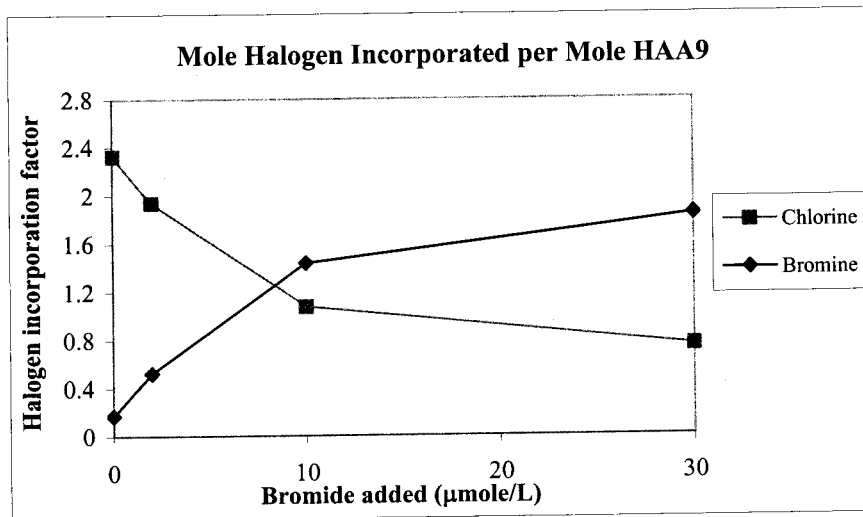
Figure 58. Tulsa Water: Halide Incorporation in HAAs versus Added Bromide.

96
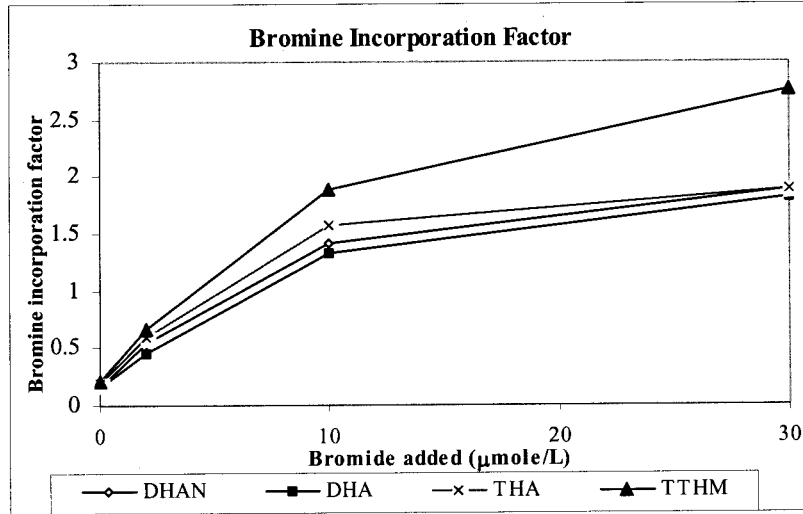
Figure 59. Tulsa Water: Halide Incorporation in DBP Families versus Added Bromide.
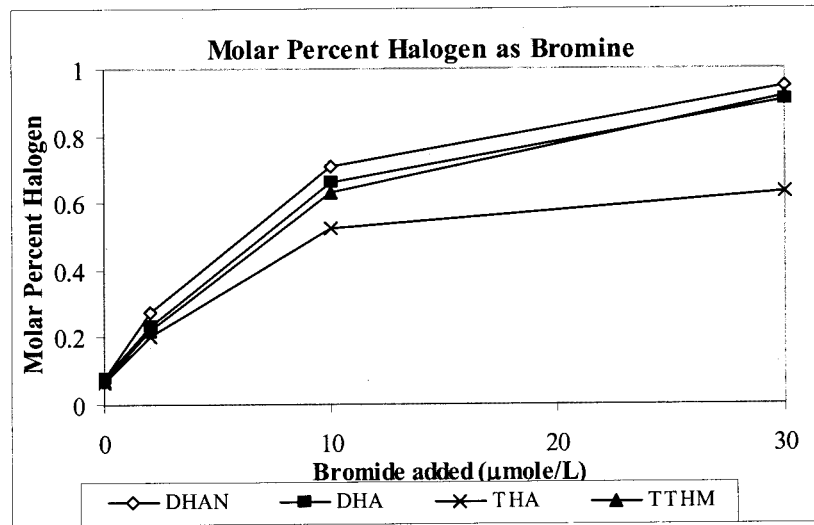
Figure 60. Tulsa Water: Bromine/halogen Molar Fraction versus Added Bromide

Addition of iodide produces iodinated byproducts much like bromine leads to brominated compounds. There is a substantial speciation shift in the THMs as iodide is added with chloroform declining and the iodinated forms becoming more prominent (Figure 61). With small ambient levels of bromide, there is a persistent presence of bromodichloromethane, however no other brominated forms were detected in these experiments. It is interesting to note that the species profile at the highest iodide level appears anomalous and bimodal. This behavior is not

generally seen with analogous bromide spiking studies. Careful review of the data failed to indicate any systematic error; and a similar phenomenon was noted in the Winnipeg sample. There may be some kinetic reasons for the relatively low level of chlorodiiodomethane as compared to the iodoform and Dichloroiodomethane.
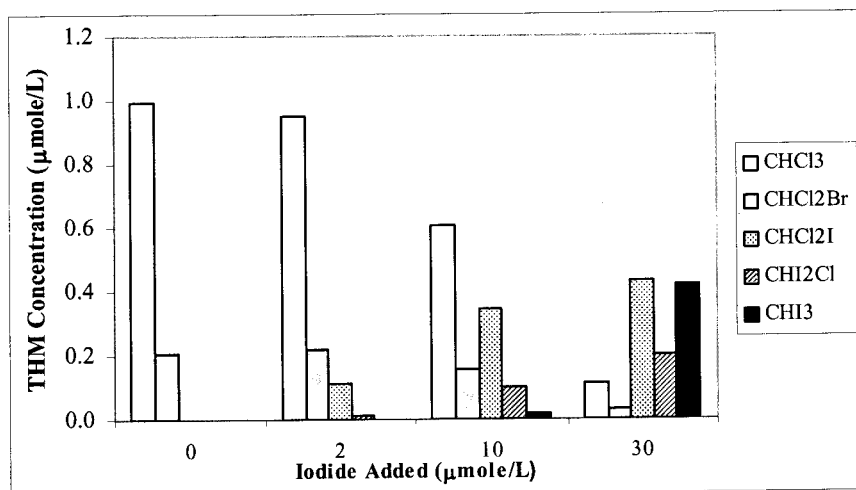
Figure 61. Tulsa Water: THM Concentrations versus Added Iodide.

Data from the Winnipeg tests indicates that iodide is somewhat less reactive than bromide as measured by tendency to form THMs. First, there is almost no change in molar TTHM level with increasing iodide (Figure 62). This is in contrast to the bromide tests. Second, the chlorine incorporation factor requires higher levels of iodide (molar basis) before it starts to decline as compared to the case with bromide (Figure 63). This may be a kinetic effect, a steric effect, or a manifestation of the instability of some iodinated intermediates.
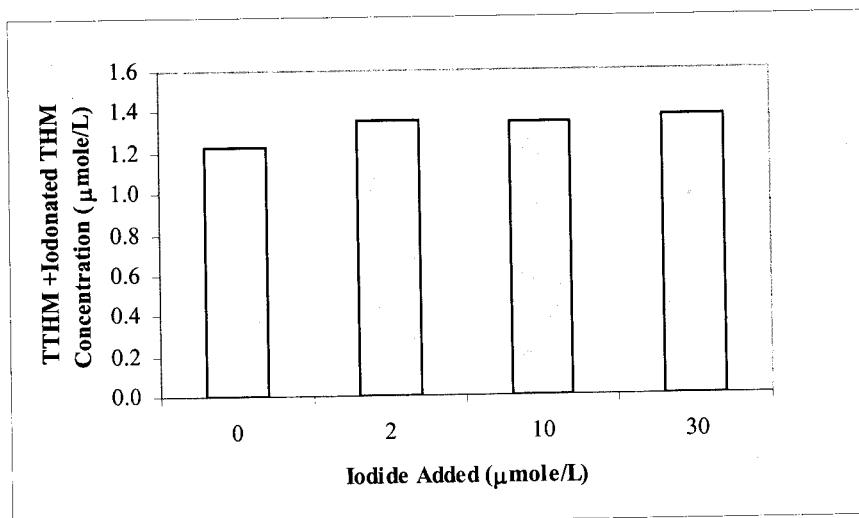
Figure 62. Tulsa Water: Molar TTHM versus Added Iodide
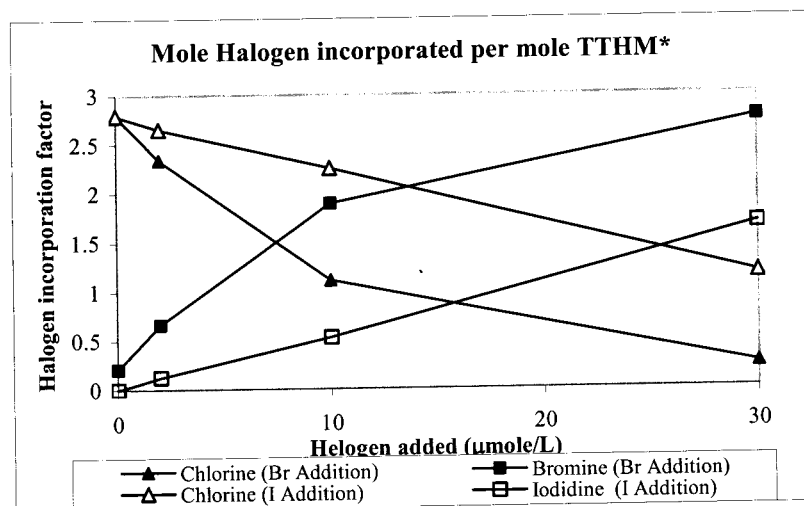
Figure 63. Tulsa Water: THM Halogen Incorporation versus Added Halide

At present we do not have authentic standards for the iodinated HAAs, so we can only reliably quantify the chloro-bromo species. Nevertheless, the HAA data show a clear decline in TCAA and DCAA with increasing iodide levels (Figure 64 and Figure 65). This is analogous to the decline in chloroform in Figure 61. Note that TCAA drops more abruptly than DCAA, as expected based on the larger number of possible iodinated trihaloacetic acids.
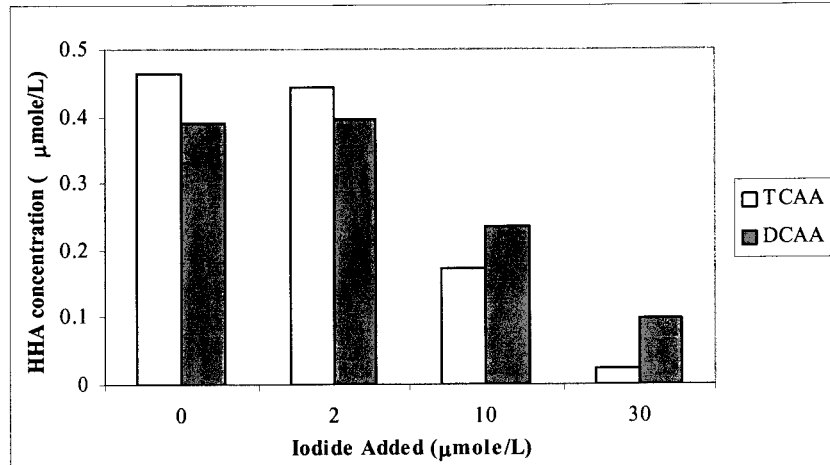
Figure 64. Tulsa Water: TCAA and DCAA Concentrations versus Added Iodide.
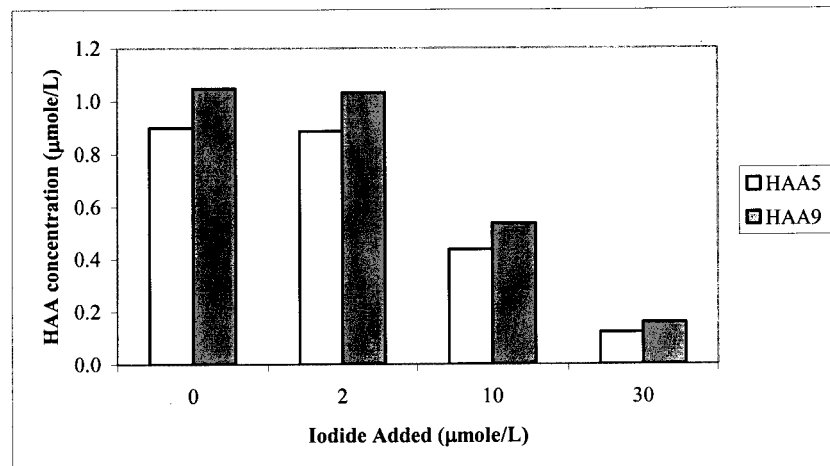
Figure 65. Tulsa Water: HAA5 and HAA9 Concentrations versus Added Iodide.

Total Organic Halides

Table 16 presents the classical TOX data from this experiment. As with the Winnipeg samples, the brominated byproducts tend to show less breakthrough than the chlorinated ones (compare 2nd GAC columns for raw and 30 uM bromide).. It also appears that the iodinated compounds are even better retained on the GAC than the brominated ones.

Table 16. TOX results for Tulsa water by Microcoulometric Detection

Samples	Carbon Type	Analyzer	TOX (μg Cl/L)		
			1st Column	2nd Column	Total
Raw water/Cl$_2$	CPI-002	Euroglas	481	42	523
	CPI-002	Dohrmann	495	34	528
Br/Cl$_2$ 2μM	CPI-002	Euroglas	447	32	479
	CPI-002	Dohrmann	433	46	479
Br/Cl$_2$ 10 μM	CPI-002	Euroglas	455	25	481
	CPI-002	Dohrmann	434	20	453
Br/Cl$_2$ 30 μM	CPI-002	Euroglas	525	13	537
	CPI-002	Dohrmann	487	11	498
I/Cl$_2$ 2μM	CPI-002	Euroglas	462	53	515
	CPI-002	Dohrmann	468	34	502
I/Cl$_2$ 10 μM	CPI-002	Euroglas	335	27	362
	CPI-002	Dohrmann	346	13	359
I/Cl$_2$ 30 μM	CPI-002	Euroglas	315	25	340
	CPI-002	Dohrmann	344	10	354

In addition to the classical TOX measurements, halide specific measurements were made on these samples. This was done using both the Eurglass and Dorhmann analyzer and by trapping the off gas as described previously and analyzing by ion chromatography. Data show that as bromide dose increases the TOCl gives way to TOBr (Figure 66). The same phenomenon is apparent in the experiments where iodide was added (Figure 67).

The overall TOX data are summarized in Figure 68. While addition of bromide has mixed or subtle effects on the TOX, addition of iodide appears to result in a net decrease in the molar concentration of organic-bound halogen. Once again, this reinforces the observations based on THM data that iodide is slightly less reactive than bromide in forming DBPs. The TOX speciation in Figure 66 and Figure 67 also support earlier observations that the same level of incorporation required higher levels of iodide as compared to bromide.
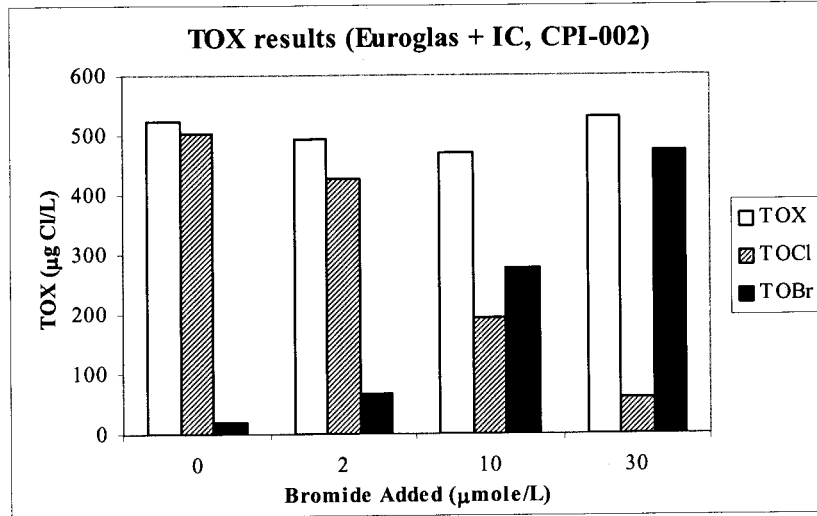
Figure 66. Tulsa Water: TOX , TOCl and TOBr concentrations versus Added Bromide
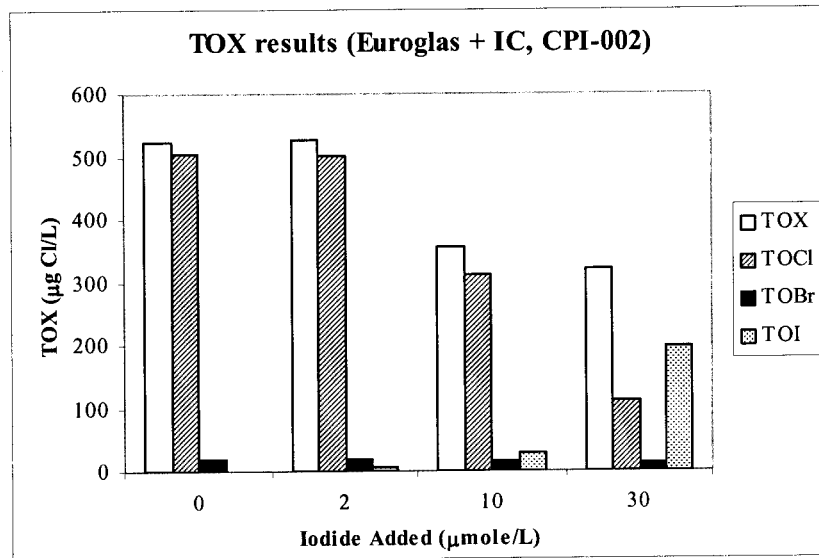
Figure 67. Tulsa Water: TOX , TOCl and TOBr concentrations versus Added Bromide

102
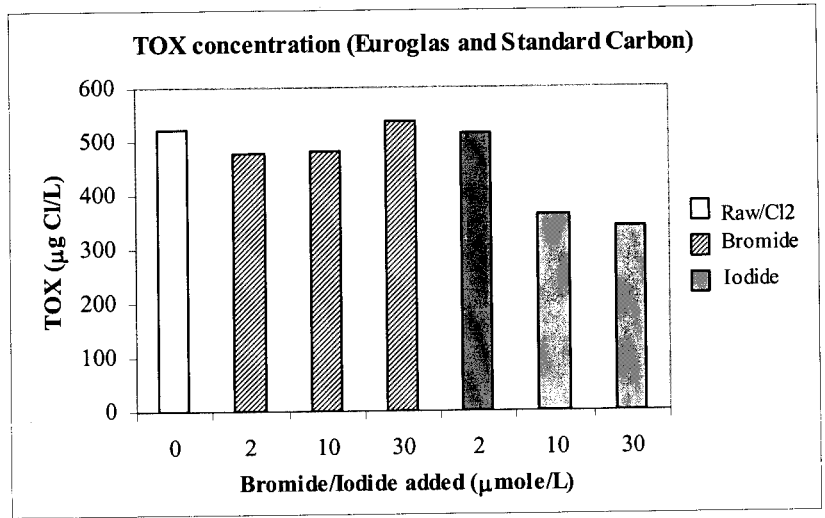
Figure 68. Tulsa Water: TOX Concentrations versus Added Bromide and Iodide.

Table 17 Shows the results of calculations concerning the total amount of analytically identified DBPs, expressed as TOX, and designated as KnTOX. These are calculations based on the THM and HAA data. From this, a measure designated as the unknown TOX or UTOX is determined as the difference between the measured TOX and the calculated KnTOX. This shows that the ratio of known to unknown TOX increases as either the bromide or iodide level increase. The implications are that bromide and iodide produce smaller amounts of non-regulated DBPs. Nevertheless, there is still a substantial amount of these compounds present, even at the highest bromide and iodide levels.

Table 17. Known and Unknown TOX Results Tulsa water (Euroglas+CPI-002)[‡]

Sample	KnTOCl	KnTOBr	KnTOI	KnTOX	KnTOCl/ UTOCl	KnTOBr/ UTOBr	KnTOX/ UTOX
Raw/Cl2	218.4	15.9		234.3	0.76	5.09	0.81
Br/Cl2 (2mM)	202.6	55.9		258.6	0.91	4.67	1.10
Br/Cl2 (10mM)	127.6	201.2		328.8	1.95	2.71	2.35
Br/Cl2 (30mM)	55.6	307.3		362.8	18.32	1.86	2.16
I/Cl2 (2mM)	222.0	17.3	6.0	245.3	0.79		0.87
I/Cl2 (10mM)	155.0	15.6	25.1	195.6	1.00		1.23
I/Cl2 (30mM)	69.4	9.1	81.3	159.8	1.61		0.98

[‡] Kn prefix signifies "Known" and is a reference to the total amount of identified DBPs (THMs, HAAs) in TOX units; the U prefix signifies "Unknown" and is a reference to the difference between the analytical TOX and the KnTOX.
[*] May be underestimated due to lack of quantitative iodinated HAA data

Comparative Performance of Different TOX Protocols

The standard carbon (CPI-002) tends to result in higher levels of TOX than the alternative carbon (CPI-001; Figure 69). Furthermore, for all but the highest iodide level, Filtrasorb 600 gives slightly lower levels than CPI-002.
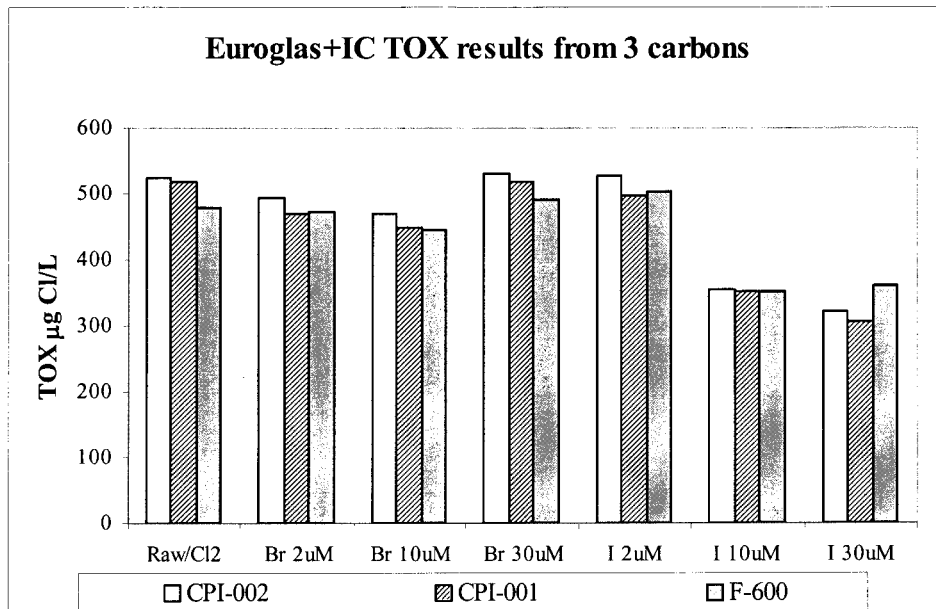
Figure 69. Tulsa Water: Effect of Carbon Type on TOX Value

Based on the Winnipeg samples, the Euroglas instrument gave nearly identical results when comparing standard mode microcoulometric detection) with IC mode (Figure 70). This was not the case for the Dohrmann instrument (Figure 71). We suspect that the transfer and trapping of inorganic halide was not as efficient when applied to the Dohrmann architecture than with Euroglas. This is supported by the lack of any obvious bias in the comparison of the two instruments by standard microcoulometry (Figure 72). In contrast the analogous comparison of IC based measurements does show a negative bias on the part of the Dorhmann setup (Figure 73).
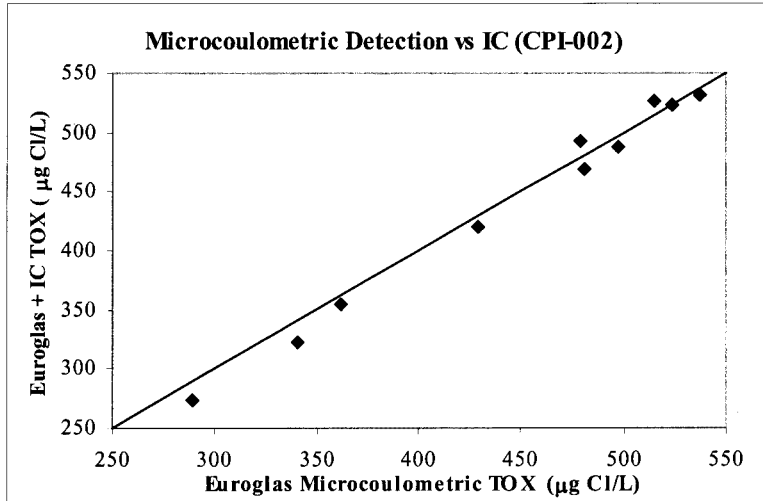
Figure 70. Tulsa Water: Microcoulometric Detection versus IC Detection: Euroglas Instrument
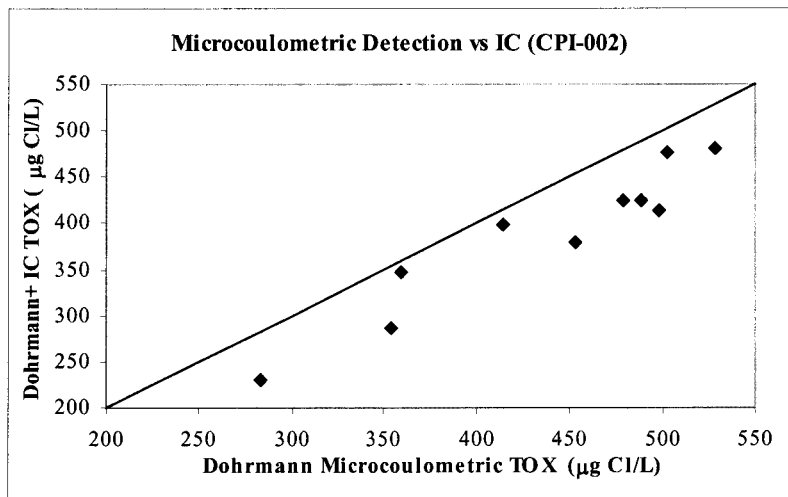
Figure 71. Tulsa Water: Microcoulometric Detection versus IC Detection: Dohrmann Instrument
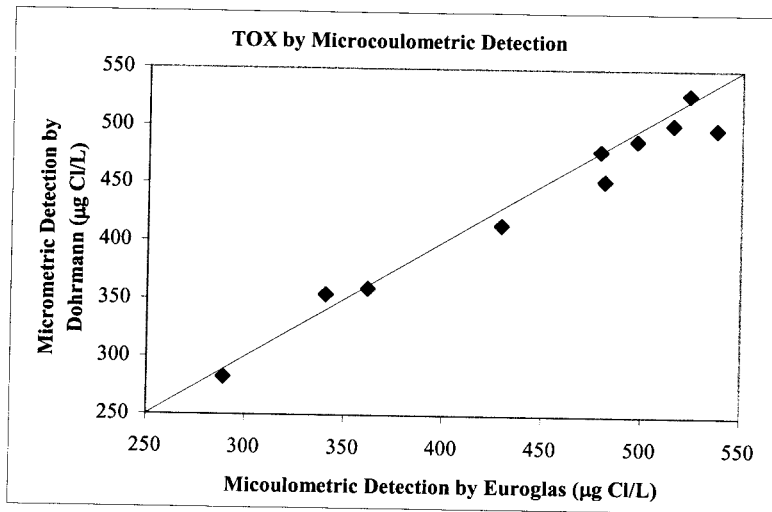
Figure 72. Tulsa Water: Euroglas Microcoulometric Detection versus Dohrmann Microcoulometric Detection
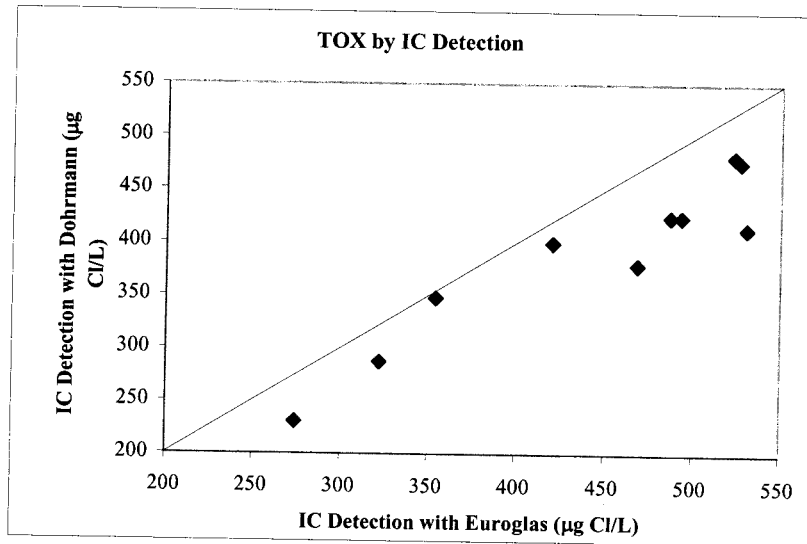
Figure 73. Tulsa Water: Euroglas IC Detection versus Dohrmann IC Detection

CHAPTER 6: SURVEY OF UNKNOWN TOX

This chapter incorporates work performed under Tasks 2 and 3. Task 2 was intended to generate data on the range of UTOX values that may be observed in waters across North America. At the same time, a characteristic distribution water sample is to be collected and analyzed for a range of NOM characteristics. For example, a portion of the sample will be fractionated based on molecular size (ultrafiltration) and hydrophobicity (hydrophobic resin adsorption). The intention is to develop a database on the general character (e.g., hydrophobicity and apparent molecular weight) of UTOX in North American waters.

The purpose of task 3 is to determine the impact of a variety of treatment conditions (disinfection conditions) on UTOX concentration. In Task 3, a smaller set of water samples will be selected from the task 2 plants. These waters will be treated with the same combinations of disinfectants as used in Task 2, but some additional experimental variations will be used. These include variations in pH, and possibly bromide level and iodide level.

UTILITY SELECTION

Utility selection should be based on known characteristics that help to identify waters of contrasting behavior. The intent is to capture the full range of relevant characteristics found within North American waters. Given the types of information available from large numbers of utilities, the following selection criteria seem to hold the greatest promise:
- High and low SUVA at a given TOC
- High and low ratios of known byproducts to other TOX (e.g., (TTHM+HAA9)/UTOX)
- High and low nitrogen contents or HAN/TOX ratios
- Contrasting watershed characteristics and watershed ecoregion locations

Synergy with other on-going projects

We are especially interested in taking advantage of other on-going surveys regarding NOM and DBPs so as to maximize relevant data collection for this project. Some AWWARF-sponsored projects that could provide some synergy to this survey are summarized in the table below. We welcome input from AWWARF and the PAC on this.

Table 18: Projects and Utilities with Potential for Synergistic Collaboration

Project Name	PI	Key Measurements	Study Locations	Flow (MGD)	Raw Water Quality TOC	Raw Water Quality SUVA	Comments
Dissolved Organic Nitrogen (DON) in Drinking Water and Reclaimed Waste Water	Paul Westerhoff	DON, inorganic N, protein analysis, ^{15}N-NMR, pyrolysis-GC/MS	4 Phoenix plants				
			8 mid-west AWWSC plants				
			7 other plants				MWD, Boston, NNWW, etc.
			11 Algal sites				From AWWARF project #2775
Formation and Decay of DBPs in the Distribution System	Hélène Baribeau	THMs, HAA9, distribution system modeling and monitoring	Fort Eustis, VA	1.1			Conventional plant with chloramination
			So. Calif Water. Co.				Conventional treatment with chloramines
			Durham, NC	52 (2 plants)	4.4 & 5.1	4.8 & 3.7	Brown & Williams Plants: both conventional with chloramination
			Marion, OH	5.9			Lime-soda softening, chlorination
			Columbus, GA	32			Conventional with chlorine, may switch to chloramines
Watershed Sources and Long-term Variability of BDOM and NOM as Precursors[6]	David Reckhow	BDOC, AOC; Acylhetero-polysaccharides, extensive analysis of watersheds, CuO degradation	Salt Lake City, UT	Many plants using this source			Source:Deer Creek Reservoir
			Akron, OH	46	6.2	2.8	Source: Lake Rockwell, conventional WTP
			Seattle, WA	88	1.2	4.7	Source: Lake Youngs, etc.
			Cambridge, MA	15	4.2		Source: Cambridge Res. Etc.
			Boston, MA	270	2.3	2.5	Source: Wachusett reservoir

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[6] Study locations will include additional cities; final list not yes established

SUVA Criteria

A set of High and low SUVA waters have already been identified, and these were integrated into the original research proposal. Winnipeg was selected as a low SUVA site. This one was particularly interesting, because it has an abnormally high TOC given its SUVA (Figure 74). This utility is also quite interested and knowledgeable about water quality research and was recently a participant in a tailored collaboration on impacts of UV treatment.
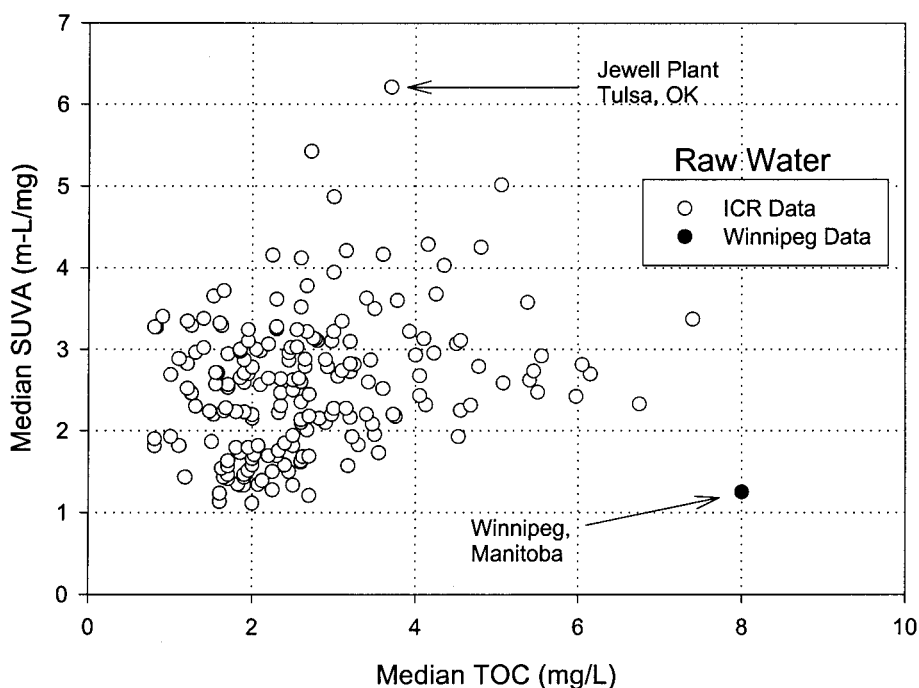
Figure 74: Distribution of Raw Water NOM Characteristics for 195 Large US Plants (Summarized from ICR data), also showing Winnipeg.

The high SUVA water that was identified is Tulsa's Jewell Plant. This plant also comes from a region of the US that is not well represented in many national studies of NOM and DBPs. For this reason, it will help us capture some of the geographical variability that is the subject of a subsequent criterion. Table 19 presents a comparison of these two waters.

Table 19: Comparative Raw Water Quality for High & Low SUVA Waters

Parameter	Tulsa, OK (Jewell Plant) Raw Water	Winnipeg, Manitoba, Raw Water
TOC (mg/L)	3.8	8.0
SUVA (L/mg-m)	**5.5**	**1.3**
UV abs (cm^{-1})	0.21	0.10
Hardness (mg/L)	142	83
Alkalinity (mg/L)	113	81
Bromide (mg/L)	0.065	low
pH	7.9	8.2
Turbidity (NTU)	22	1.0

Criteria based on ratios of known to unknown TOX

To help in the selection of utilities for task 2, an analysis of existing data on TOX and calculated UTOX was to be conducted. This analysis is still in progress, but some of the important findings and summary statistics are presented below and in the appendix.

Based on averaged data from the Information Collection Rule, about 30% of the measured TOX is accounted for in the major byproducts (THMs and HAAs). However, individual systems show a broad range in the ratio of known TOX (essentially TTHM + HAA9) to the unknown TOX (i.e., the TOX not accounted for in these compounds). To determine which systems represent extremes, data from the ICR were carefully selected. To avoid systems that were not practicing free chlorination, only samples exhibiting 75% of the total residual chlorine in the free form were included. Furthermore, systems with TOX values below 75 ug/L were excluded in the interest of avoiding high relative uncertainties in the TOX value. Finally, the SDS data were extracted so that confounding factors such as losses to biodegradation in distribution systems might be avoided. The remaining data, representing nearly 500 systems, are shown in the figure below. Nearly all of the data are bracketed by the two lines, representing 10% known TOX and 50% known TOX. Utilities that correspond to the data falling near the two extreme lines are good candidates for capturing variability in TOX speciation. To avoid the occasional spurious data point, we have selected only those utilities that appear more than once

in the extreme zones. These are listed in the table below.

Based on well-controlled laboratory data using aquatic humic substances (i.e., Black Lake Fulvic Acid; see appendix), the fraction of known to unknown TOX would be expected to range from 0.3 (low pH, reaction time, dose) to about 1.0 (high pH, reaction time, dose) under the full range of conditions one might expect to find in a water treatment plant. This is equivalent to about 25% - 50% known TOX accountable in the major byproducts.
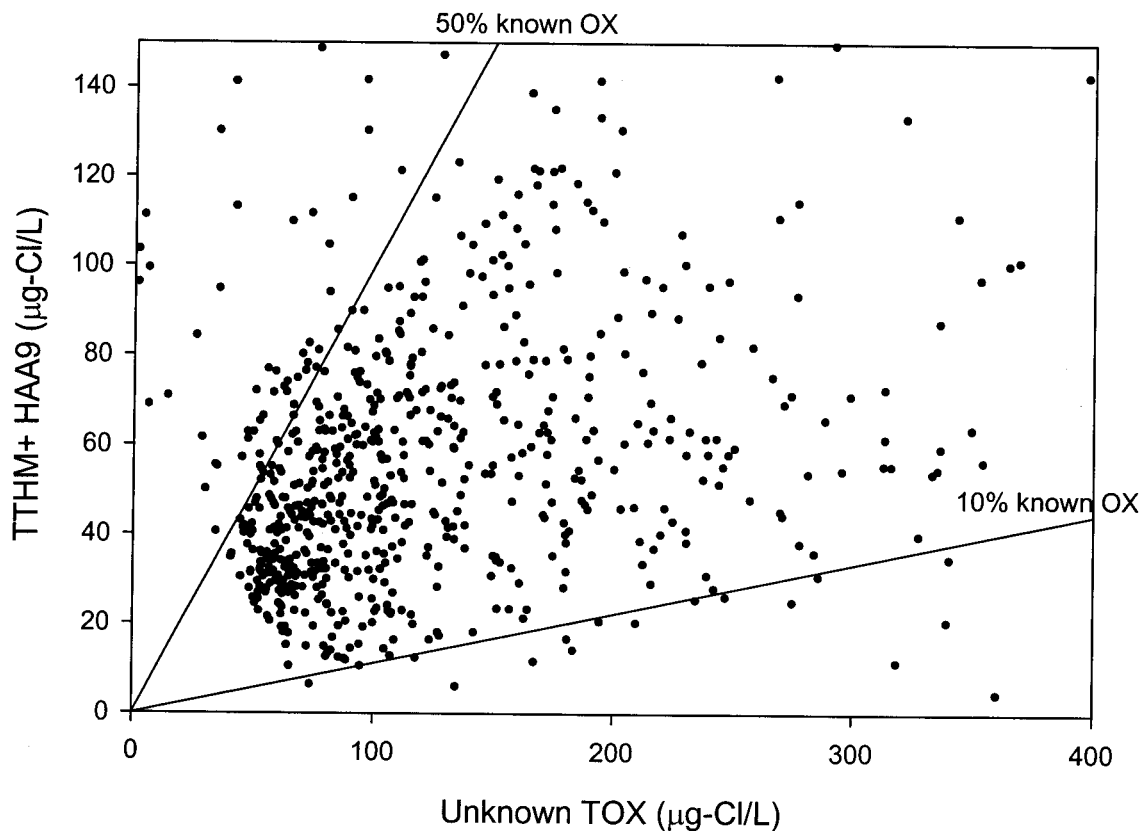
Figure 75: Known versus Unknown TOX in Selected ICR Data[7]

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[7] Selected SDS data (see text). HAA9 concentrations were either measured or estimated using the method of Roberts and Singer.

113

Table 20: Selected Utilities Representing Extremes in Known to Unknown TOX Ratios

City	State	Plant ID PWSID(ICR)	Flow (MGD)	Raw water quality		Notes
				TOC	SUVA	
Low Known/Unknown OX						
Brockton	MA	4044000(402)	11.5	3.8	3.1	Trident 210A with GAC?
Layton	UT	4900512(667)	26	3.3	3.3	Weber Basin #2; Conventional with GAC caps; UV going it to WTP#3
Rochester	NY	0004518(514)	38	2.6	1.9	Hemlock plant; direct filtration
Salt Lake City	UT	4900390(662)	100	1.3	1.7	City Creek WTP: Several rivers; Conventional
Clayton Co.	GA	0630000(324)	27	3.4	2.2	Hooper WTP
New York	NY	0003493(721)	1400	1.5	2.6	Catskill System; unfiltered
High Known/Unknown TOX						
Shrewsbury	NJ	1345001(473)	38	3.0	6.9	Swimming River WTP
Glendale	AZ	0407093(126)	42	6.8	7.3	Cholla WTP
Sacramento	CA	3410020(205)	122	1.3	2.6	Sacramento River WTP; Fairbairn WTP (ICR#204) may be similar
Durham	NC	0332010(450)	35	4.4	4.8	Brown WTP

114

The variable pH was examined as a likely factor in determining the ration of known to unknown TOX. pH is well known to play a prominent role in determining relative amounts of THMs and trihaloacetic acids in laboratory tests. This pH relationship has also been observed when comparing overall speciation of THMs versus Total HAAs, although it is usually weaker. Examination of the IRC data shows that this also holds true in full-scale, on a national level (Figure 76).
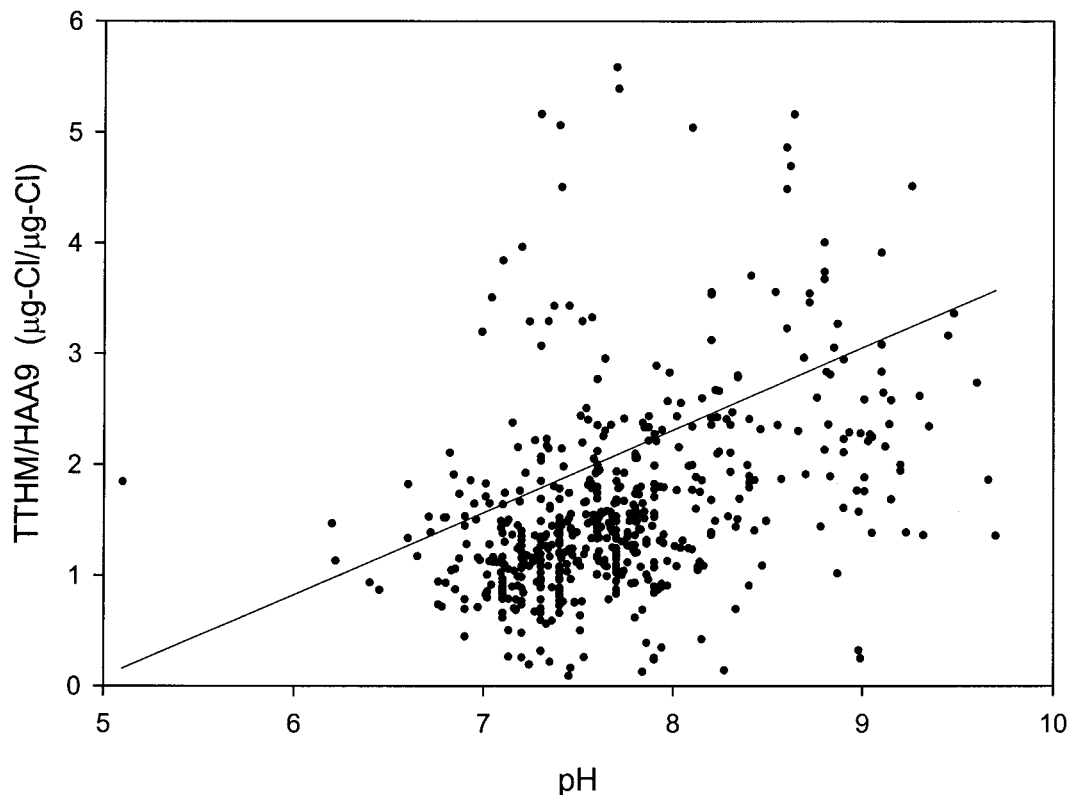
Figure 76: Relationship between TOX Speciation and pH in ICR Data (SDS subset)

Laboratory data also show a distinct increase in known TOX to unknown TOX with increasing pH (see appendix). We attempted to investigate this effect in the ICR data, and the results are summarized in Figure 77. Its clear that there is only a very weak relationship between (TTHM+HAA9)/UTOX and pH. Also shown in this figure are lines representing data from laboratory studies of TOX and DBP formation (for more, refer to appendix). These are

115

represented in the form of model predictions based on the data and formulated for two chlorine doses at contact times of 3 days. These doses were selected to cover the range of very high residuals (as expected with the 20 mg/L dose) and near zero residuals (i.e., the 3 mg/L dose). A substantial amount of the ICR data fell between these two lines, but the majority of it fell either above or below. There's little doubt that this is a reflection of the great diversity of natural waters, which a single extracted fulvic acids from one source cannot adequately capture.

One of our conclusions from this analysis is that pH is not a major factor in predicting known TOX to UTOX ratios in the ICR data. For this reason, it was not considered in this analysis represented by Figure 75.
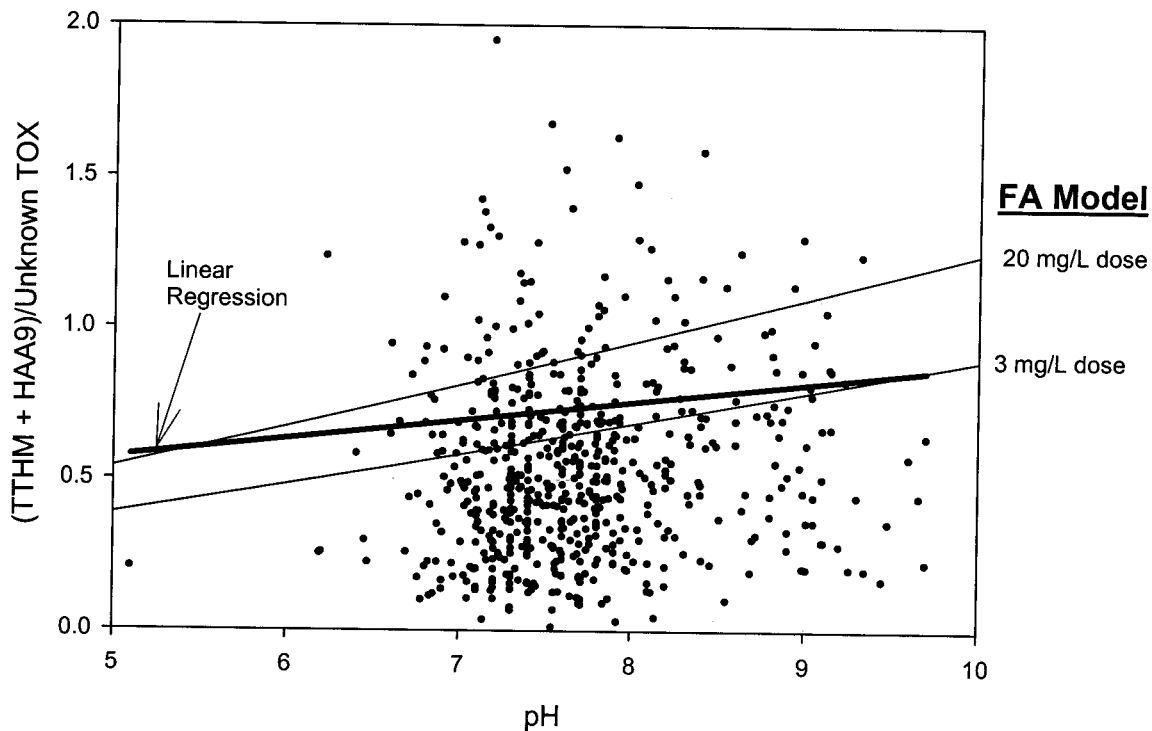
Figure 77: Relationship between Known/Unknown TOX ratio and pH in ICR Data; Comparison with Model based on Laboratory Fulvic Acid Data[8]

Other Criteria

Additional criteria including HAN formation, ecosystem regions and general watershed characteristics have only been partially explored. For example, there are many levels or types of

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[8] ICR SDS Data plotted versus pH. Model based on BLFA data, assuming 72 hour reaction time and two example doses.

ecosystem designations that are appropriate for capturing and classifying the diversity of flora, fauna, geology, climate and topography (Figure 78). The USEPA has characterized these tools as follows.

Figure 78. Level II Ecoregion Designation for North America

"Ecoregions are defined as areas of relative homogeneity in ecological systems and their

117

components. Factors associated with spatial differences in the quality and quantity of ecosystem components, including soils, vegetation, climate, geology, and physiography, are relatively homogeneous within an ecoregion. Ecoregions separate different patterns of human stresses on the environment and different patterns in the existing and attainable quality of environmental resources. They have proven to be an effective aid for inventorying and assessing national and regional environmental resources, for setting regional resource management goals, and for developing biological criteria and water quality standard."[9]

In addition to the other criteria mentioned, we will make note of the ecosystem designation for watershed from the candidate utilities. We would then strive to include samples from all of the major ecosystems, and as many secondary ones as we can accommodate given the other criteria and the limitations on site numbers.

We have also decided to consider build on the synergy of another project that is also engaged in identifying a set of diverse locations for study. A mail survey is being conducted in connection with the ongoing project "Watershed Sources and Long-term Variability of BDOM and NOM as Precursors". Written surveys were sent to all US utilities serving more than 50,000 and using some surface water. For the purposes of this TOX study, we added a few questions to the survey on TOX data. Using this information as well as the NOM-related information, we should have yet another means of assessing candidate sites for the task 2 work.

PRELIMINARY RAW WATER AND DISINFECTED WATER TESTS

During the first two project periods, several opportunities presented themselves to analyze some diverse treated drinking waters for TOX and UTOX. These samples were all collected by one of the PIs (Reckhow) and immediately transported to the UMass laboratory for analysis. While these waters were not formally selected for study, it was thought that their analysis would help to expand the existing database on UTOX. The three were from Binghamton, NY, North Brookfield, MA and Gardner, MA.

The data from Binghamton are summarized in Table 21. Table 22 contains data on

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[9] http://www.epa.gov/bioindicators/html/usecoregions.html

finished water from the two central Massachusetts communities. It's interesting that Gardner has such a low ratio of known TOX to unknown (KnTOX/UTOX). This is probably worth examining further, as this might be a suitable site representing other waters with high ratios of unknown TOX. The raw and filtered waters from Binghamton give anomalous KnTOX/UTOX ratios because these have not yet seen direct chlorination. However, the subsequent samples did show typical ratios. Binghamton treats water from the Susquehanna River, a rather turbid and moderately contaminated supply.

Table 21: Analysis of Water Samples from Binghamton, NY

Sample	pH	UV$_{254}$	DOC (mg/L)	CHCl$_3$ (µg/L)	CHCl$_2$Br (µg/L)	CHClBr$_2$ (µg/L)	TTHM (µg/L)	DCAA (µg/L)	BCAA (µg/L)	TCAA (µg/L)	HAA9 (µg/L)	TOX (µg Cl/L)	KnTOX/UTOX
Raw water	6.96	0.0715	2.42	8.8	1.9	0.2	11.8	0.3	BDL	0.3	1.6	12.5	0.89
Filtered water	6.57	0.0296	1.42	8.1	BDL	BDL	8.1	3.0	BDL	1.8	7.3	12.4	1.08
Finished water (Clearwell)	6.45	0.0215	1.52	10.6	BDL	BDL	10.6	7.5	1.2	6.4	17.3	70.4	0.34
Distribution system (Espail Res.)	6.44	0.0249	1.44	13.9	0.9	BDL	13.9	20.4	2.0	29.3	59.0	179.0	0.38

Table 22: DBPs in Finished Water Samples from Gardner and North Brookfield, MA

Source	UV$_{254}$	TOC (mg/L)	CHCl$_3$ (µg/L)	CHCl$_2$Br (µg/L)	CHClBr$_2$ (µg/L)	TTHM (µg/L)	DCAA (µg/L)	BCAA (µg/L)	TCAA (µg/L)	HAA9 (µg/L)	TOX (µg-Cl/L)	KnTOX/UTOX
Gardner. MA	0.045	2.63	34.90	7.09	1.89	44	28.38	4.17	2.84	35	284.8	0.25
N. Brookfield, MA	0.026	2.55	33.51	3.99	0.33	38	19.13	1.24	10.96	31	162.1	0.56

TASK 2 EXPERIMENTAL DESIGN

Task 2 is intended to generate data on the range of UTOX values that may be observed in waters across North America. Once the participating utilities are selected, raw waters and finished waters will be collected from each site at different points throughout the project period. These will be shipped to UMass for treatment with disinfectants and chemical analysis. At UMass each will be treated with the five disinfection scenarios (chlorine, chloramine, both with an without preozonation, and chlorine dioxide). A standard set of protocols will be used for all samples (see Table 2). All samples will then be quenched and analyzed for the full suite of DBPs (THM, HAAs, TOX, TOCl, TOBr and TOI).
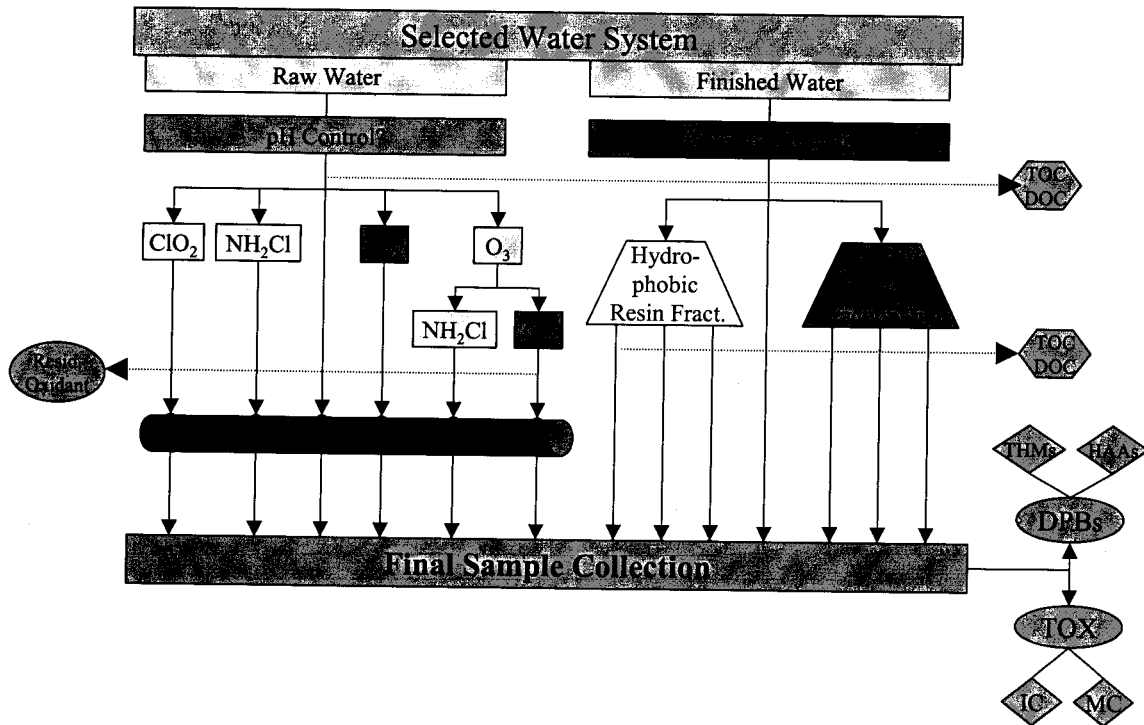
Figure 79. Task 2 Experimental Flow Diagram

The first utility formally selected for inclusion in task 2 is Cambridge, MA. This is a city with a state-of-the-art ozone plant, and an organization that is quite interested in participating in research projects such as this one.

Table 23. Task 2 Test Conditions

	Standard conditions
Bromide/Iodide	Ambient
pH	Ambient
Pre-O_3 dose	1 mg-O_3/mg-C
Free Cl_2 target residual	1.5 mg/L
Chloramine target residual	2.5 mg/L
Cl_2/N ratio	4.5 g/g
ClO_2 dose	1.5 mg/L
Disinfectant Contact Time	48 hr
Temp	20°C

FULL TESTS ON WATER FROM CAMBRIDGE, MA

Raw water quality

The Cambridge raw water comes from the Hobbs Brook and Stony Brook Reservoirs located west and north of the City of Cambridge. The raw water has a moderate TOC and SUVA (Table 24).

Table 24. Characteristics of Raw Water Sample from Cambridge

Sample Location	Date of collection	TOC (mg/L)	DOC (mg/L)	UV_{254} (cm-1)	SUVA (L/mg/m)	Br⁻ (µg/L)	pH
Cambridge, MA	02/18/04	4.39	4.20	0.141	3.35	95	6.7

Finished water quality

Cambridge treats its water by pre-ozonation, coagulant addition in rapid mix, flocculation, dissolved air flotation, intermediate ozonation, GAC filtration, pH adjust,

122

fluoridation, and chloramination. The finished water is of very high quality (Table 25) and the residual organic matter is highly oxidized as evidenced by the low SUVA.

Table 25. Characteristics of Finished Water Sample from Cambridge

Sample Location	Date of collection	TOC (mg/L)	DOC (mg/L)	UV_{254} (cm-1)	SUVA (L/mg/m)	Free Cl2 Residual (mg Cl_2/L)	Monochloramine Residual (mg Cl_2/L)	pH
Cambridge, MA	02/18/04	2.07	2.05	0.035	1.69	0	2.06	7.8

Analysis of the finished water shows low THM and HAA concentrations (Table 26), commensurate with the compliance values from the distribution system. Accordingly, the TOX, TOCl and TOBr values are quite low. The UTOX/TOX are typical for most waters (~50%).

Table 26. DBP Analysis of Finished Water Sample from Cambridge, MA

Sample	TTHM (μg/L)	HAA9 (μg/L)	TOX (μg Cl/L)	UTOX (μg Cl/L)	UTOX /TOX (%)	TOCl (μg Cl/L)	UTOCl/ TOCl (%)	TOBr (μg Cl/L)	UTOBr/ TOBr (%)
Finished Water	14.1	19.9	38.0	19.8	52.4	22.2	44.6	13.3	57.0

Fractionation of the finished water shows a nearly equivalent balance between hydrophobic and hydrophilic organics (Table 27). The TOX is similarly balanced, but in this case the transphilic shows a proportionally larger amount. Analysis of THMs and HAAs shows that most of the THMs are almost completely lost across the XAD-8 column. The HAAs are partly removed by XAD-8 and partly by XAD-4. Accordingly, the remaining (unknown) TOX completely resides in the hydrophilic and transphilic fractions.

The organic material was intermediate in size. Based on the DOC, there was a

123

disproportionate amount of TOX in the smallest size fraction. However, most of that was detectable as THMs and HAAs. Once these "known" compounds were subtracted from the overall TOX (i.e., the UTOX), the distribution of halogenated compounds matched well the DOC in the three smaller size fractions. The larger fraction still has a lower halide content than the others.

Table 27. Hydrophobicity and Molecular Size Analysis of Finished Water Sample from Cambridge

Sample	DOC (mg/L)	DOC fractions(%)	TOX (μg Cl/L)	TOX fractions (%)	UTOX (μg Cl/L)	UTOX fractions (%)
Hydrophobic	0.80	39.0	11	27.9	0.2	1.2
Hydrophilic	0.76	36.9	10	27.4	9.3	47.1
Transphilic	0.49	23.9	17	44.7	10.3	51.7
MW>10K	0.25	12.3	1	2.5	1.0	5.0
10K<MW<3K	0.63	30.5	12	30.4	8.8	44.5
3K<MW<0.5K	0.80	38.9	11	28.9	9.0	45.3
MW<0.5K	0.37	18.1	14	38.2	3.6	18.4
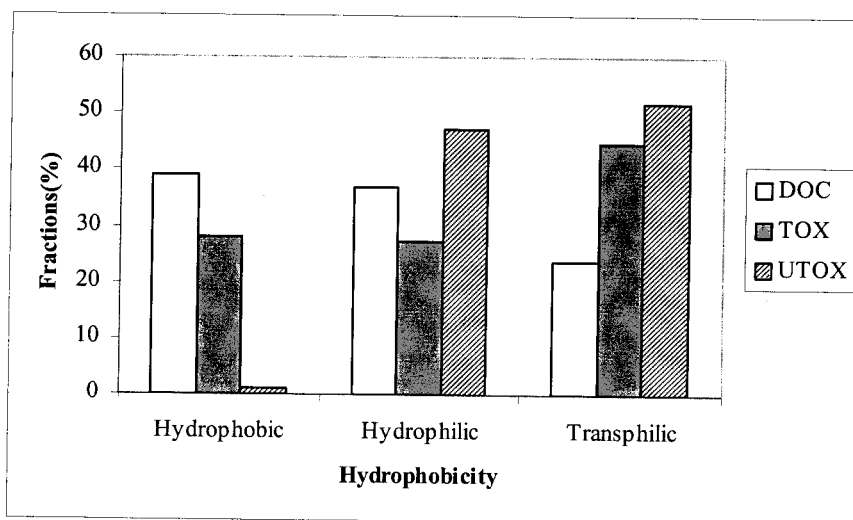
Figure 80. Cambridge Finished Water: Hydrophobic and Haloorganic Properties
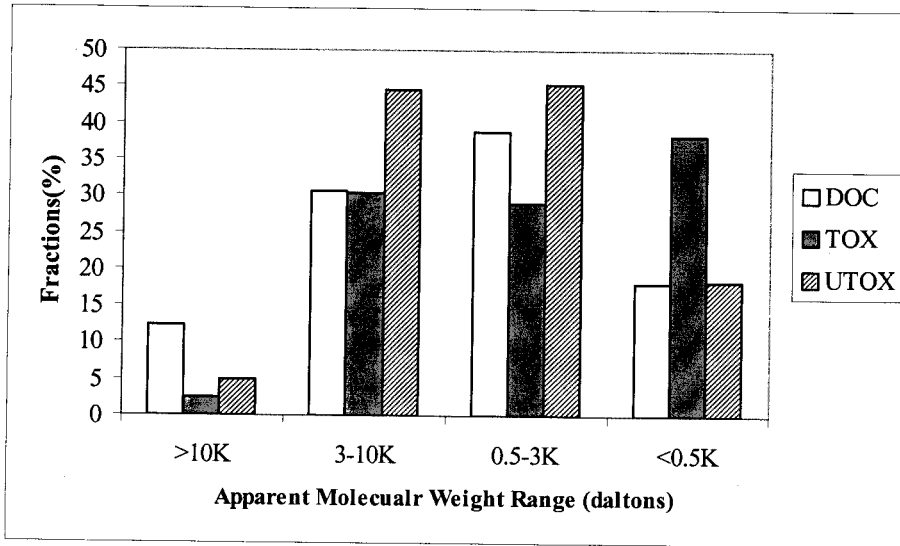
Figure 81. Cambridge Finished Water: Molecular Size and Haloorganic Properties

Raw Water Disinfection Tests

Chlorine and chloramine demand tests were necessary to select the proper doses for this particular water (Figure 62 and Figure 63). Given the target residuals, doses of 5.2 mg/L and 2.6 mg/L were selected for chlorine and chloramines respectively.
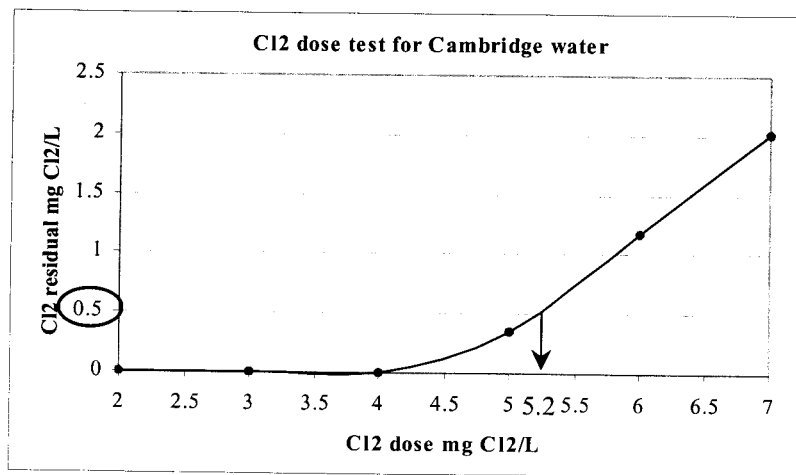
Figure 82. Chlorine Demand Test Results for Cambridge Raw Water
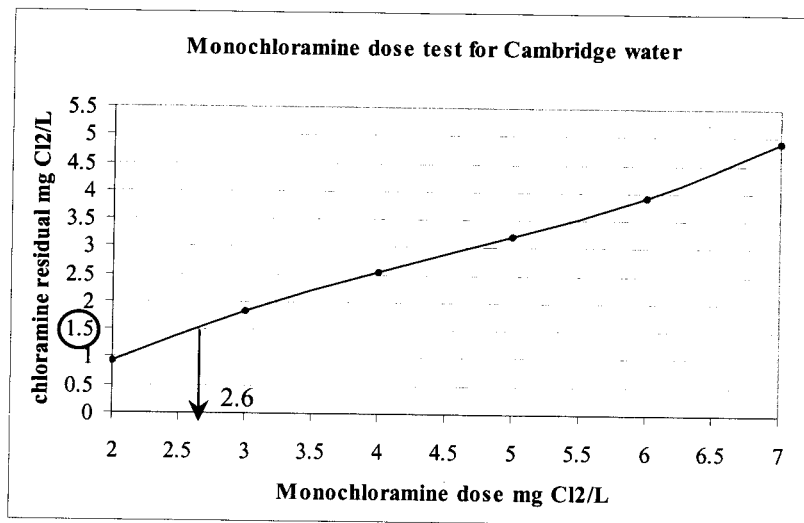
Figure 83. Chloramine Demand Test Results for Cambridge Raw Water

Considering the raw water DOC, it is not surprising that free chlorine produces well over 100 ug/L of THM and HAA (Table 28). However the total unknown DBPs amount to an additional 59.4% (Table 29). As expected, preformed chloramines, and chlorine dioxide produce almost no THMs and relatively small amounts of HAAs. They do, however, produce substantial TOX. Most of this is "unknown" (i.e., >80%).

Table 28. DBP Analysis for Cambridge Raw Water Test

Samples	TTHM (µg/L)	HAA9 (µg/L)	TOX (µg Cl/L)	UTOX (µg Cl/L)	TOCl (µg Cl/L)	TOBr (µg Cl/L)
Cl₂	173.1	190.4	643	382	595.7	37.1
PFCLM	3.2	30.6	118	98	118.3	5.1
O₃/Cl₂	90.0	158.0	335	160	296.1	25.2
O₃/CLM	1.7	22.1	79	64	65.7	4.6
ClO₂	0.7	15.4	52	43	40.1	8.9

An error occurred in these ozone tests such that the samples were overdosed by an indeterminate amount. For this reason, the magnitude of the impact of pre-ozonation is probably overstated in the data. Nevertheless, it is clear that ozone can destroy precursors THM, HAA

126

and "unknown" TOX, whether followed by chlorine or chloramines.

Table 29. TOX & UTOX Percentages

Samples	UTOX/TOX (%)	TOCl/TOX (%)	TOBr/TOX (%)	UTOCl/TOCl (%)	UTOBr/TOBr (%)
Cl_2	59.4	94.1	5.9	59.8	41.9
PFCLM	83.0	95.9	4.1	84.1	73.8
O3/Cl_2	47.6	92.1	7.9	47.1	25.3
O_3/CLM	81.6	93.5	6.5	80.8	59.2
ClO_2	83.7	81.8	18.2	82.9	82.5

CHAPTER 7: ADVANCED CHARACTERIZATION OF UNKNOWN TOX

All of the task 4 work during the first two project periods involved training and method refinement/development. During the most recent project period, work began on analysis of raw and chlorinated drinking water samples. This work was done at both Ohio State University and the University of Massachusetts.

CUO OXIDATION METHOD

Lignin is a complex biomacromolecule composed of methoxylated phenol units linked by ether and carbon bonds. Alkaline CuO oxidation is one technique commonly used to analyze the composition of lignins in complex samples (Lobbes 1999; Louchouarn 2000). More generally it has been used to characterize natural organic material (NOM), with special application to lignin-type structures incorporated into the NOM.

Initial work at UMass focused on selection, testing and refinement of existing CuO degradation methods and analysis of products. These involve chromatographic separation and identification by mass spectrometry. The method of choice is one based on the classical Hedges & Ertel protocol with some important modifications (Figure 84 and appendix). Among these are the use of ethyl acetate instead of diethyl ether as the primary extraction solvent. We've also been using both LC/UV and LC/MS in separating and identifying CuO degradation products (GC/MS remains an option). Finally, we have adopted the microwave digestion method as it has greater promise for consistent reaction conditions (temperature and pressure), which should translate to more reproducible results. The full methodology is attached as Appendix B. Note that this is still in draft form, and some sections have not been finished as of this writing.
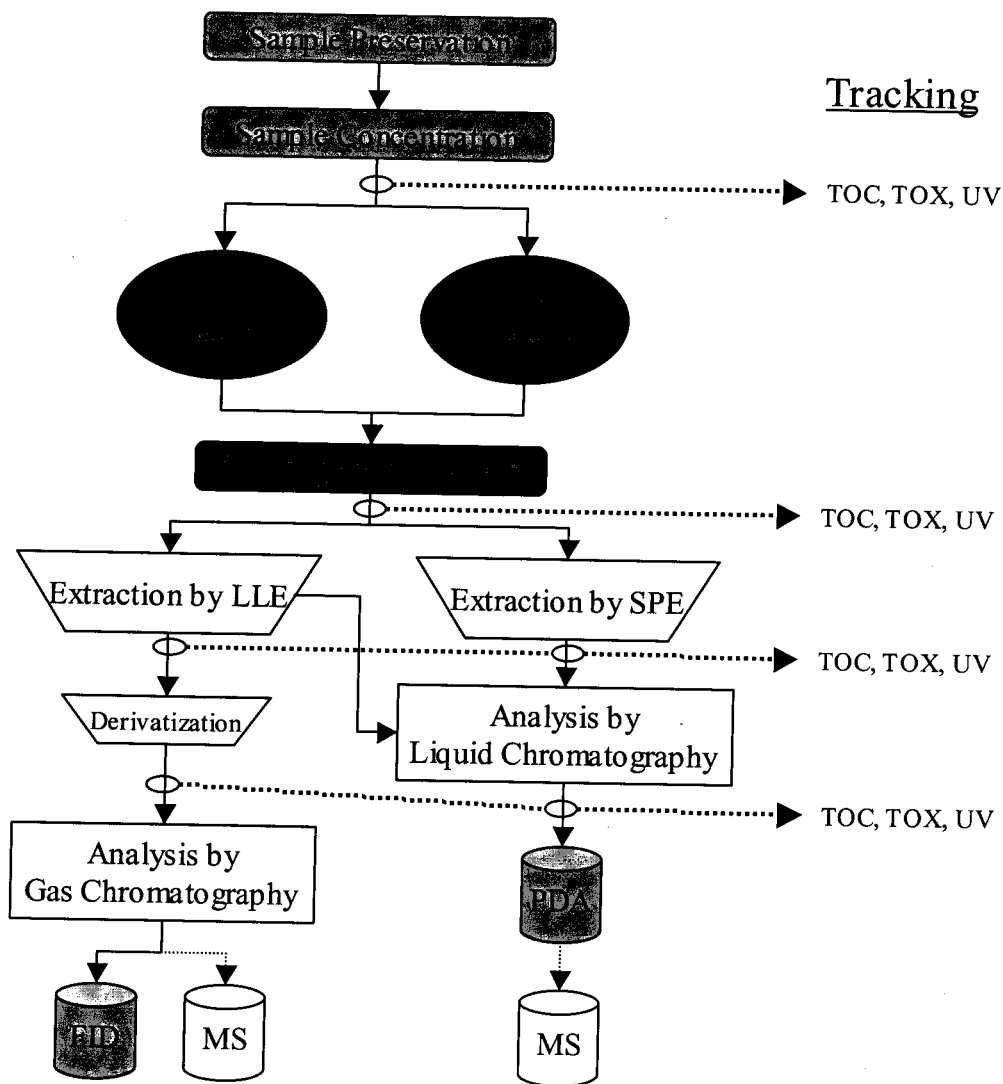
Figure 84: Schematic for CuO Method incorporating both GC and LC (figure also shows tracking options)

As originally developed by Hedges and Ertel (1988), CuO oxidation methods have several drawbacks that have limited their wider utilization. Important constraints include the relatively few samples that can be analyzed during a single oxidation, the long duration of the reaction, and the analyst-intensive nature of the procedure.

A CuO oxidation method with microwave digestion has been developed by Goni and Montgomery(2000). We have selected many features from this method and we have modified it for analysis with HPLC-MS.

Degradation was preformed with the help of a CEM MARS-X microwave digestion

129

system fitted with up to 12 all-Teflon vessels (CEM) designed for liquid-phase hydrolysis reactions (Figure 85).
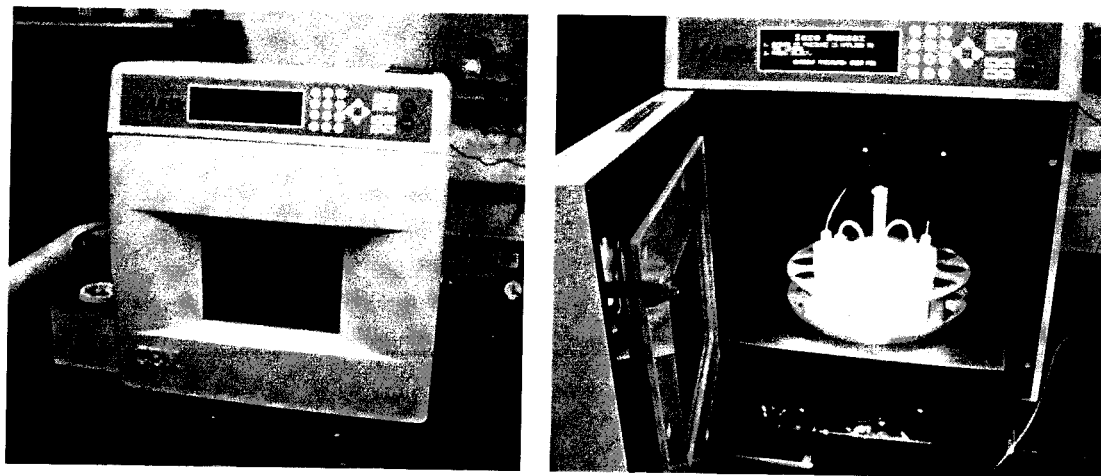
Figure 85. Microwave Digestor, showing exterior and interior

Lignin compounds in water were analyzed in 4 principal steps; sample concentration, microwave-assisted digestion using alkaline CuO, analysis HPLC and finally UV or MS.

I. Sample concentration for CuO degradation

a. Disk Method
1. Bring analytical samples to room temperature, and prepare surrogate standard and QC sample

2. Condition C18 extraction disks and place in extraction apparatus
   - 3M Empore disk; 47 mm(St. Paul, MN)
   - follow manufacturer's instructions
     - Soak with 10mL of MeOH
   - standard 47 mm filter apparatus consisted of a stainless steel mesh support on a Teflon base
     - An aspirator or vacuum pump is used to draw the water samples through the extraction disk

3. Filter and acidify water sample to be extracted
   - Use 0.3 um nominal pore size glass filters (Whatman, Clifton, NJ)
   - Then adjust pH to between 2 and 2.5 with tetrafluoroacetic acid (Applied Biosystems, Foster City, CA) or hydrochloric acid (ACS grade, Fisher Scientific)

4. Pump 0.3 to 7 Liters of acidified sample through the extraction disk
   - Volume is selected so that DOC loading is about 5 mg-C

5. Elute disk
   - Use 10mL of 90:10 MeOH:H2O eluent

130

- Repeat with a second 10 mL volume of eluent

6. Bring eluent to dryness under N2
   - Evaporator setup

b. Disk method (Louchouarn 2000)

1. Bring analytical samples to room temperature and prepare surrogate standard and QC samples

2. Condition SPE (C18) extraction disks
   - C18-SPE Mega-Bond Elut; Varian
   - Pretreated with 100mL methanol
   - Before extraction, followed by acidified (pH2) Milli-Q Plus UV water (50 mL)

3. Filter and acidify water sample to be extracted
   - Filtered water samples were acidified to pH 2 with HCl
   - Pumped through the SPE cartridge with a peristaltic pump and silicone tubing (Cole Parmer)

4. Pump (1-50 L) of acidified sample though the SPE disk
   - Volume is selected so that DOC loading is about 5 mg-C
   - Flow rate: 50 ± 2 mL/min
   - The cartridges were stored at 4 ℃ or in the freezer after extraction

5. Elute SPE disk
   - Use 50 mL of methanol

6. Bring eluent to dryness under vacuum
   - Collected into a muffled glass flask
   - Evaporated to dryness under vacuum

II. CuO method with microwave digestion

1. Add 2-5 mg of the dried sample to an all-teflon PFA reaction vessel.
   - A Teflon-lined mini-bomb

2. Add regents:
   - 0.50 g of fine CuO powder
   - 0.050 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (binds any remaining oxygen)
   - 15 mL of N_2-sparged (overnight) 2N NaOH
   - 13.3 µL of the 5.49 mM stock solution of Ethylvanillin
   - Stir Bar

3. Cap vessels, place in microwave oven, and flush with N2
   - Cap using automatic capping station (CEM)
   - Place them in rotating tray
   - Interconnect with Teflon tubing
   - Install temperature and pressure probes (Temp probe goes in first tube, pressure in last one)
   - Leak check with 60 psi N2
   - Purge system several times with new N2
   - Establish a slight positive pressure of N2 (~10 psi)

131

4. Run microwave oven for 90 min at 150 ℃
   - Temperature is reached in 10 min
   - Pressure is held at 60-70 psi

5. Allow samples to cool and open
   - Open using the capping station

III. Post-Digestion Treatment

1. Add known amount of 2° recovery standard to each reaction vessel
   - 40 µL trans-cinnamic acid (40 µL of 6.75 mM stock)
   - phenylacetic acid

2. Transfer contents to a 50 mL centrifuge tube
   - Rinse with small amount of 1N NaOH

3. Centrifuge samples to remove solids
   - 3000 rpm for 10 minutes

4. Transfer supernatant to extraction vial
   a) Decant supernatant from reaction vessel into an appropriate vial
   - For classical method use 50 ml Pyrex tubes fitted with Teflon-coated caps, or larger Pyrex and Teflon vessels as needed
   - For microwave digestion use 50 mL Pyrex tubes fitted with Teflon-coated caps
   b) Add additional 1N NaOH to each tube to help with quantitative transfer
      - For classical method use about 20 mL of 0.1 N NaOH
      - For microwave digestion use about 5 mL of 0.1 N NaOH
   c) Repeated centrifuge step
   - 3000 rpm for 10 minutes

5. Acidify solution to about pH 1
   - For classical method add about 4 mL of conc. HCl
   - For microwave digestion add between 4 and 40 mL of conc. HCl, depending on total volume

IV. Microwave Hydrolysis System Operation
1. Add the appropriate amount of sample to the 120 mL vessel body containing the sample.

2. Close the cap
   - Place the seal ring
   - Cap onto the vessel body
   - Thread the vessel cap onto the vessel body "finger tight".

3. Seal the vessel cap using the electronic Capping Station
   - Carefully lower the vessel into the capping socket
   - Push and hold the toggle switch upward to the position marked "Tighten".
   - Hold the toggle switch in the "Tighten" position until the needle of the torque meter reaches the blue colored region indicating
   - Hold the Toggle switch 5 times

4. Place it into the turntable that total number of samples to be run

5. Connect the tube to the vessel
   - Loosen the ferrule nets on the sample vessels
   - Insert a 0.125 in. O.D. tube into a vessel
   - Connect it to the vessel in the position beside it
   - Adjust the ferrule nets finger tight
   - Using a small wrench, slightly turn the net (no more than 1/4 turn)
   Note: Do not connect the port of the last vessel to the port of the first vessel. These two ports will be used for temperature and pressure sensors.

6. Connect the temperature
   - Carefully slide the ferrule nut on the a Teflon coated pyrex thermowell
   - Insert it into the open port of the last vessel until the tip nearly touches the bottom of the vessel
   - Insert the temperature probe into the thermowell
   - Plug the temperature probe into its receptacle located in the cavity ceiling

7. Connect the pressure sensing line
   - Connect the pressure sensing line to the open port in the first vessel
   - Tighten the ferrule not to secure the pressure line

8. Rotate the turntable
   - Secure the pressure and temperature sensing line into the standoff located in the center of the turntable
   - With the instrument door open, press the turntable key to rotate the turntable
   - Allow the turntable to rotate 2 or 3 times to ensure that probes do not become entangled
   - If necessary, adjust and/or reposition tubing, recheck turntable rotation
   - Close the instrument door

9. Close the vent valve and open the sample isolation valve

10. Load the program a hydrolysis method
    - Press CUO 1-2 VSLS-PFA when there will be 1-2 samples
    - Press CUO 3-6 VSLS-PFA when there will be 3-6 samples
    - Press CUO 7-12 VSLS-PFA when there will be more 7 samples

11. Put nitrogen gas in the vessel
    - Turn the circular valve handle to the "Nitrogen" position
    Note: isolation valve must be open and vent valve must be closed to blanket samples with nitrogen
    - Open the nitrogen cylinder valve
    - Adjust the pressure regulator to deliver 15 psig to the sample vessels for a period of 10 seconds
    - Turn on the vacuum pump
    - Turn the circular handle on the valve panel to the "Vacuum" position
    Note: Sample isolation valve must be open and vent valve must be closed during vacuum evacuation
    - Evacuate the sample vessels down to 1.0 Torr as indicated on the vacuum pump gauge
    - When the vacuum stabilized, turn the circular valve handle to the "Nitrogen" position
    - Turn the circular valve handle between "Nitrogen" and "Vacuum" positions a minimum of five times.

12. Close the sample isolation valve
    - Sealing the samples under a 15 psig nitrogen atmosphere

13. Press "Start" to run the programmed hydrolysis procedure

14. Release the vessel
- At the conclusion of the hydrolysis cycle, Remove the temperature probe form the connector in the ceiling of the instrument cavity
- Lift the turntable of the drive lug
- Cool the vessels by lowering the entire turntable assembly into a plastic basin containing an ice water bath
- The acid vapor pressure shown on the display will decrease
- Remove the turntable from the ice water bath five minutes after the pressure reads <50 psig.
- Place the turntable on the drive lug in the microwave cavity and close the instrument door

15. Disconnect the tube
- Open the vent valve to release the remaining atmosphere of N_2 gas
- Disconnect the pressure sensing line from the fitting of turntable handle
- Remove the turntable form the cavity, loosen the ferrule nut and remove the tube from each sample vessel

16. Loosen the vessel
- Carefully lower each sample vessel into the socket of the Capping Station
- Briefly push the toggle switch to the position marked "Loosen"
- The vessel cap will loosen by turning in a counterclockwise direction

Caution: Do not completely unthread the vessel cap in the Capping Station. Removing an uncapped vessel from the Capping Station may permit acid spillage and/or contamination of the samples

17. Lift the vessel from the Capping Station and remove the cap
- The hydrolysate should be separated from any remaining solids
- Prepared for analysis

After about 3 months into the testing and validation phase of this work the microwave digestor self ignited and was damaged beyond repair. This occurred at about 30 minutes into a routine run, and resulted in destruction of the Teflon vessels, loss of the samples and severe damage to the oven. After examining the charred, damaged oven, the manufacturer proposed that a runaway reaction occurred initiated by the NaOH solution used in these tests. They suspect this encouraged arcing that could have caused a small amount of charring in one of the Teflon vessels. Once this happened, the char could serve to focus microwave energy, lead to more charring, and initiate a runaway reaction. The possible arcing problem from a 1N NaOH solution was not initially recognized by the manufacturer, but in retrospect they believe that this contributed to the catastrophe. For this reason, we decided that the risks of continuing with a new microwave digestor from this vendor was unacceptable. We are now using a conventional laboratory oven for this work. We will report on the exact oven protocol in the next progress report.

Analysis of Fragments

Lignin Monomers

The monomeric phenols listed in Table 30 are the major classical products of the alkaline CuO oxidation of lignin. They are referred to as lignin phenols in the text.

Table 30. Lignin Phenolic compounds

Peak no.	Lignin phenols	RT (MIN)	M.W. (G)	CAS No
1	4-hydroxybenzioc acid	12.57	138.12	
2	4-hydroxy-3-methoxy-benzoic acid (Vanillic acid)	16.33	168.15	121-34-6
3	4-hydroxybenzaldehyde	17.36	122.12	123-08-0
4	3,5-dimethoxy-4-hydroxy-benzoic acid (Syringic acid)	19.10	198.28	530-57-4
5	4-hydrixyacetophenone	23.23	136.15	99-93-4
6	4-hydroxy-3-methoxy-benzaldehyde (Vanillin)	23.23	152.15	121-33-5
7	3,5-dimethoxy-4-hydroxy-benzaldehyde (Syringaldehyde)	28.26	182.18	134-96-3
8	4-hydroxy-cinnamic acid (*p*-coumaric acid)	29.06	164.16	501-98-4
9	4-hydroxy-3-methoxy-acetophenone (Acetovanillone)	30.08	166.18	498-02-2
10	3,5-dimethoxy-4-hydroxy-acetophenone (Acetosyringone)	34.53	196.20	2478-38-8
11	4-hydroxy-3-methoxy zimt acid (Ferulic acid)	35.52	194.19	537-98-4
12	4-hydroxy-3-ethoxy-benzaldehyde (Ethyl vanillin)	40.40	166.18	121-32-4
13	3-phenyl-propenoic acid (Cinnamic acid)	56.51	148.16	140-10-3

Commercially available standards of the lignin phenols were obtained from Aldrich Chemical Company (Milwaukee, WI). All chemicals and standards were of HPLC, or the highest available, grade.

1. Preparation of Lignin compounds

- Weigh the desired amount of lignin compound
- Dissolved in methanol: 0.125M
- Acetosyringone was dissolved in methanol using a sonicator
- Keep frozen at -30℃

2. Preparation of stock standard mixtures
- Stock standard mixtures of each lignin phenol (1.25mM) were prepared in water
- In a 100 mL volumetric flask containing some water
  - Add 1 ml of 0.125 M lignin compounds stock
- Keep in a refrigerator in 18 mL polyethylene vials (Beckman, poly-Q vial)
Note: Lignin compounds were stable for more than 3 months except ethyl vanillin was stable for one month.
- Internal standards, 5.49 mM of ethyl vanillin and 6.75 mM of trans-cinnamic acid, were prepared in water.

3. Preparation of calibration standards
- Internal standards: 5, 10, 50, 250, 500, 1000, 5000, 10000, and/or 25000nM
- Add 20 uL of each internal standard to the samples without further dilution

Separation by HPLC

For separation and detection of the lignin phenols, gas chromatography and HPLC methods are generally used. The combination of gas chromatography with mass spectrometry results in a good resolution and unambiguous identification of the phenols. The disadvantage of this method is the additional derivation of the phenols required following the CuO oxidation step (Lobbes, 1999). HPLC methods allow for analysis of the underivatized product. Most published methods using HPLC employ a single absorption wavelength (e.g., 275 nM; Da Cunha 2001). The HPLC method used in this work employs a photodiode array detector, permitting detection from wavelengths of 220 nm to 370 nm. The method enables the reliable determination of the composition and quantity of small amounts of lignin in water samples.

Chromatography and detection of the lignin phenols were carried out on an Alliance Waters 2890 HPLC system consisting of an autosampler, and a PDA detector. A discovery C18 column (5 um particle diameter, 250 mm X 4 mm, Supelco Co.) with a guard column was used for separation. For elution, a gradient program was used (Table 31). This method was modified from Cunha (2001) and Charriere (1991). Flow rate of the gradient was 1.0 mL/min. Injection (100 uL) was made automatically by the autosampler.

Table 31. HPLC Gradient Program

Time (Min)	% A	% B
0	90	10
13	67	30
20	65	35
40	50	50
45	0	100
55	0	100
60	90	10

Eluent A: water/acetonitrile/acetic acid (97.5:0.5:2)
Eluent B: water/acetonitrile/acetic acid (75:25:2)
A discovery C18 column (5 um particle diameter, 250 mm X 4 mm, Supelco Co.)
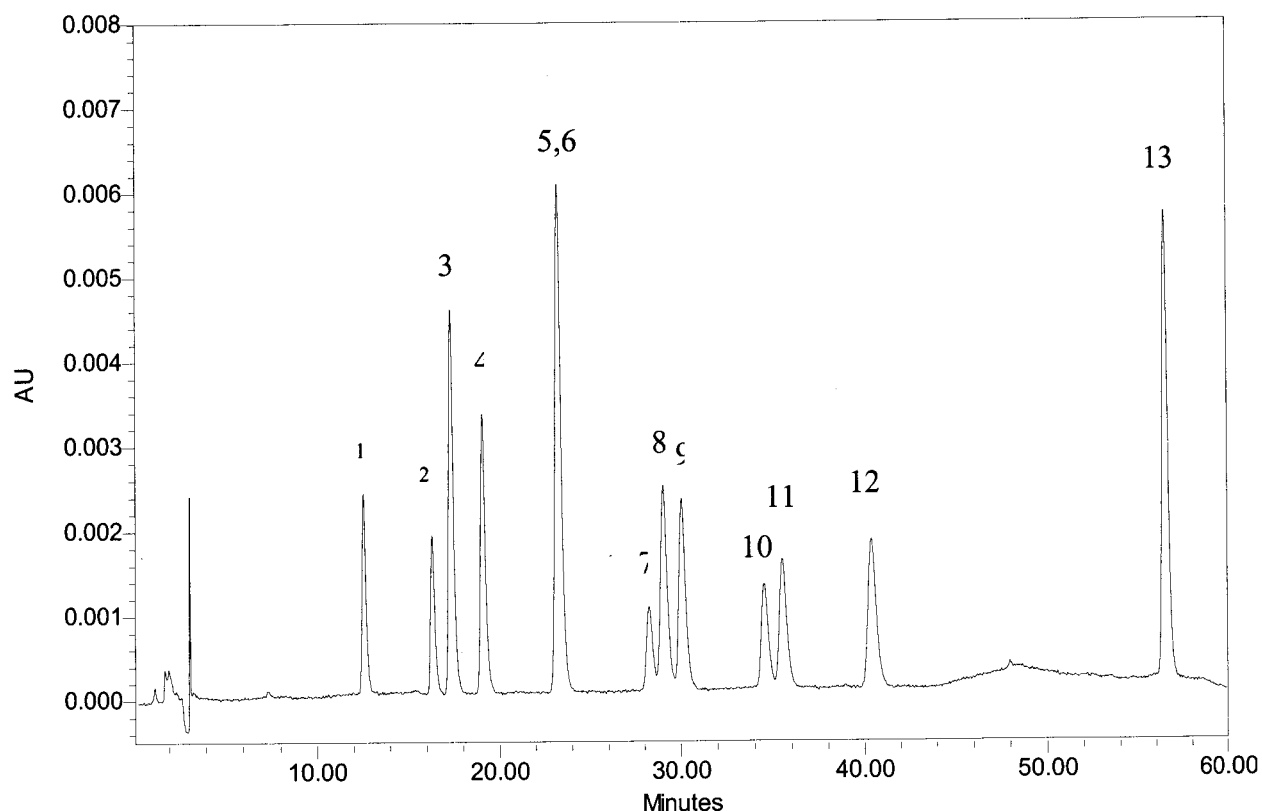
Figure 86. Reversed-phase chromatograms of the lignin and standard phenols (1000nM each); Phenols are numbered by their order of elution; for chemical nomenclature refer to Table 30.

Figure 86 shows a chromatogram of lignin standard phenols. Peaks 12 and 13 are internal standard peaks. Figure 87 shows a 3D UV chromatogram from 220 nm to 370 nm. A

137

wavelength of 275 nm was selected for quantification and presentation as all peaks show a good absorption in this region.
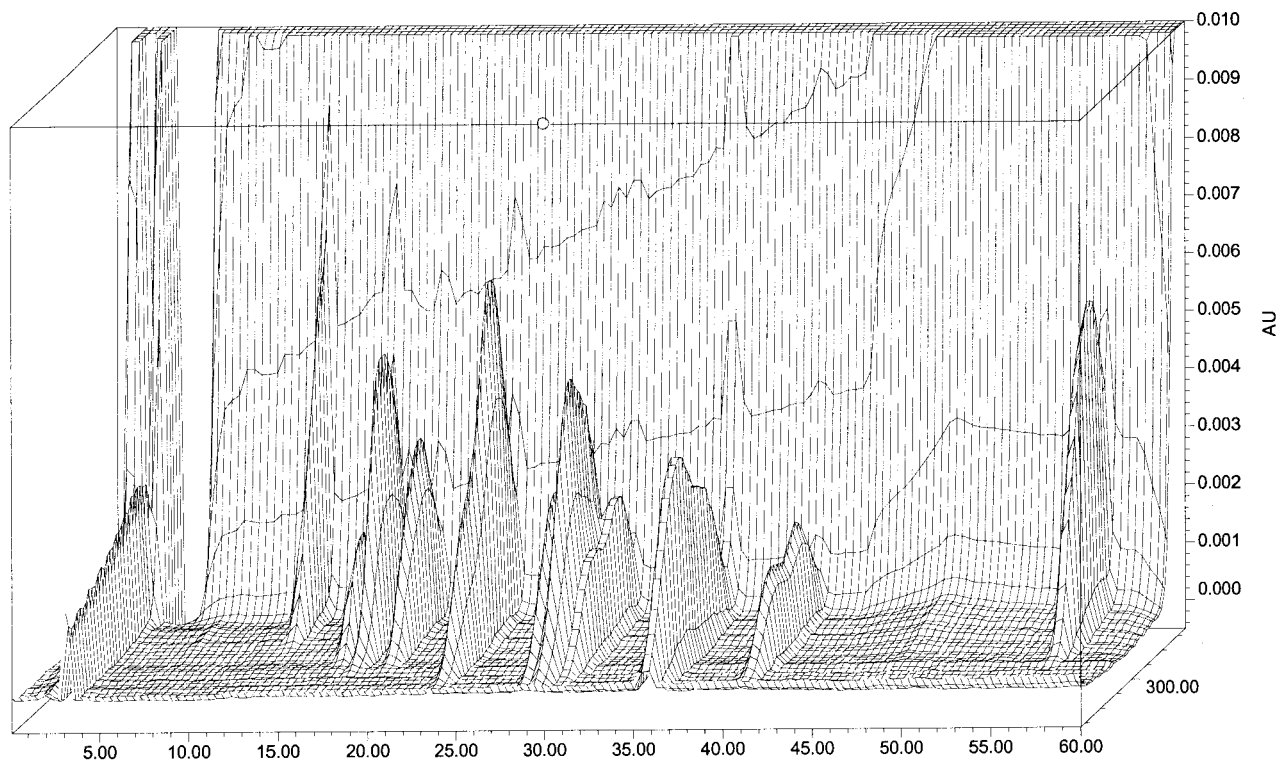
Figure 87. 3D of chromatograph of UV (220 ~ 370 nm)

D. Relative absorption spectra of the lignin compounds

The purity of peaks of the lignin phenols from environmental samples was determined by comparing their absorption spectra with those of the standard phenols. If the spectrum of a lignin-derived phenol was subject to interference by a contaminant, the spectrum of the impurity can be detected at the beginning or end of the peak or by subtraction of the lignin-phenol spectrum form that of the contaminated one. Then the peak areas of the lignin phenol can be integrated separately at the preferred wavelength of 275 nm.

A) Vanillyl-phenol groups

16.325
23.225
30.075

B) Syringyl-phenol groups

19.092
28.258
34.508

C) p-hydroxy-phenol groups

12.575
17.358
23.225

D) cinnamyl-phenol groups

E) internal standard phenols
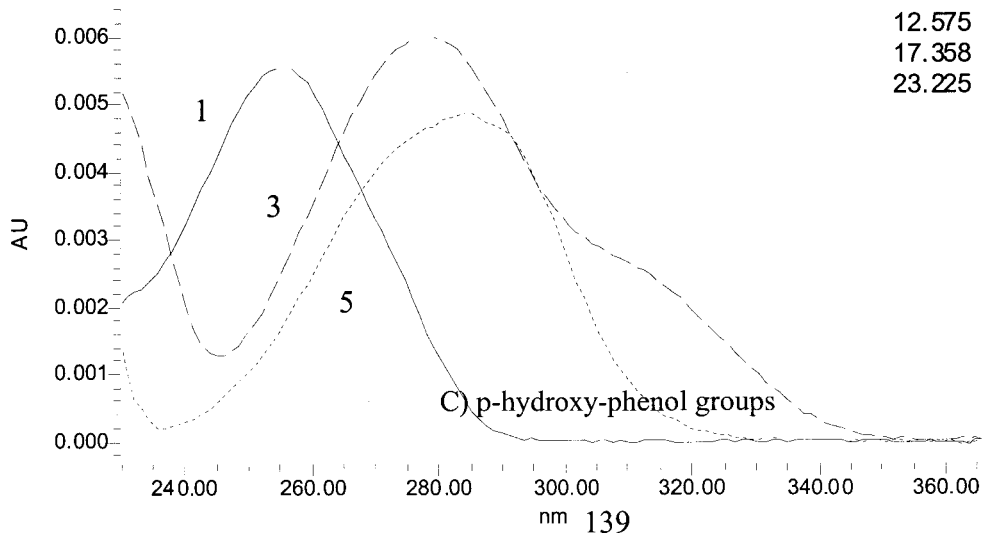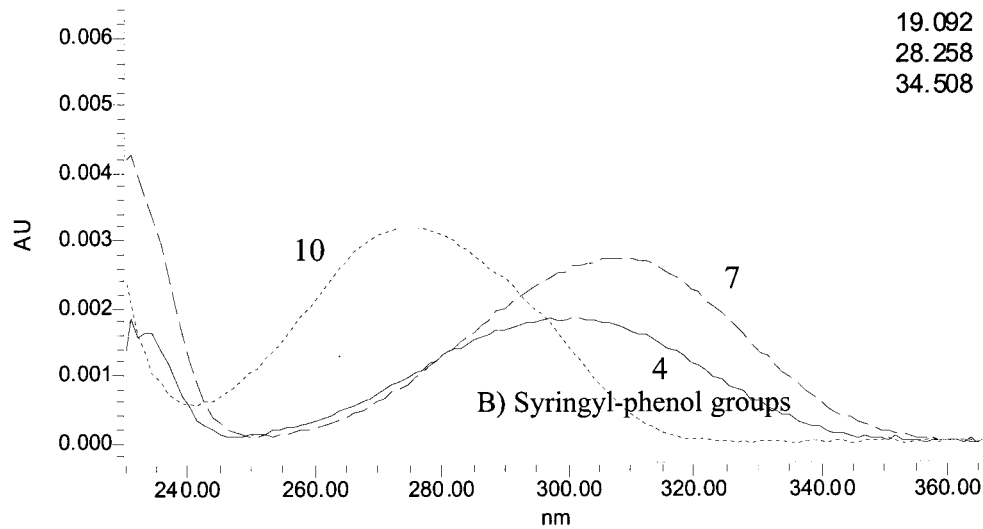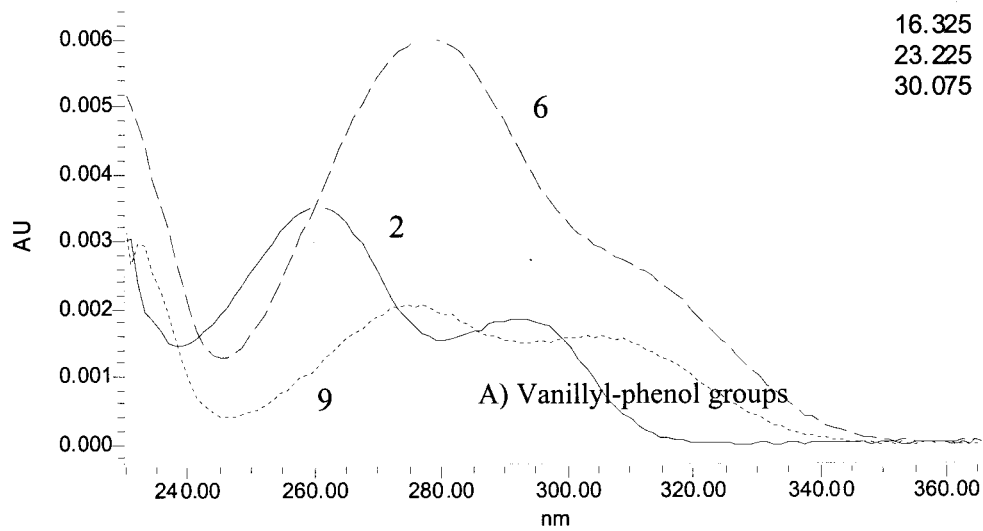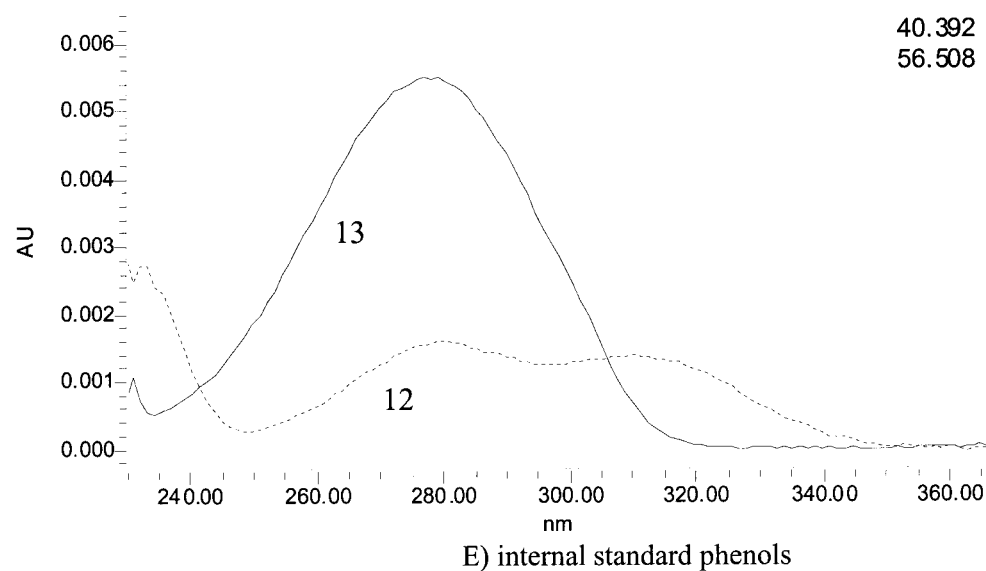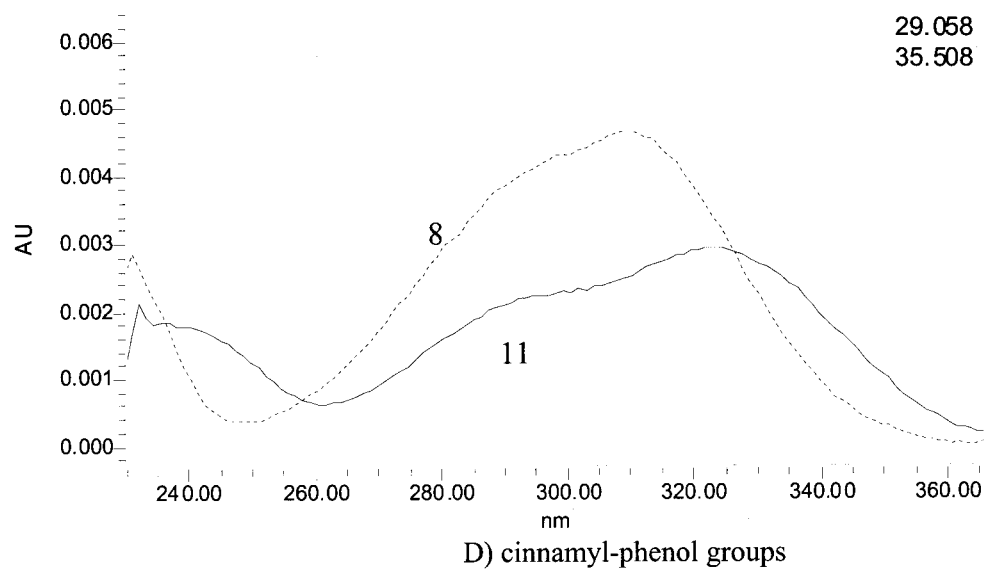
Figure 88. Relative absorption spectra of the lignin phenols and the internal standard phenols

The spectrum of each monomer was measured in the wavelength range from 220 nm to 370 nm, which covers the maximum of the absorption (Figure 88). For quantification, all peaks were integrated at the wavelength of 275 nm where all phenols show a good absorption.

140

E. Calibration of the lignin compounds

The lignin standards were analyzed with HPLC. This method was easily capable of detecting levels down to 10nM, and lower levels were not investigated. Figure 89 to Figure 99 shows the excellent precision and linearity of the lignin quantification.
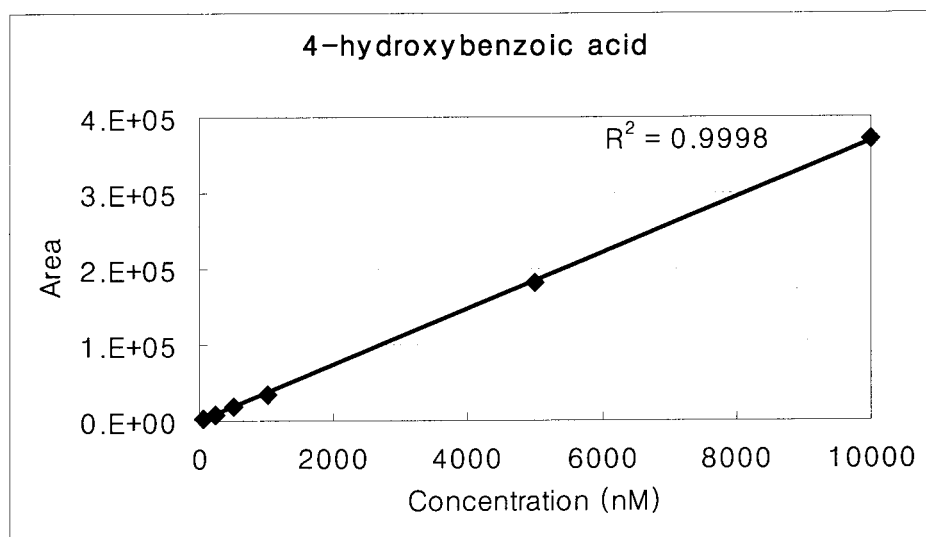
Figure 89 . Calibration of 4-hydroxybenzoic acid
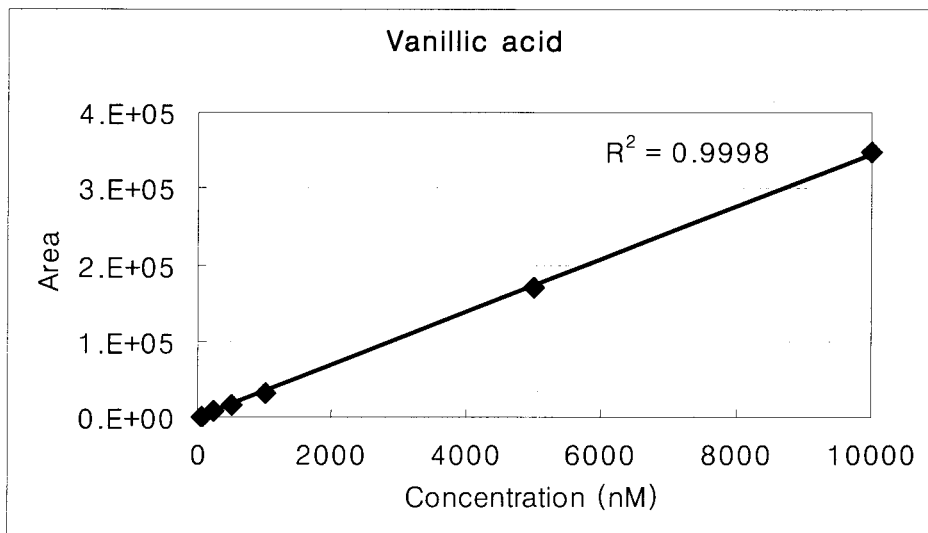
Figure 90. Calibration of Vanillic acid
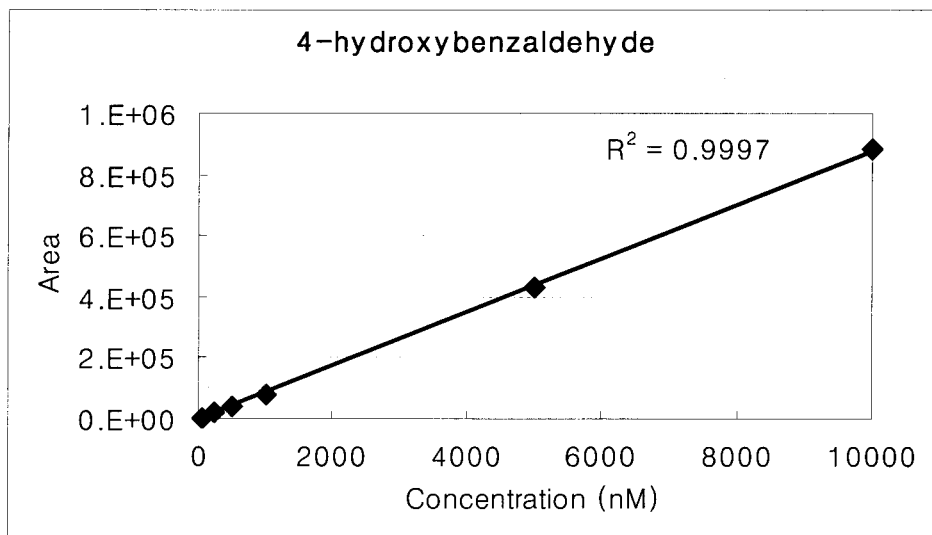
Figure 91. Calibration of 4-hydroxybenzaldehyde
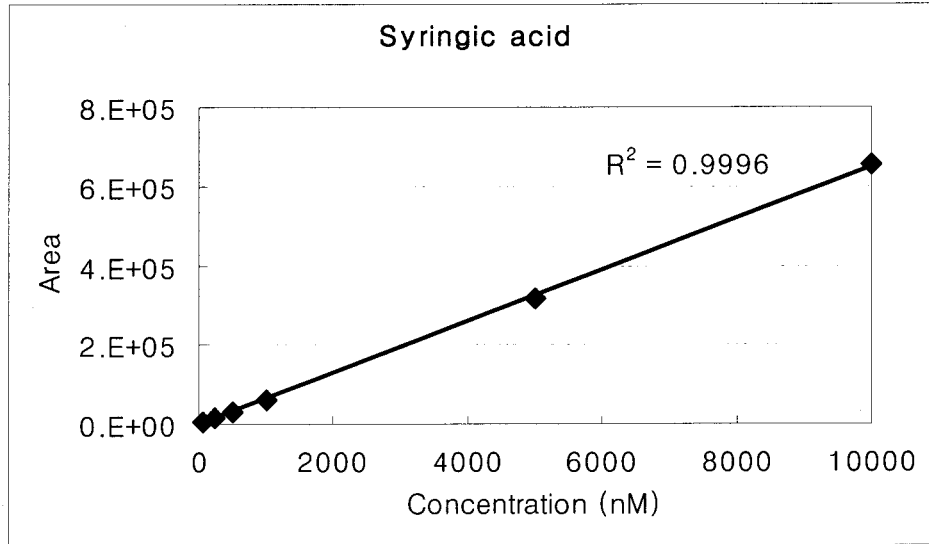
Figure 92. Calibration of Syringic acid
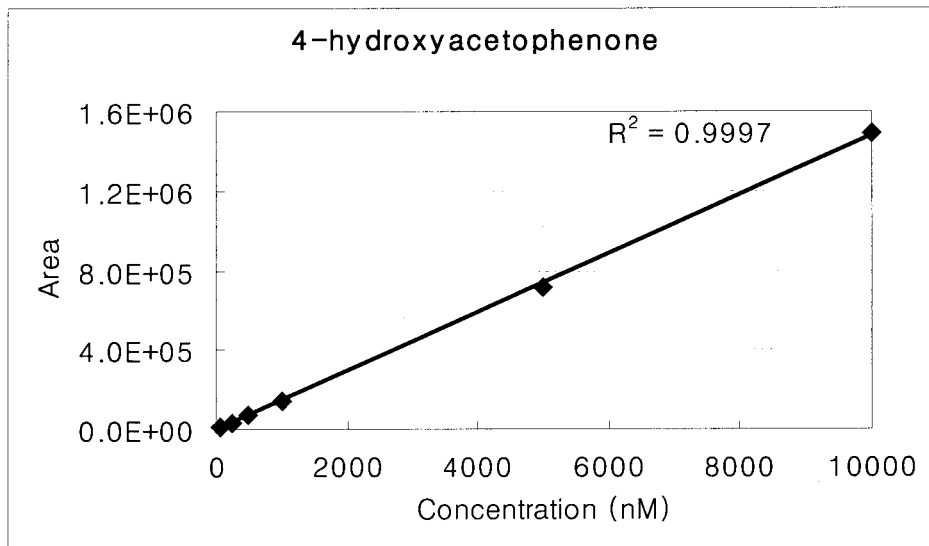
Figure 93. Calibration of 4-hydroxyacetophenone
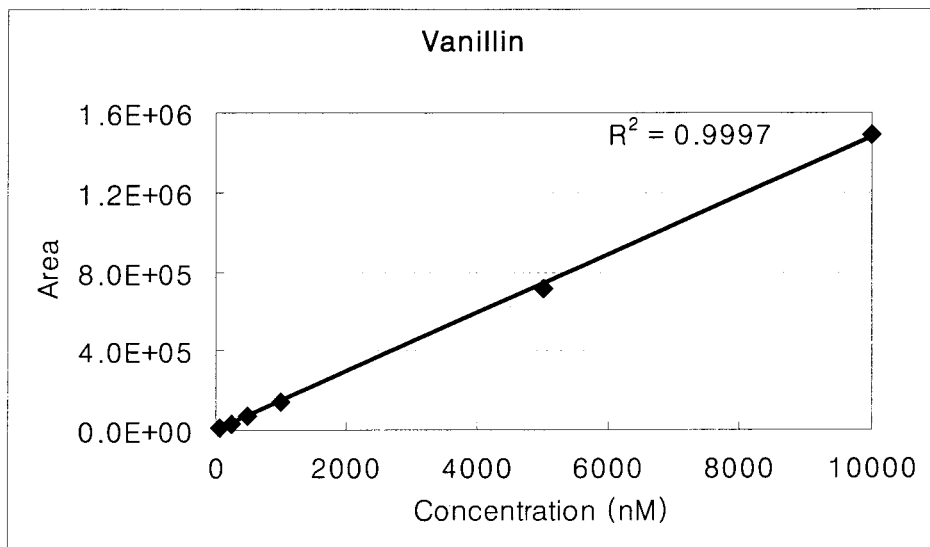
Figure 94. Calibration of Vanillin
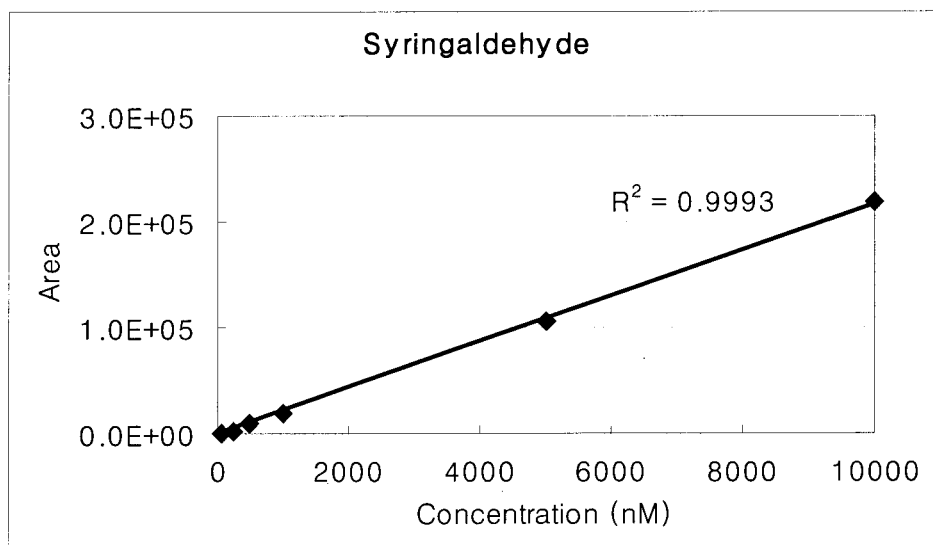
Figure 95. Calibration of Syringaldehyde

144
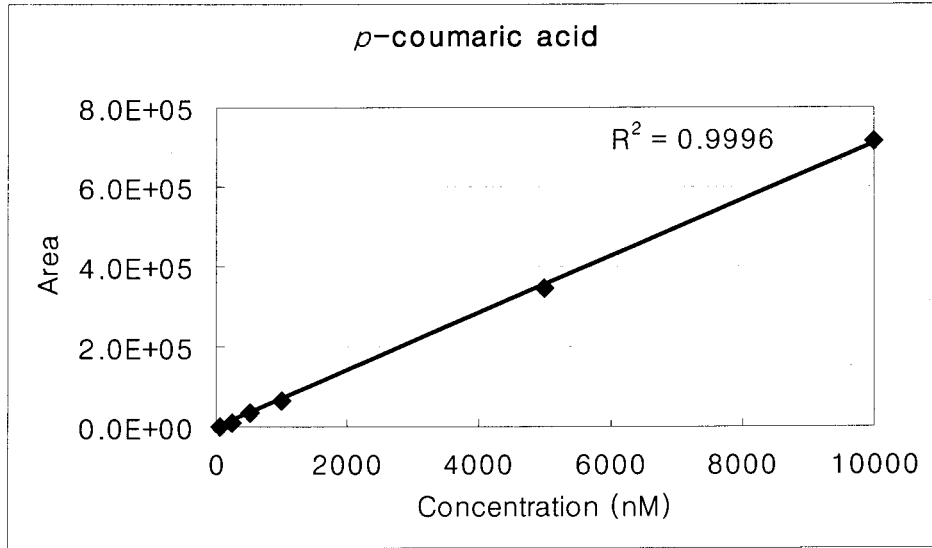
Figure 96. Calibration of p-coumaric acid
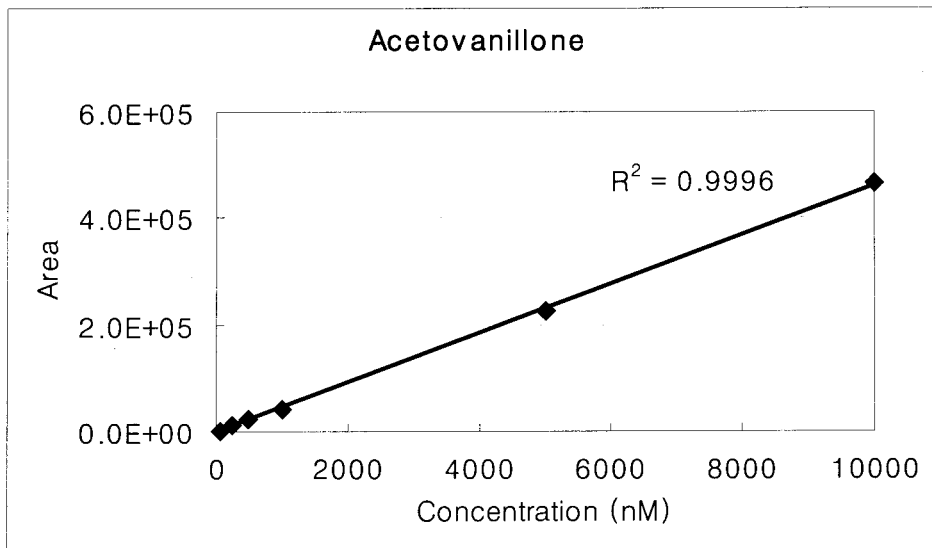
Figure 97. Calibration of Acetovanillone
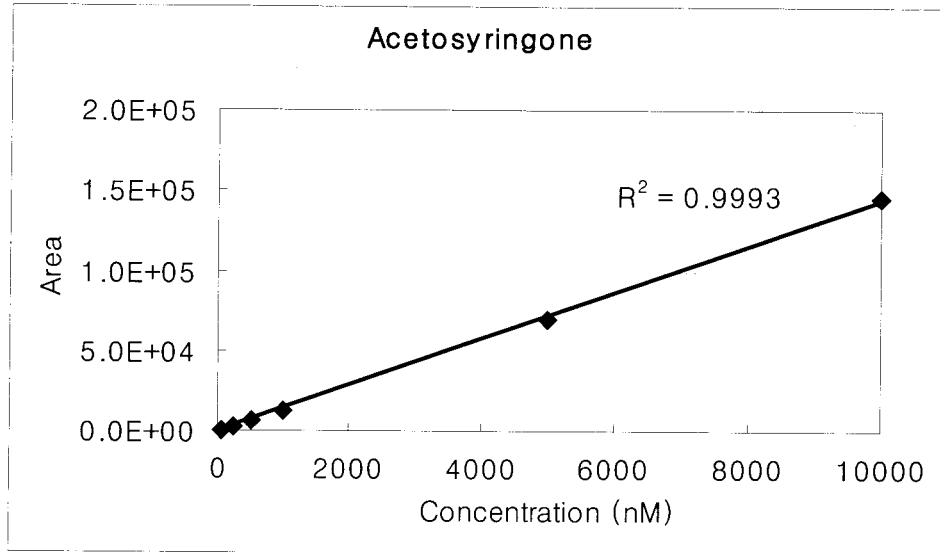
Figure 98. Calibration of Acetosyringone
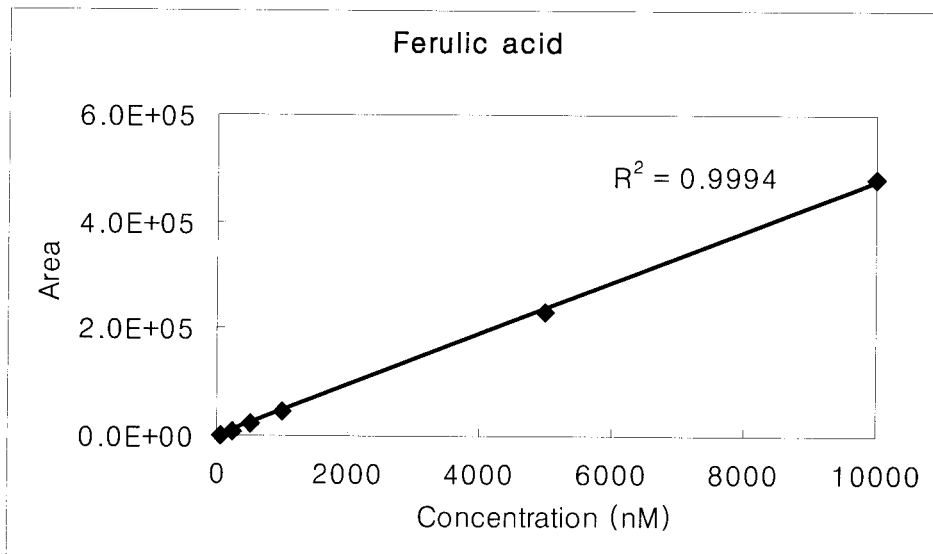
Figure 99. Calibration of Ferulic acid

Mass spectra of lignin compounds were analyzed with a Finnigan LCQ MS system. Lignin compounds were injected with syringe and spayed by He gas and N_2 gas in the ESI source. Mass spectra were analyzed in the negative mode. Table 32 shows the operating condition of the ESI-MS.

Table 32. Conditions of ESI-MS

ESI SOURCE		VACUUM	
Source Voltage (kV):	3.96	Vacuum OK:	TRUE
Source Current (µA):	8.59	Ion Gauge Pressure OK:	TRUE
Vaporizer Thermocouple OK:	FALSE	Ion Gauge On:	TRUE
Vaporizer Temp (°C):	-0.00	Ion Gauge (Torr x10e5):	1.60
Sheath Gas Flow Rate ():	67.82	Convectron Pressure OK:	TRUE
Aux Gas Flow Rate ():	4.35	Convectron Gauge (Torr):	1.49
Capillary RTD OK:	TRUE		
Capillary Voltage (V):	-6.44		
Capillary Temp (°C):	195.30		
8 kV supply at limit:	FALSE		
TURBO PUMP		**ION OPTICS**	
Status:	Running	Octapole Frequency On:	TRUE
Life (hours):	42376.00	Octapole 1 Offset (V):	5.85
Speed (rpm):	60000.00	Octapole 2 Offset (V):	8.34
Power (Watts):	56.35	Lens Voltage (V):	27.51
Temperature (°C):	40.00	Trap DC Offset (V):	10.17
		Analyzer Temperature (°C):	24.95
MAIN RF		**SYRINGE PUMP**	
Reference Sine Wave OK:	TRUE	Status:	Running
Standing Wave Ratio Failed:	FALSE	Flow Rate (µl/min):	3.00
Main RF DAC (steps):	31.00	Infused Volume (µl):	259.00
Main RF Detected (V):	0.01	Syringe Diameter (mm):	2.30

Figure 100 through Figure 112 show individual mass spectra of each of lignin compounds using the LCQ. In each case we were able to observe a strong molecular ion. Figure 113 and Figure 114 show mass spectra of the mixture of lignin compounds at injected concentrations of 1000 nM and 25000 nM, respectively. These last two figures are without LC separation.

147
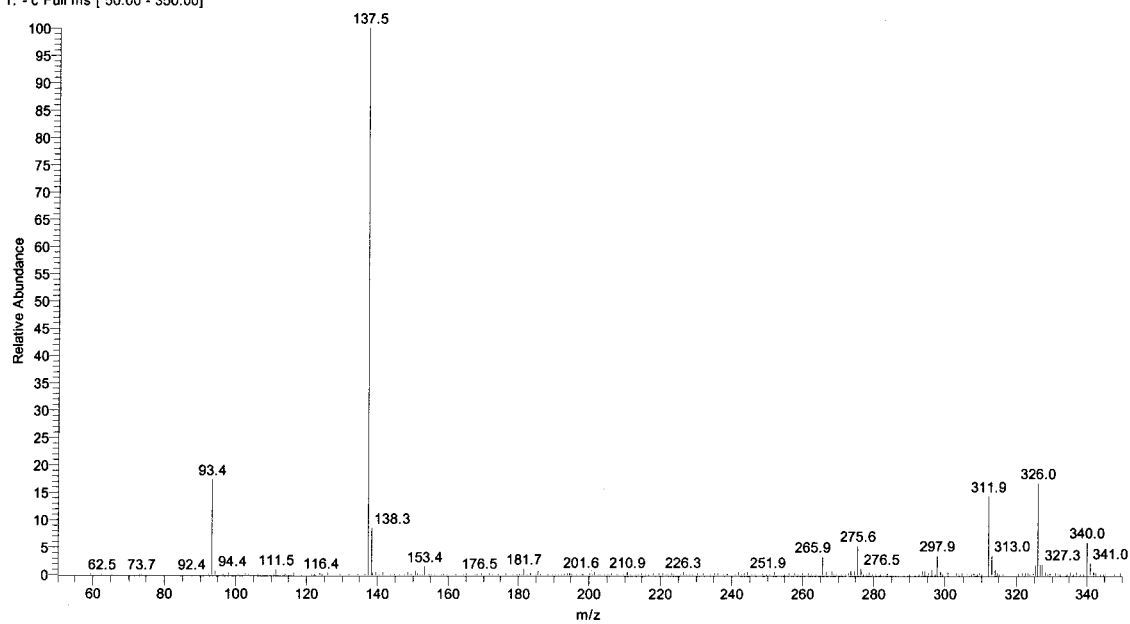
Figure 100. ESI Mass Spectrum of 4-hydroxybenzoic acid
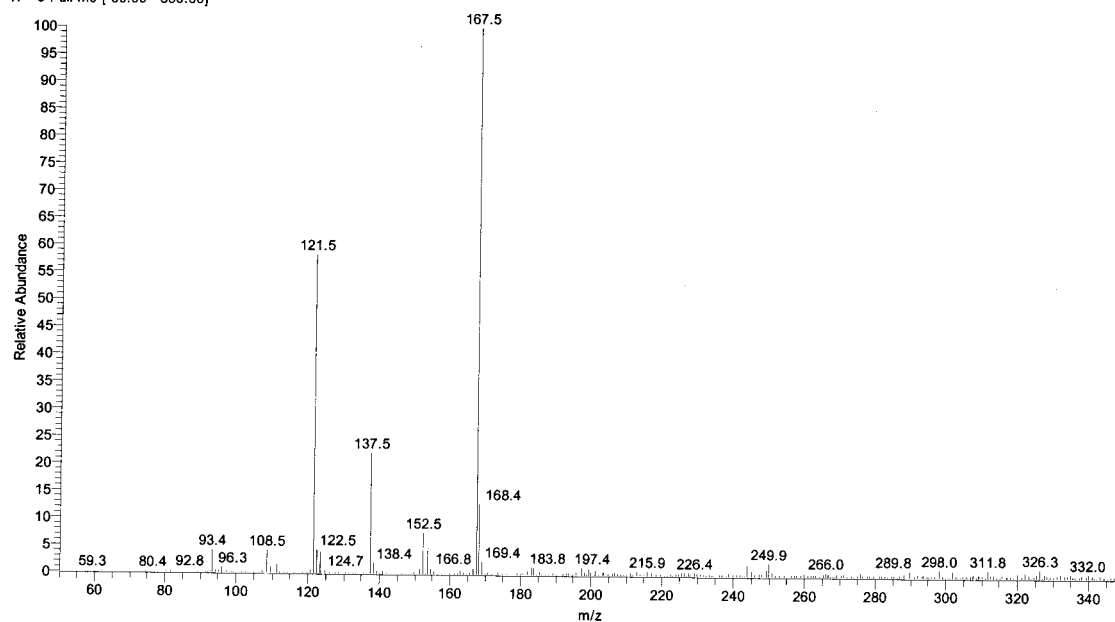
Figure 101. ESI Mass Spectrum of 4-hydroxybenzaldehyde
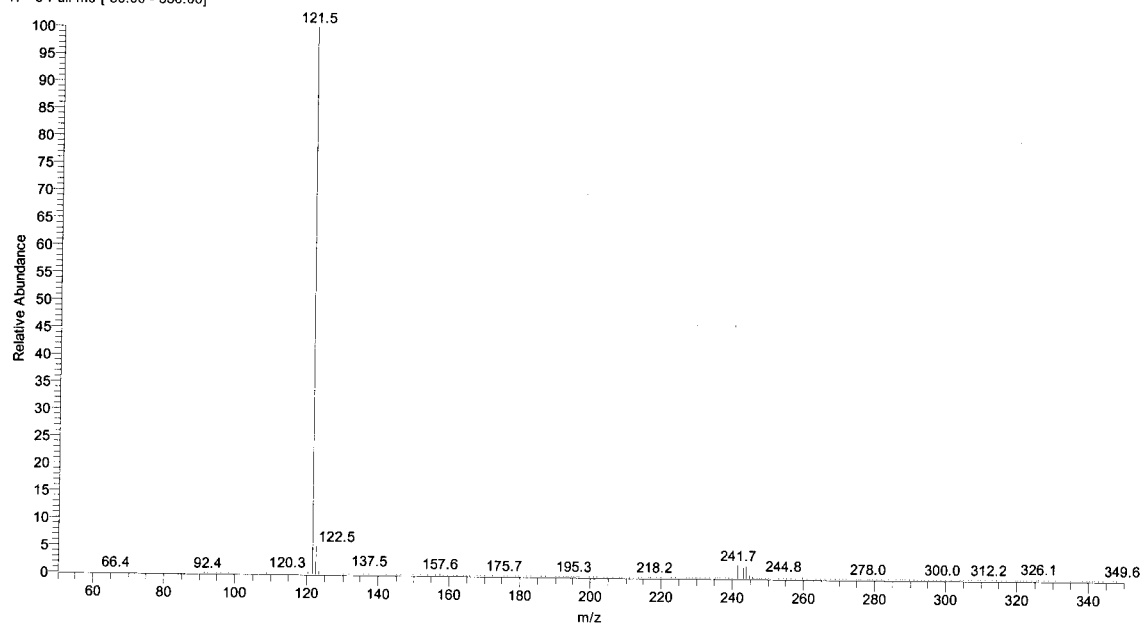
Figure 102. ESI Mass Spectrum of Vanillic acid

149
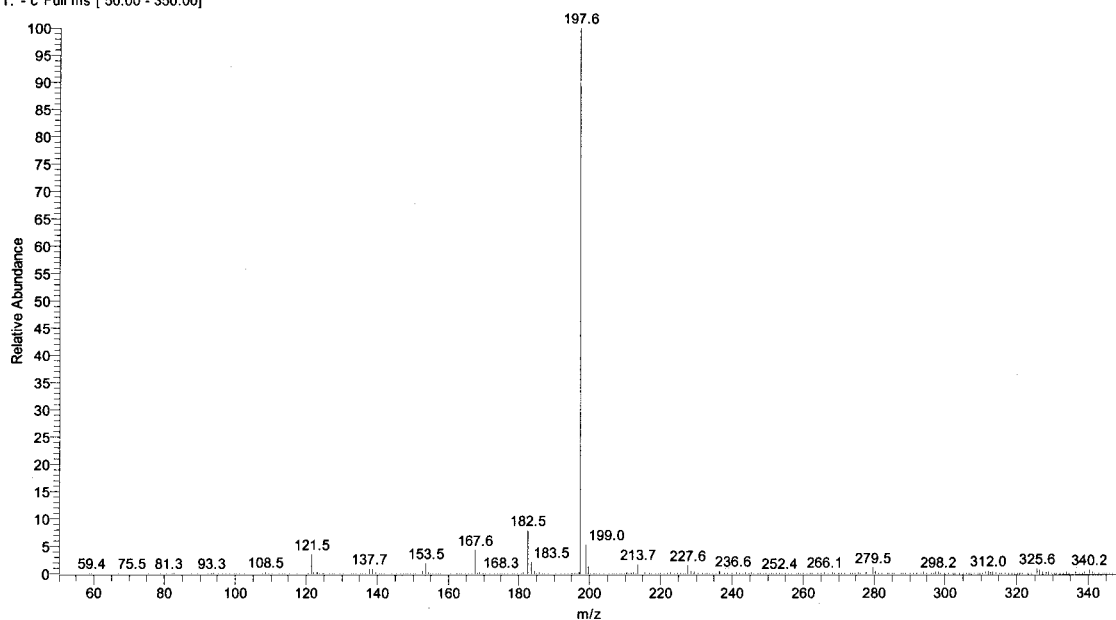
Figure 103: . ESI Mass Spectrum of Syringic acid
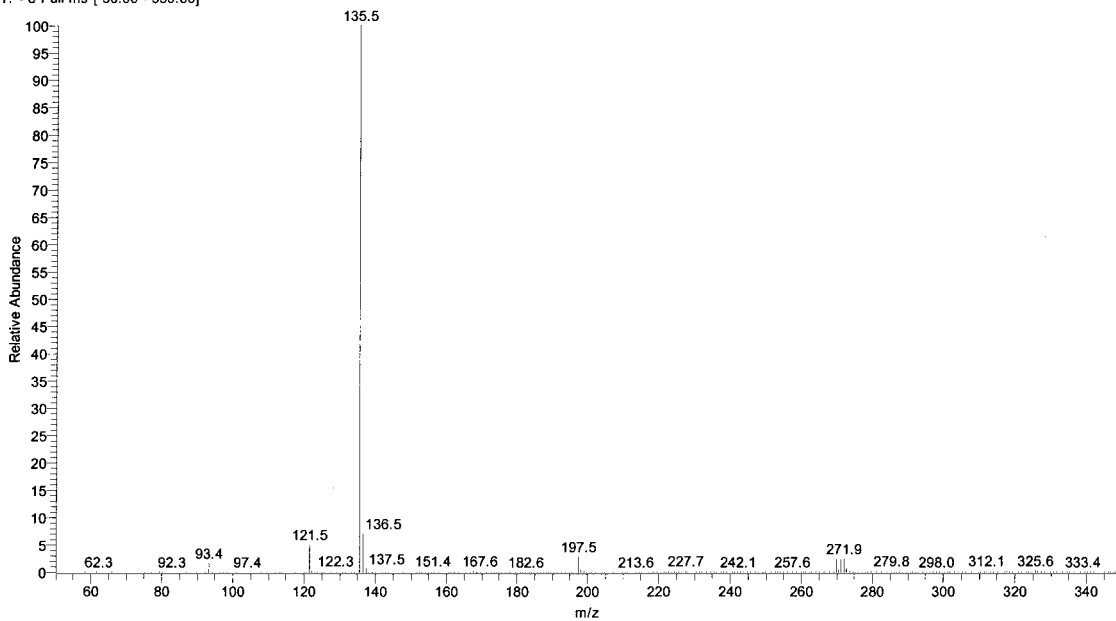
Figure 104. ESI Mass Spectrum of 4-hydroxyacetophenone

150
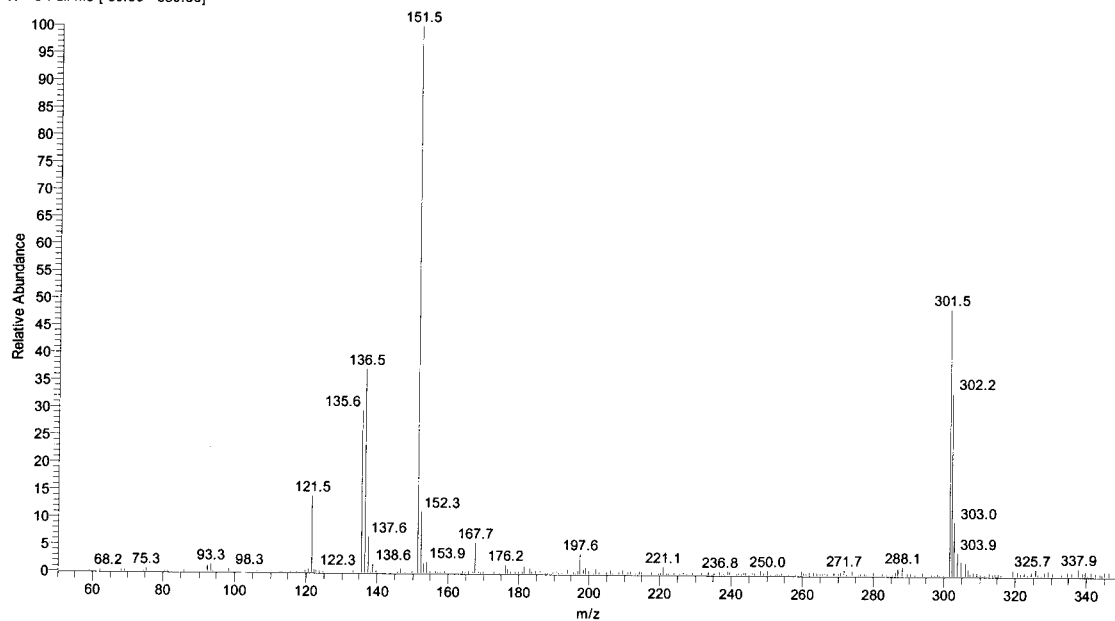
Figure 105. ESI Mass Spectrum of Vanillin
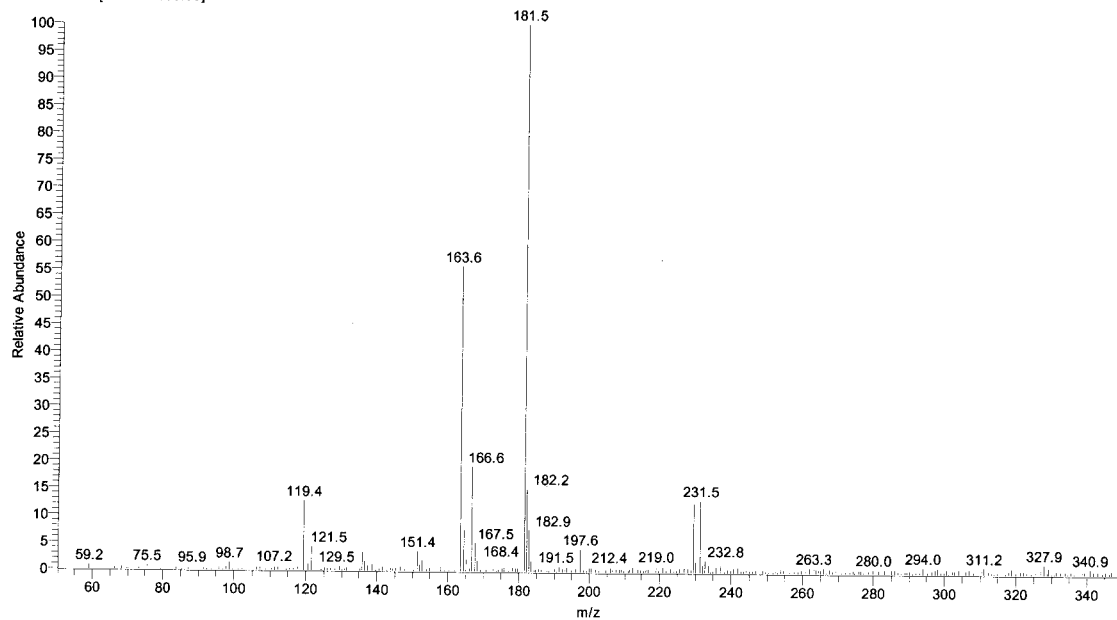
Figure 106. ESI Mass Spectrum of Syringaldehyde

163.6

119.5

164.7

66.9 73.5   93.5 102.3   120.4   151.5   165.7 179.5 197.6 211.7 225.8   241.7 252.7 266.0   285.9 301.3   325.7 328.9 344.1

328.0

m/z
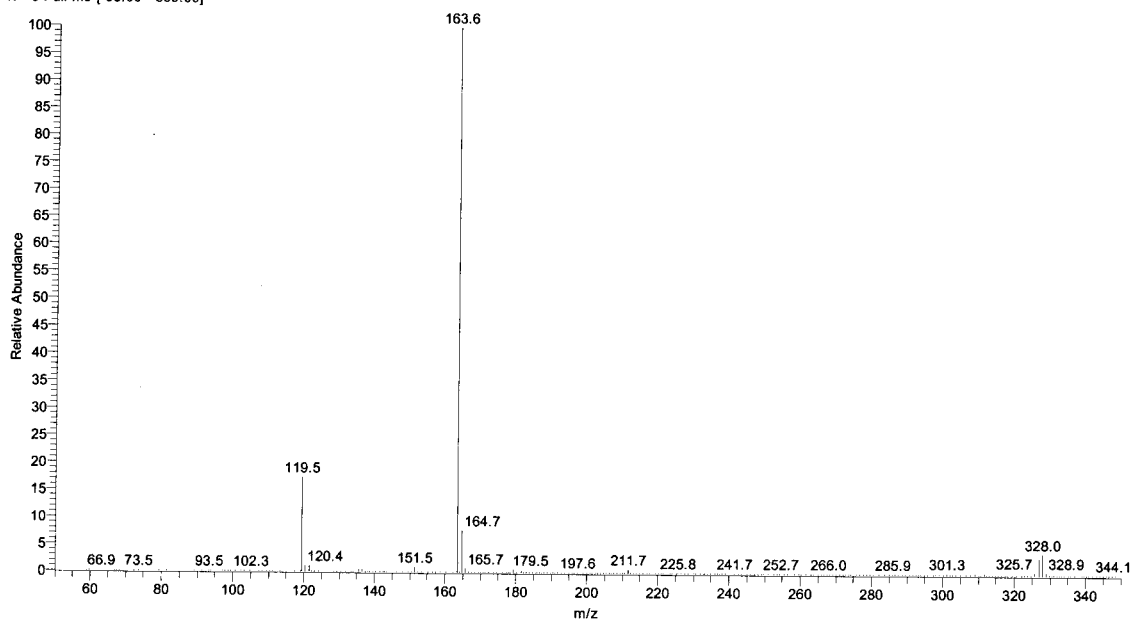
Figure 107. ESI Mass Spectrum of p-coumaric acid

165.6

150.6

151.6

135.6

329.6

121.5

330.4

163.7

166.7 181.3

136.6

167.8

198.2

331.4 347.7

59.4   84.6 92.8 98.5 106.2   127.5   215.0   238.5   246.1   258.0   277.9 297.0 305.2 318.6

m/z
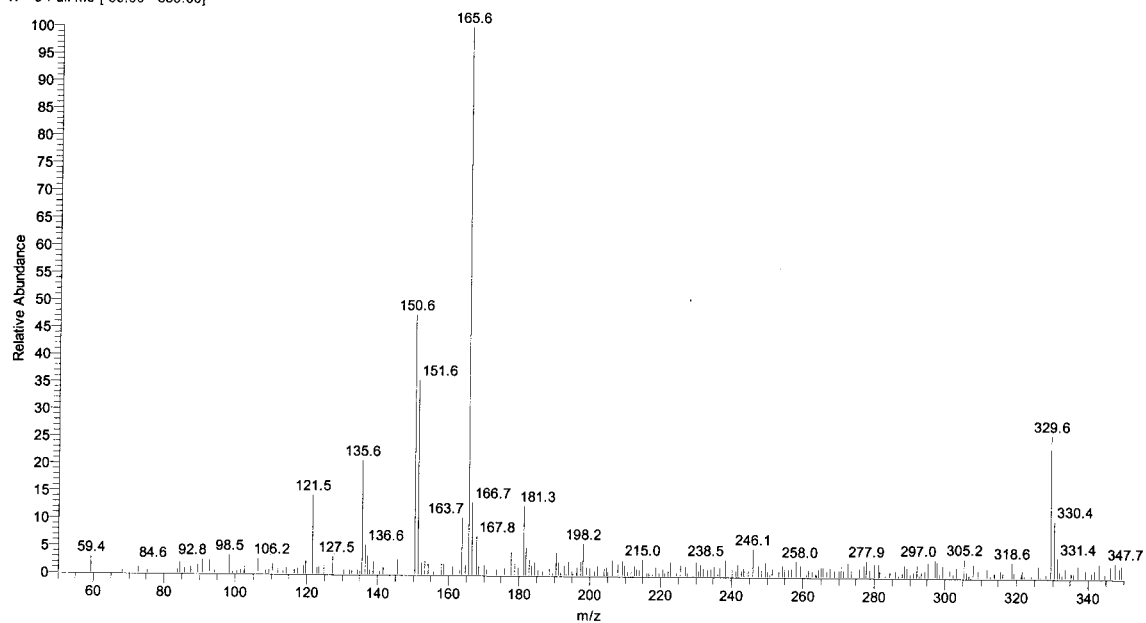
Figure 108. ESI Mass Spectrum of Acetovanillone

152
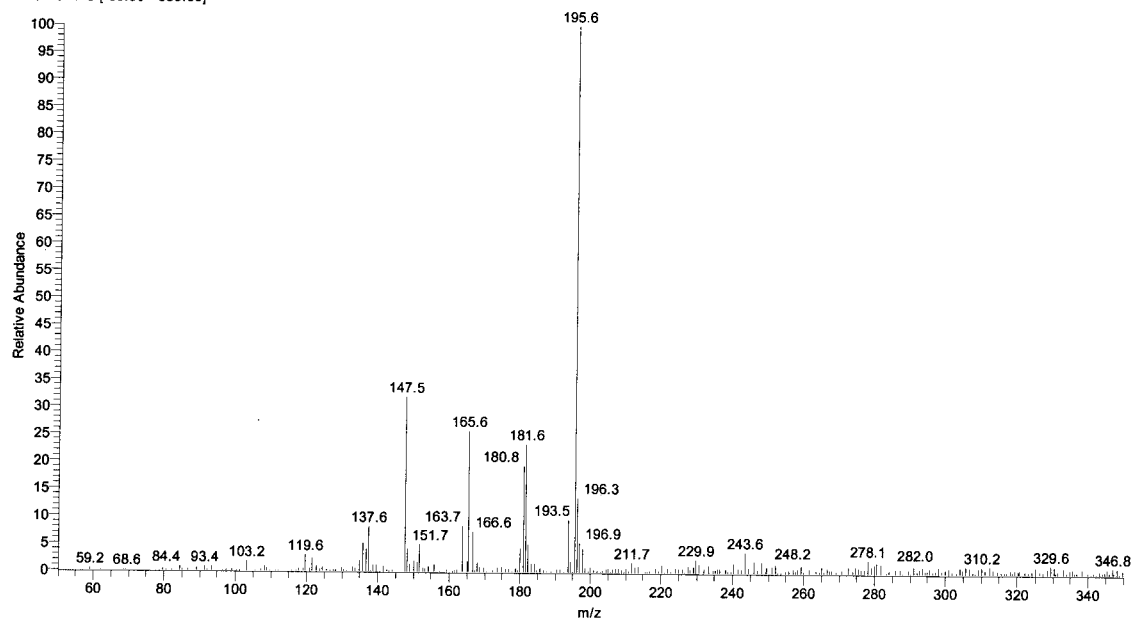
Figure 109. ESI Mass Spectrum of Acetosyringone
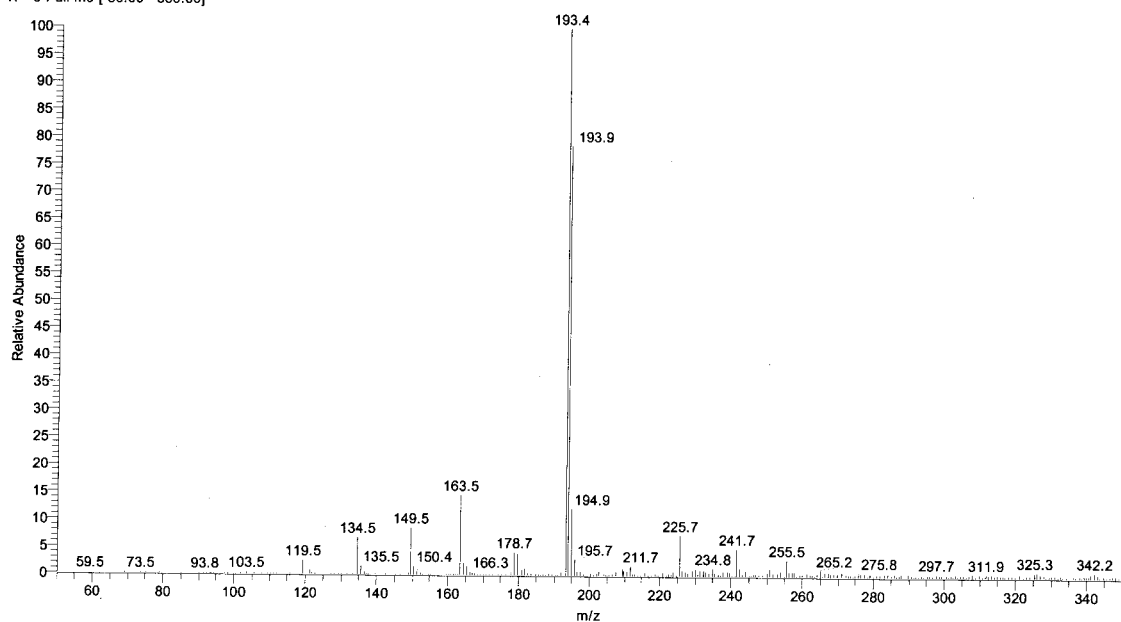
Figure 110. ESI Mass Spectrum of Ferulic acid
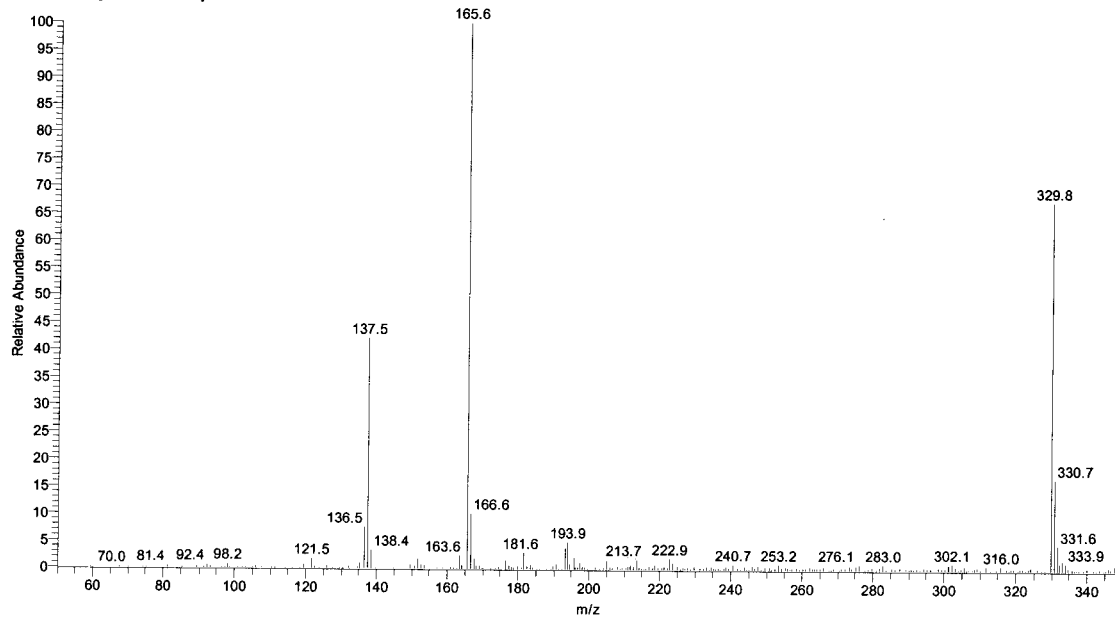
Figure 111. ESI Mass Spectrum of Ethyl Vanillin
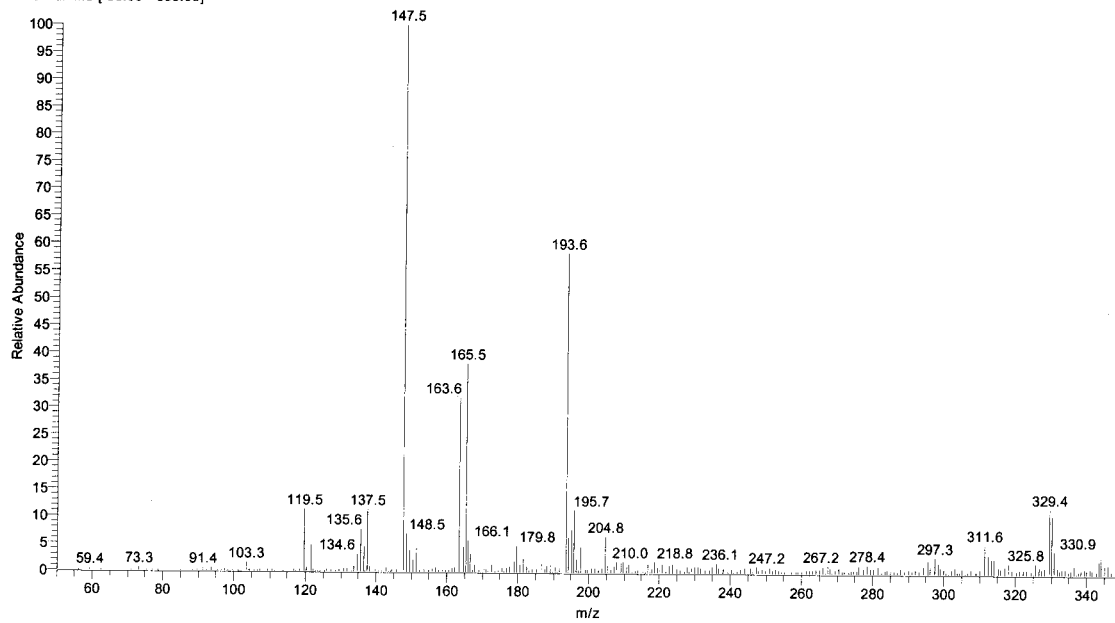
Figure 112. ESI Mass Spectrum of Cinnamic acid

154
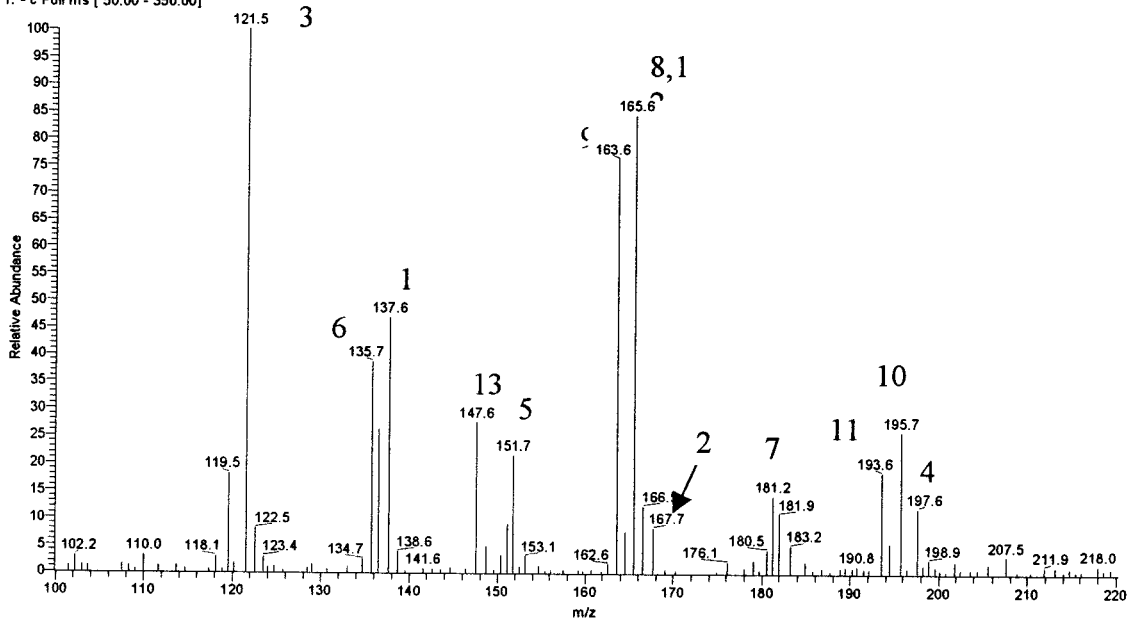
Figure 113. Lignin compounds and internal standards (1000nM)
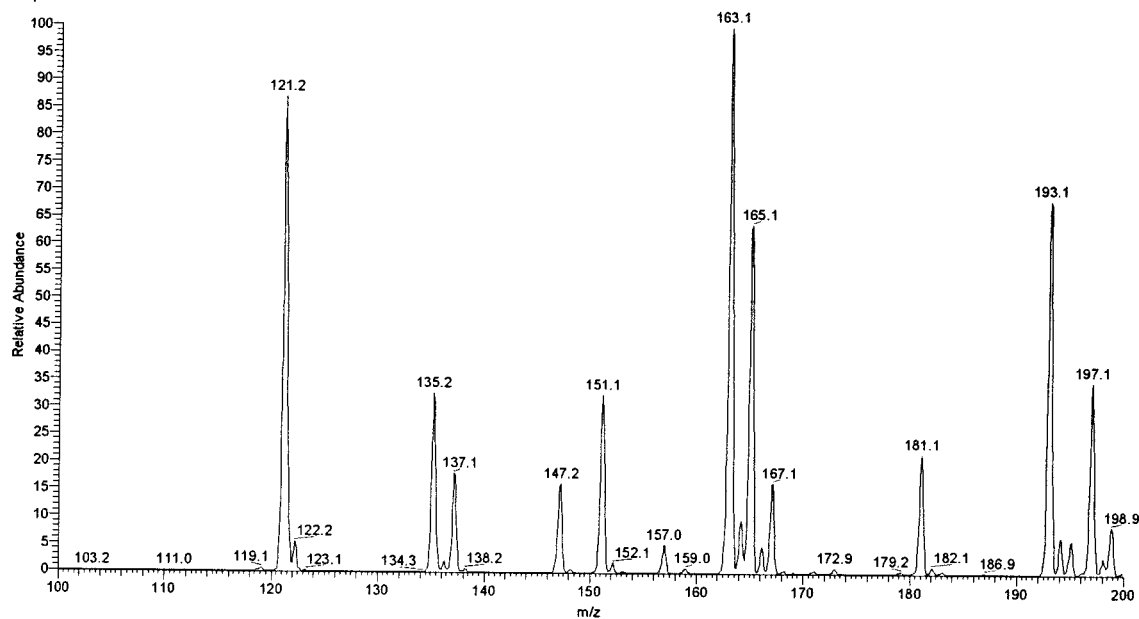
Figure 114. Lignin compounds and internal standards (25000nM)

155

ESI-MS

During the first period of the project, research at OSU focused on the identification of natural dissolved organic matter (DOM) molecules by high-resolution electrospray ionization mass spectrometry (ESI-MS). DOM in natural water systems is comprised of a complex mixture of compounds including degradation products from plants and animals. Understanding the chemistry and origin of DOM, specifically riverine DOM, is important for this study to characterize TOX molecules. However, there is currently little molecular level information available for DOM (Hedges et al., 2000), attributed mainly to analytical difficulties arising from DOM's complexity, high polarity, low concentration in natural water (ppm or ppb level) and lack of well-suited non-invasive analytical methods.

Electrospray ionization (ESI) is a soft ionization technique that has been recently used to analyze trace amounts of biomolecules. ESI-MS is becoming an important technique for identification and characterization of natural organic mixtures such as humic substances (Brown and Rice, 2000; Fievre et al., 1997; Hatcher et al., 2001; Kujawinski et al., 2002; Leenheer et al., 2001; Plancque et al., 2001; Solouki et al., 1999; Stenson et al., 2002a). Since riverine DOM is composed of polar natural organic mixtures including humic substances (Thurman, 1985), it is reasonable to assume that ESI-MS can be an important analytical method to obtain molecular level information on DOM. McIntyre studied organic material from ground water and showed that ESI-MS could be used to study the material (McIntyre et al., 1997). Because relatively small amounts of organic material exist in natural water, sample pre-treatment is necessary to concentrate the organic material, before ESI-MS analysis can be performed. In this study, C_{18} solid phase extraction (SPE) has been used to concentrate and desalt trace organic molecules from natural water samples. A sample can be isolated and concentrated in a considerably shorter amount of time with a disk because higher flow rates can be used (Liska, 2000). Since extraction rate is relatively independent of flow rate (Liska, 2000), the experimental setup for disk SPE is more flexible than for cartridge SPE. For example, a simple filtration setup with an aspirator as a vacuum source can be used to extract a sample. Because of this flexibility and simple setup, disk SPE can be easily adapted to field studies. Absorbance spectra from McDonalds Branch raw water and eluent were compared to evaluate the extraction efficiency of chromophoric substances (Figure 115a). To calculate extraction efficiency of the disk, the absorbance values

for each trace were integrated from 250 to 400 nm and the values were compared. Overall, 70 % of chromophoric material was retained on the disk after sample acidification. During sample loading, the retained organic material changed the color of the disk from white to brown. The color of the disk reverted to white after elution with 90:10 MeOH:H_2O. The recovery of the extracted material into the eluent solvent (90:10 MeOH:H_2O) was calculated from absorbance spectra (Figure 115b). To calculate the recovery efficiency, the integrate A values from 250 to 400 nm of the diluted and volume reconstituted retentate were compared to the integrated absorbance differences of the same range from Figure 1a. The absorbance difference in Figure 1a represents the amount of chromophoric molecules extracted by the C_{18} disk SPE. About 90% of the colored material was recovered from the disk into eluent solvent. Overall, over 60 % of the original DOM in water is recovered without the interference of salts
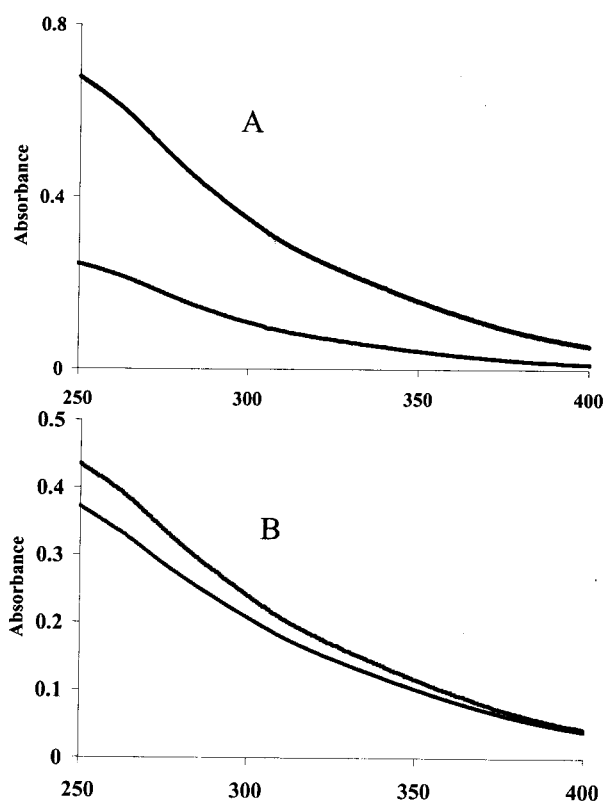
Figure 115: Extraction efficiency (a) and recovery rate (b) of C_{18} disk measured by absorbance spectroscopy

The extracted samples were analyzed by 7 T ESI-Fourier transform ion cyclotron

157

resonance mass spectrometer for enhanced resolution and sensitivity. A high resolution positive ion spectrum (mass resolving power of $m/\Delta m_{50\%} > 80,000$ at $m/z < 600$) was obtained (Figure 116). This spectrum shows the molecular complexity of DOM. Not only are there clusters of peaks at every nominal mass unit up to 1000 m/z, but each cluster is further resolved into several peaks. Similar results were observed in ESI FT-ICR mass spectra of other humic substances (Kujawinski et al., 2002; Stenson et al., 2002a). Nested within the spectrum are series of intense peaks at odd mass to charge ratio (m/z) and weak peaks at even numbered m/z. This pattern was previously reported (Brown and Rice, 2000) from negative ion spectra of fulvic acid and interpreted as either chloride adducts or a homologous series of molecules.
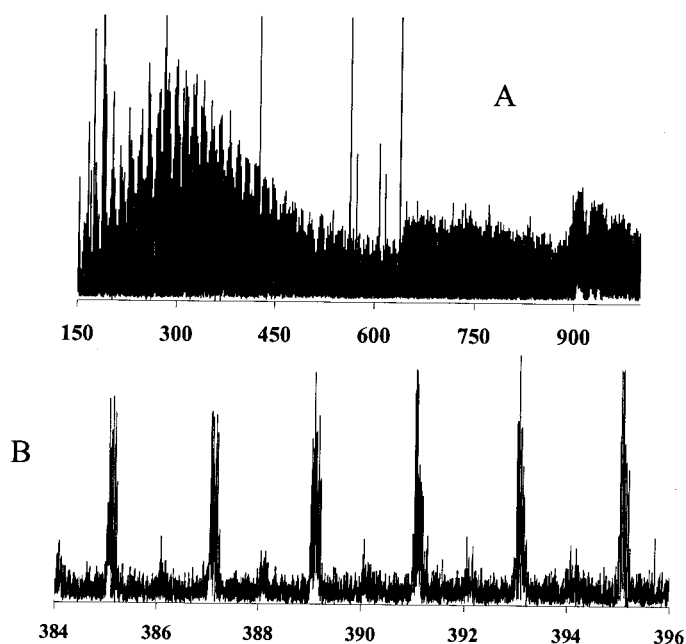
Figure 116: Positive ion mode ESI 7 T FT-ICR mass spectrum on DOM (a) and expanded view of selected region (b).

Kendrick mass defect (KMD) analysis (Kendrick, 1963) can be used to identify patterns of elemental composition within high resolution mass spectra of complex mixtures (Hughey et al., 2001; Stenson et al., 2002). The concept of Kendrick mass is to change the mass scale into a CH_2 mass-normalized scale (equation 1). Kendrick mass defect is then calculated as the difference between the normalized Kendrick mass and the nominal observed mass (equation 2). The values for Kendrick mass defect are a reflection of the deviation of an exact mass from that

of homologous structures varying only by CH_2 groups. In other words, the exact mass of molecules varying by the same functional group (CH_2) would be different by multiples of the exact mass of CH_2, and, as a result, they will have the same Kendrick mass defect value. Thus, two fatty acids with elemental compositions of $C_{20}H_{40}O_2$ and $C_{21}H_{42}O_2$ will have the same Kendrick mass defect. Kendrick mass defect can be used to identify patterns of masses having the same compositional differences (Stenson et al., 2002).

Kendrick mass = observed m/z x (14/14.01565) (1)
Kendrick mass defect = (nominal observed mass - Kendrick mass) x 1000 (2)

Kendrick mass defects for the many peaks in the DOM sample were calculated from the FT ICR MS data and plotted (Figure 117a).
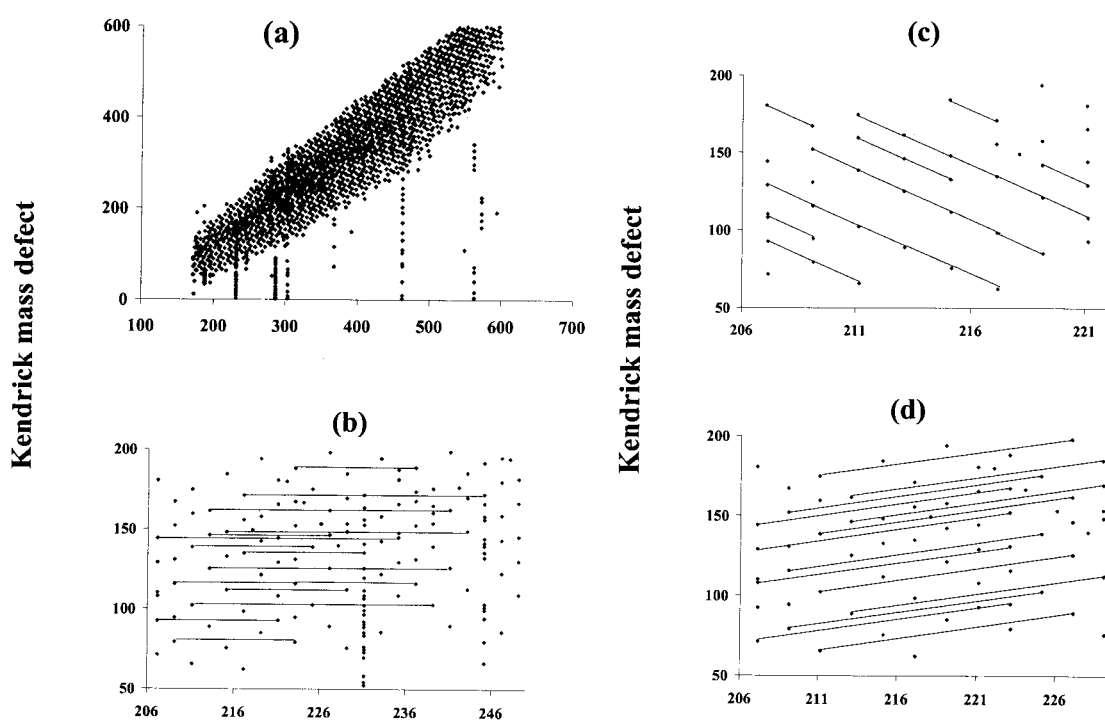
Figure 117: Kendrick mass defect plot for the entire mass region (170 < m/z < 600) (a) and expanded plots with lines denoting the series of peaks separated by ch_2 (b), h_2 (c) and o (d).

159

Four significant figures after the decimal point were used to calculate the mass defect. In a plot of Kendrick mass defect versus nominal observed mass, molecules differing by a specific elemental composition (due to exact mass of the contributing atoms) are connected by lines. The slope of the lines can be determined by the following equation:

$$\text{Slope} = (\,\delta \text{Kendrick mass defect} / \delta \text{nominal observed mass}) \times 1000 \qquad (3)$$

where δ is used to represent a difference.

Molecules separated by CH_2 units will have same Kendrick mass defect (the numerator in equation 3 will be zero in these cases) and be connected by horizontal lines with a slope of 0. The lower expanded mass range of the spectrum of DOM is shown in Figure 117b and numerous series of molecules differing by CH_2 can be identified. Other series of molecules differing by H_2 and O were also identified (Figure 117c,d). In these plots, the peaks differing by the corresponding masses of H_2 or O can be identified by parallel lines, each with a specific slope (a slope of -6.7 and 1.4, respectively). The series are further verified by calculating and comparing the exact mass difference between the peaks to the theoretical mass of H_2 and O. The series representing CH_2, H_2 and O result in even number differences between peaks and this, along with the added H^+ from ionization, is what primarily contributes to the pattern of predominantly odd mass peaks observed for DOM.

The DOM samples were further analyzed with a 9.4 T FT-ICR mass spectrometer to achieve better resolving power and sensitivity. Considering the complexity of the spectra of DOM, it is clear that resolving power is very important. At the time of analysis, internal standard was also analyzed along with sample for exact mass measurement. Figure 118 displays the calibrated mass spectrum of McDonalds Branch DOM. Over 5000 peaks with > 4% relative abundance (corresponding to a signal to noise ratio of 5) are detected in the mass range from 300 to 700 m/z. The peak resolving power ($m/\delta m_{50\%}$) is calculated to be over 300,000 at around 300 m/z with an average resolving power of over 200,000 for the entire mass range ($300 < m/z < 700$). The complexity, also shown in previous studies(Brown and Rice, 2000; Kujawinski et al., 2002; Stenson et al., 2002b), derives from the fact that DOM contains a multitude of natural products or their biodegraded residues resulting in a multitude of peaks can be observed at each nominal mass (see Figure 118). As previously observed(Kim et al., 2002; Stenson et al., 2002a),

peaks in the vicinity of odd nominal m/z are dominant. Considering the low content of nitrogen (around 1 %) in this sample, it is very unlikely that a significant part of even numbered peaks are from molecules with even numbers of nitrogen atoms. Rather, the majority of peaks at even m/z can be assigned to the ^{13}C isotope of peaks at odd m/z since, in most cases, peaks at even m/z can be identified readily by adding the mass of a neutron to the mass of odd m/z peaks. A similar dominance of ^{13}C isotope peaks was previously used to explain the even m/z peaks in high resolution mass spectra of NOM.(Stenson et al., 2002a) Accordingly, only peaks at odd m/z are considered for processing in this paper. By the same token, most of the observed peaks at odd m/z are found to be from singly charged ions, since corresponding ^{13}C isotope peaks can be found at a unit mass difference. Therefore mass instead of mass to charge ratio is used to indicate peaks in the spectrum through the rest of this report.
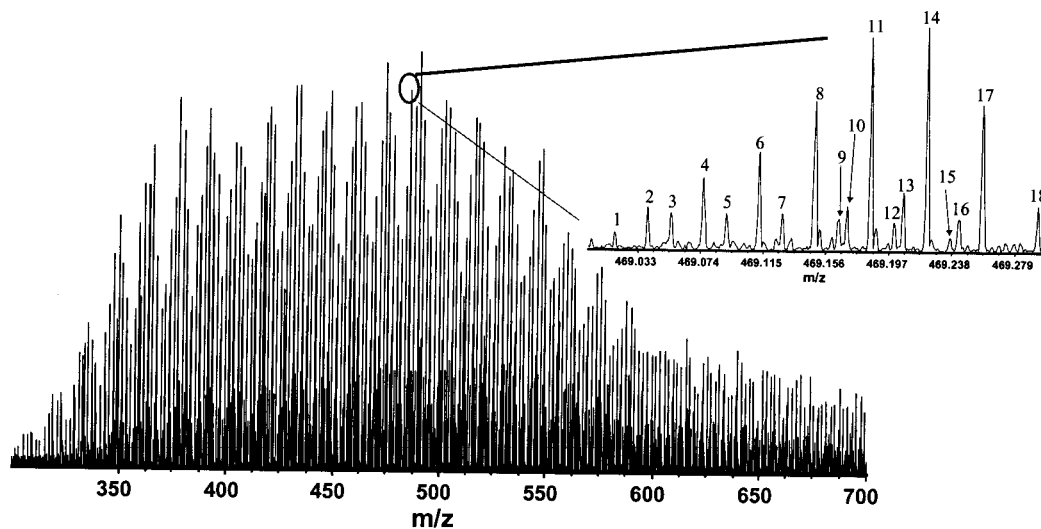
Figure 118: Negative ion mode ultra-high resolution mass spectrum of McDonalds Branch DOM and the expanded view of the 469.0 – 469.3 m/z region of the ultra-high resolution mass spectrum of McDonalds Branch DOM. The numbers above peaks are used for identification in Table 1.

161

Table 33: List of peaks identified in the expanded spectrum (Figure 118).

Peak #	Proposed molecular formula	Observed values	Theoretical values	Difference from Theoretical value (ppm)
1	$C_{25}H_{10}O_{10}$	469.02018	469.02012	-0.1
2	$C_{22}H_{14}O_{12}$	469.04118	469.04125	0.1
3	$C_{26}H_{14}O_9$	469.05646	469.05651	0.1
4	$C_{23}H_{18}O_{11}$	469.07763	469.07764	0
5	$C_{27}H_{18}O_8$	469.09288	469.09289	0
6	$C_{24}H_{22}O_{10}$	469.11401	469.11402	0
7	$C_{28}H_{22}O_7$	469.1293	469.12928	0
8	$C_{25}H_{26}O_9$	469.15042	469.15041	0
9	$C_{29}H_{26}O_6$	469.16576	469.16566	-0.2
10	$C_{22}H_{30}O_{11}$	469.17151	469.17154	0.1
11	$C_{26}H_{30}O_8$	469.18681	469.18679	0
12	$C_{30}H_{30}O_5$	469.20201	469.20205	0.1
13	$C_{23}H_{34}O_{10}$	469.20789	469.20792	0.1
14	$C_{27}H_{34}O_7$	469.22316	469.22318	0
15	$C_{31}H_{34}O_4$	469.23838	469.23843	0.1
16	$C_{24}H_{38}O_9$	469.24423	469.24431	0.2
17	$C_{28}H_{38}O_6$	469.25949	469.25956	0.2
18	$C_{29}H_{42}O_5$	469.29584	469.29595	0.2

The elemental compositions of peaks exceeding a 4 % of base peak threshold at odd mass are calculated from the corresponding exact mass numbers obtained from the calibrated spectrum. C, H, O, and N atoms are used to assign the most probable elemental formulas. The compositions can be assigned with usually less than 1 ppm error. Some of the peaks, especially in the higher molecular weight (m > 480) portion of the spectrum, have more than one possible elemental formula. In those cases, Kendrick mass defect analysis is used to determine the assigned elemental formula as it was done in previous studies.(Hughey et al., 2001; Stenson et al., 2002b) To show the complexity of the spectrum, one mass unit region is selected and expanded (Figure 118). Eighteen peaks can be detected and assigned (Table 33). Therefore, examining individual peaks in the entire mass range (300 < m < 700) and extracting information such as the distribution of classes of compounds directly from the conventional display of spectra represents a tremendous time and effort involved. This is going to be even a bigger problem in this study because inclusion of TOX molecules will make the spectra even more complex. herefore, it would be advantageous to have a tool that can simplify the spectrum so that the interpretation and further the identification of TOX molecules would be easier. To Reduce and

visualize the complex ultra-high resolution mass spectra, van Krevelen diagram has been applied to complex mass spectrometric data(Van Krevelen, 1950). The van Krevelen diagram can facilitate information retrieval from assigned formulas. The plot, constructed from the assigned elemental compositions of each peak in the mass spectrum, is displayed in Figure 119. Most of the odd numbered peaks (greater than 95 % of all odd-numbered peaks detected) in the mass spectrum are visually displayed in a single plot. The outliers possibly derived from noise spikes were excluded in the diagram. For the DOM sample, data are distributed in the form of a pattern. In the pattern, there are obvious blank spaces (for example line A in Figure 119). One of the reasons for the pattern can be attributed to limitations in numbers of carbon, oxygen and hydrogen atoms in the observed peaks. Given the mass range, the maximum numbers of carbon, oxygen and hydrogen atoms in the compositions are 41, 20 and 58 respectively. Also, the numbers of hydrogens are only even-numbers. Due to these limitations, points can't exist in certain areas of the plot. For example, since there is a maximum of 41 carbon atoms, the nearest points from any point with O/C ratio of 0.5 are 20/41 and 20/39. In other words, there cannot be any points either between the lines defined by O/C = 0.5 and O/C = 20/41 or between lines defined by O/C = 0.5 and O/C= 20/39 in the plot, resulting in the empty space parallel to O/C = 0.5 line (line B in Figure 119). Obviously, the pattern evolves from the fact that elemental compositions of the variety of peaks differ from each other by quantized ratios of the elements C, H and O. In the van Krevelen plot, trends along the lines can be indicative of structural relationships among families of compounds brought about by reactions which involve loss or gain of elements in a specific molar ratio.(Van Krevelen, 1950) Lines from each reaction path have characteristic slopes or intercepts that can be easily demonstrated from mathematical calculations.(Van Krevelen, 1950) The characteristics of the lines are summarized in Table 34. From these lines, a series of peaks, possibly products from various chemical reactions, can be visually identified. For example, a trend line representing methylation/demethylation reactions always intersects the ordinate at an H/C value of 2 (e.g., line A in Figure 119). Hydration/condensation reactions induce changes along a trend line with a slope of 2 (e.g. line C in Figure 119). It is apparent from Figure 119 that numerous trend lines in DOM can be clearly discerned. It is important to point out that the genetic relationships among compounds identified by their elemental formulas is tenuous at best, unless one has prior knowledge of a diagenetic reaction pathway leading to the transformation of precursors to products.
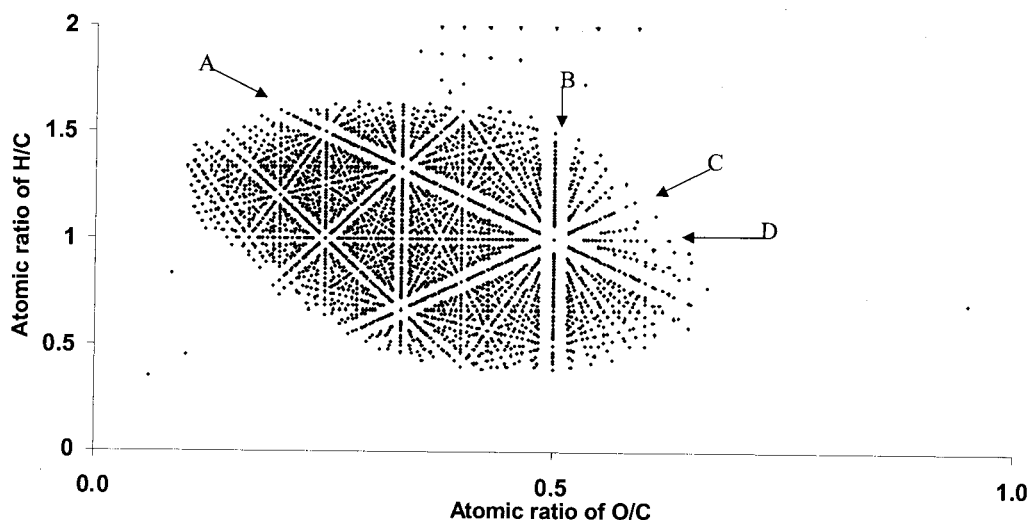
Figure 119: The van Krevelen plot for elemental data calculated from the ultra-high resolution mass spectrum of McDonalds Branch DOM. Distinctive lines in the plot representing chemical reactions are noted as; A: methylation, demethylation, or alkyl chain elongation B: hydrogenation or dehydrogenation, C: hydration or condensation, and D: oxidation or reduction.

Table 34: Characteristics of lines identified **in the van Krevelen plot.**

Chemical reactions	Characteristic of the line
Methylation or demethylation[1]	b = 2
Hydrogenation or dehydrogenation[2]	Vertical line
Hydration or dehydration[3]	a = 2
Oxidation or reduction[4]	b = 0
Decarboxylation	Pass (2,0)

'a' and 'b' each designate slope and intercept of a line defined by the equation $\frac{H}{C} = -a\ \frac{O}{C} + b$. 1,2,3 and 4 each correspond to line A,B,C and D in Figure 119.

164

Van Krevelen (Van Krevelen, 1950) used this diagram to examine reactions of a series of coals that could be viewed as diagenetic homologs. Thus, one could excise specific reaction pathways based on the knowledge that product-precursor relationships existed by nature of the way coal is formed (e.g., sequential burial after deposition). In DOM, all reaction states (products and precursors) exist in the same sample because DOM is an integrated accumulation of organic matter derived from a multitude of sources at various levels of diagenetic history. Thus, we might expect to visualize in the van Krevelen plot a complete diagenetic series and, no doubt, some of the trendlines observed may indeed reflect such series.

The van Krevelen diagram can not only be used to examine possible reaction series but it can also be used to identify the types of compounds that comprise different types of natural organic matter.(Hedges, 1990; Reuter and Perdue, 1984; Van Krevelen, 1950; Visser, 1983) This is possible because major bio-molecular components of source materials, mainly the products derived from plants, occupy fairly specific locations on the plot. In previous studies (Hedges, 1990; Reuter and Perdue, 1984; Van Krevelen, 1950; Visser, 1983), the positions of classes of biologically-derived compounds – lipids, cellulose, lignins, proteins and condensed polyaromatic type carbons – have been noted and the positions are reproduced on the plot shown in Figure 120. A qualitative analysis of the major classes of components contributing to DOM can be made using the locations of the peaks on the van Krevelen diagram.
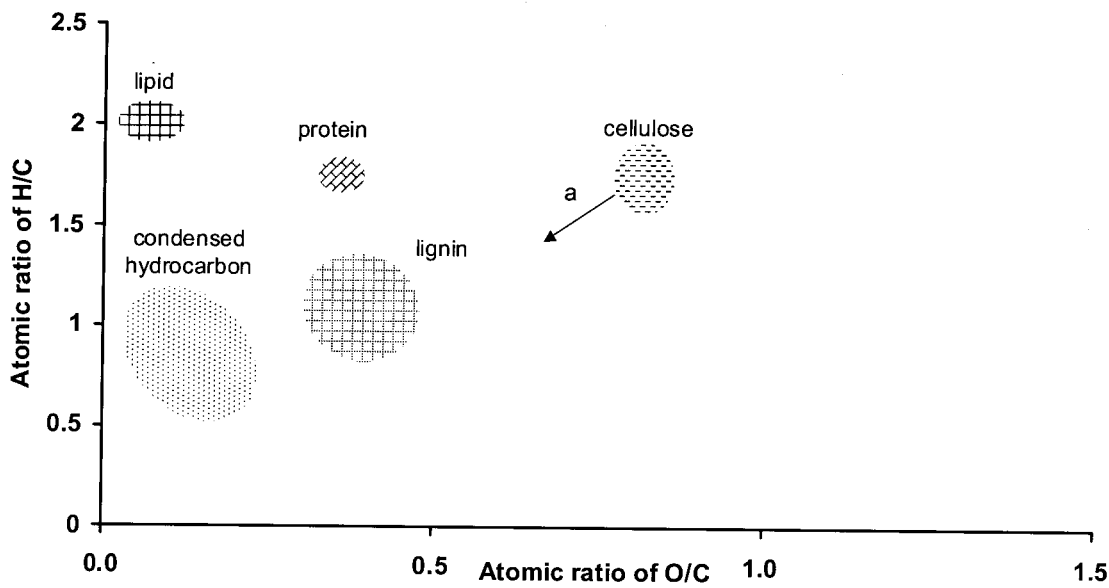
Figure 120: Regional plots of elemental compositions from some major bio-molecular components on the van Krevelen diagram, reproduced from previous studies.[15-17, 25] The arrow designates a pathway for an condensation reaction

The van Krevelen diagram of compounds in the DOM (Figure 119) is complicated. Peaks are located in a broader region with O/C ratios between 0.1 and 0.7 and H/C ratios between 0.4 and 1.7. Comparison with Figure 120 shows that this region corresponds to mainly lignin-type molecules. The water sample from McDonalds Branch had a dark tea color and relatively high total organic carbon (TOC) values (averages 16-18 mg/L). Organic rich soil materials leaching from the soils in the area(Maurice and Leff, 2002) are primarily responsible for the color and high TOC values. Therefore, humic substances associated with the surrounding terrestrial vegetation would compose the major part of the analyzed DOM sample. The strong contribution from lignin-type molecules to the mass spectrum is understandable, as lignin has been widely considered to be a major portion of humic substances.(Stevenson, 1994) In fact, Stenson et. al.(Stenson et al., 2002b) examined fulvic acid from a similar black water river by FT-ICR MS and found peaks that could be structurally tied to modified lignin molecules. The peaks in the area could also be derived from tannin-like molecules since tannin molecules would have similar H/C and O/C ratios with those of lignin-type molecules. Some of the points in the

166

diagram can also be related to condensed (e.g., dehydrated) cellulose-type molecules, because the condensation reaction would move the points in the cellulose region toward the direction noted as an arrow in Figure 120. There could also be contributions from lipid-type structures that have undergone extensive oxidation. Other major bio-molecules, proteins, were not considered as major contributors because of the low nitrogen content of the analyzed DOM sample.(Kim et al., 2002) Another aspect of the plot shown in Figure 119 that should be pointed out is the existence of molecules with apparently low H/C ratios (e.g., around H/C ratio of 0.5). A significant number of points are found in that area of the plot. The low H/C ratio indicates a significant deficiency in H among the molecules that could indicate presence of condensed ring structures.

In addition to the mass-to-charge ratios and calculated elemental formulas, the peak intensities or relative intensities are important pieces of information offered by mass spectrometric analysis. Intensities or relative intensities can be used for in a semi-quantitative way to differentiate between similar types of compounds or samples with same conditions. Therefore, it is beneficial to retain peak intensity information in the display. The relative intensity of peaks in the mass spectrum can be added to the van Krevelen diagram as a z-axis resulting in a 3D display. The 3D contour van Krevelen plot of ultra-high resolution data from McDonalds branch DOM was constructed and displayed in Figure 121. Colors of points are varied according to their relative peak height in the mass spectrum to make the plot more readable. A possible application of the 3D van Krevelen plot is for an intersample comparison. Conventional 2D van Krevelen plots that appear to be very similar to one another may in fact be different when expanded to 3D by inclusion of the peak intensities. The relative significance of each class of compounds among samples can be different. This approach could be limited to compare similar types of compounds or samples until more is known about the electrospray ionization process. Because there are numerous parameters which could affect the relative intensities of peaks (ionization efficiencies, mobile phase composition, data acquisition parameters, etc.), specific use of intensities is at best a relative approach. Nonetheless, 3D plots can provide another dimension when multiple spectra are compared. Therefore, interpretation and comparison of multiple spectra could be more complete with a 3D display.
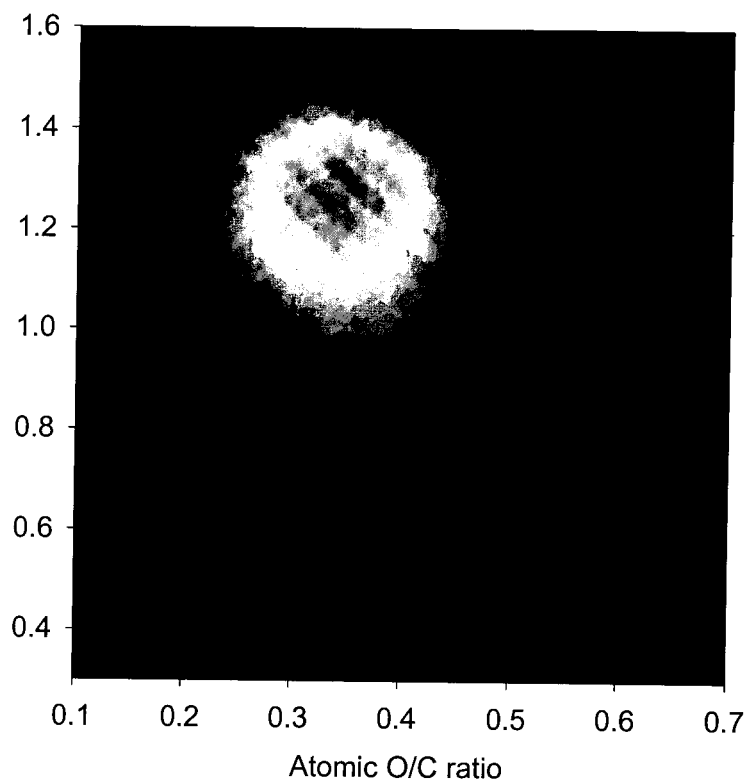
Figure 121: 3D contour display of van Krevelen diagram of DOM

During the second major project period, research at OSU was focused on isolation of unknown disinfection by-products (DBPs). In this regard, a method was developed in which chlorine gas is bubbled through drinking water spiked with humic acid. After the chlorination, the disinfection by-products are extracted following EPA method 551.1. The extracts are then preliminarily characterized by mass-spectrometry. Preparative capillary gas chromatography is used for the isolation and purification of the by-products. Nuclear magnetic resonance will then be used for further characterization of the pure fractions isolated.

Experimental Section:

All glassware including PFC sample traps was baked at 300 °C for 30 min. prior to use. Preparative GC separations were done on a HP6890 GC coupled to a Gerstel Preparative Fraction Collector using a DB5MS capillary column (30m, 0.53mm ID, 1.5um phase thickness) from Restek. The separations were done with the inlet programmed to run in solvent vent mode.

Analysis of the fractions were done using HP6890 GC with FID detector and Pegasus II GC-TOF MS from Leco using a DB5 capillary column (30m, .25mm, 0.25um film thickness).

Chlorine Gas Treatment and Sample Preparation. A setup was used in which chlorine gas is generated by a reaction of 12 g potassium permanganate with 60 mL of hydrochloric acid, and bubbled through a 100 mL sample of the lake Drummond dismal swamp water spiked with 0.8 g of Everglades peat humic acid. Excess gas was quenched in a sodium hydroxide solution. The $KMnO_4$ was slowly added so that a constant stream of gas is bubbled through the constantly stirred water solution until the $KMnO_4$ is completely used. The chlorinated solution was subsequently let to sit in the dark at 25 °C for 48 hours. After the 48 hour period the chlorinated disinfection by-products were extracted with 100 mL of pentane which is then rotavapped down to ~0.250 mL.

GCPFC Analysis. A sequence of 50 injections @ 20 uL per injection of the extract was injected into the preparative GC and PFC programmed to collect cuts as shown below.

Trap No.	Start Time	End Time
1	3.65	4.30
2	6.11	6.52
3	7.26	7.63
4	9.31	9.78
5	12.37	12.76
6	All other signals	

Sample Workup. The sample traps were rinsed with 1 mL CD_2Cl_2 and dried with nitrogen to a final volume of 0.5 mL. Samples have been checked for purity using capillary GC-FID. The fractions have also been analyzed by GC-MS. The mass spectral interpretations are currently underway. Further, the isolated fractions will also be analyzed using NMR for structural identification of the compounds.
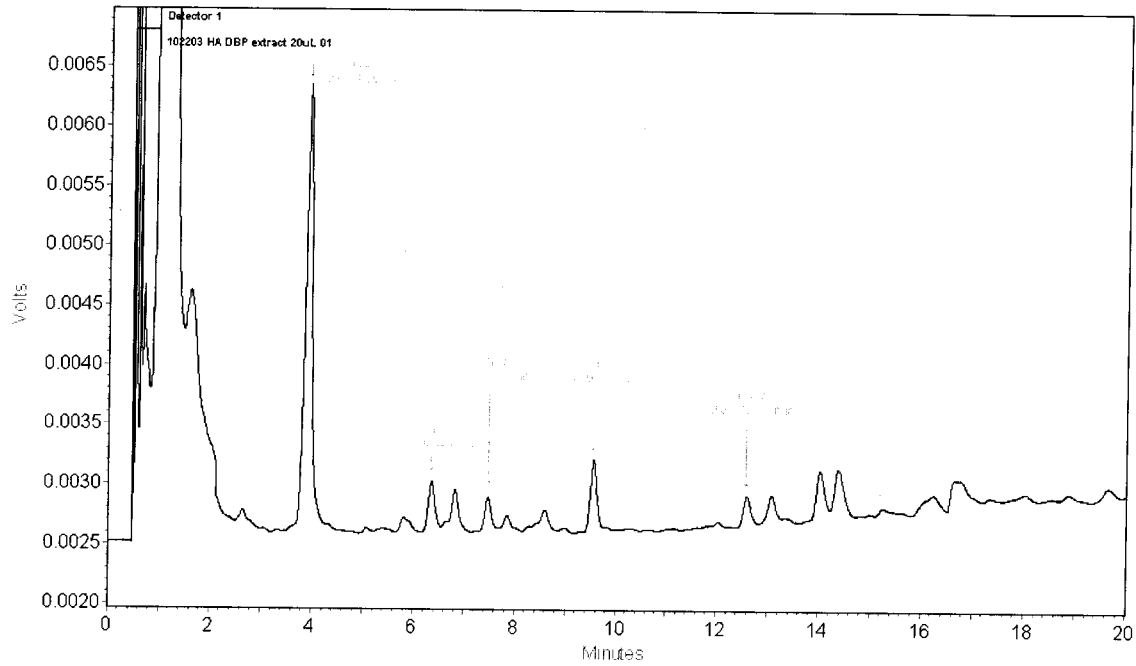
Figure 122: Gas Chromatograph of the sample on GC-PFC
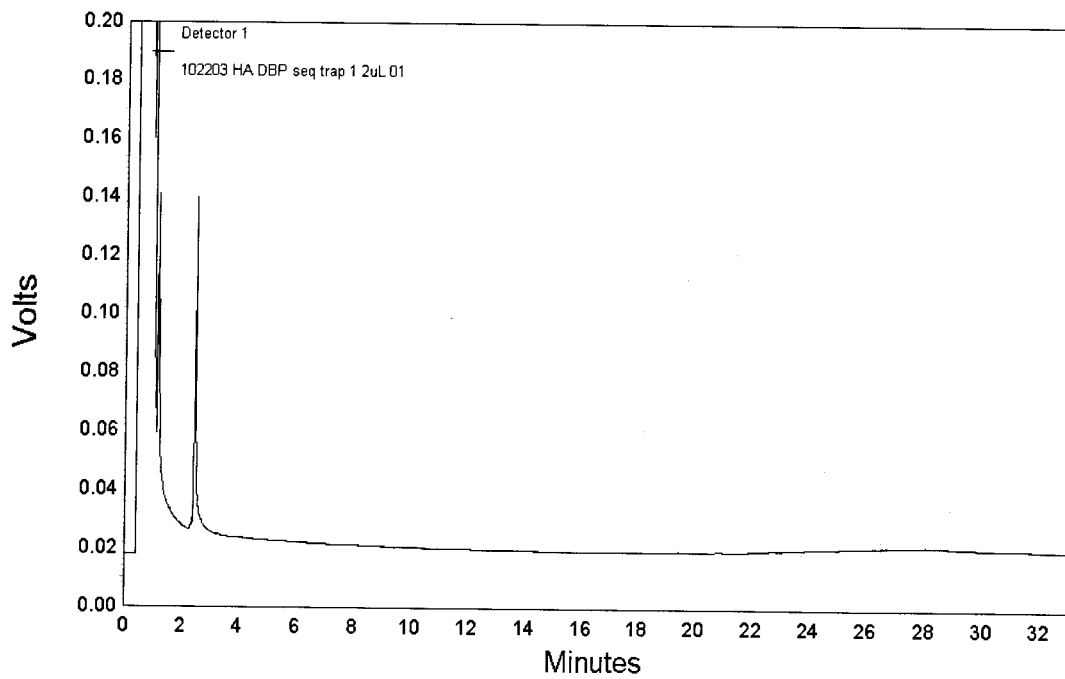
Figure 123: Gas Chromatograph of the fraction collected in Trap 1

170
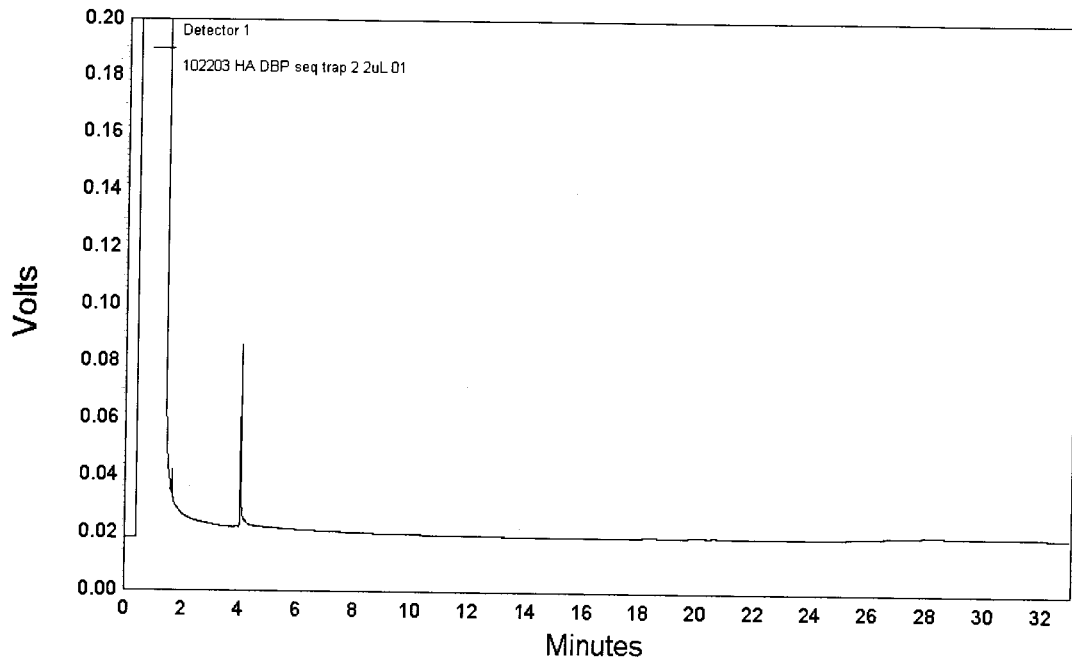
Figure 124: Gas Chromatograph of the fraction collected in Trap 2.
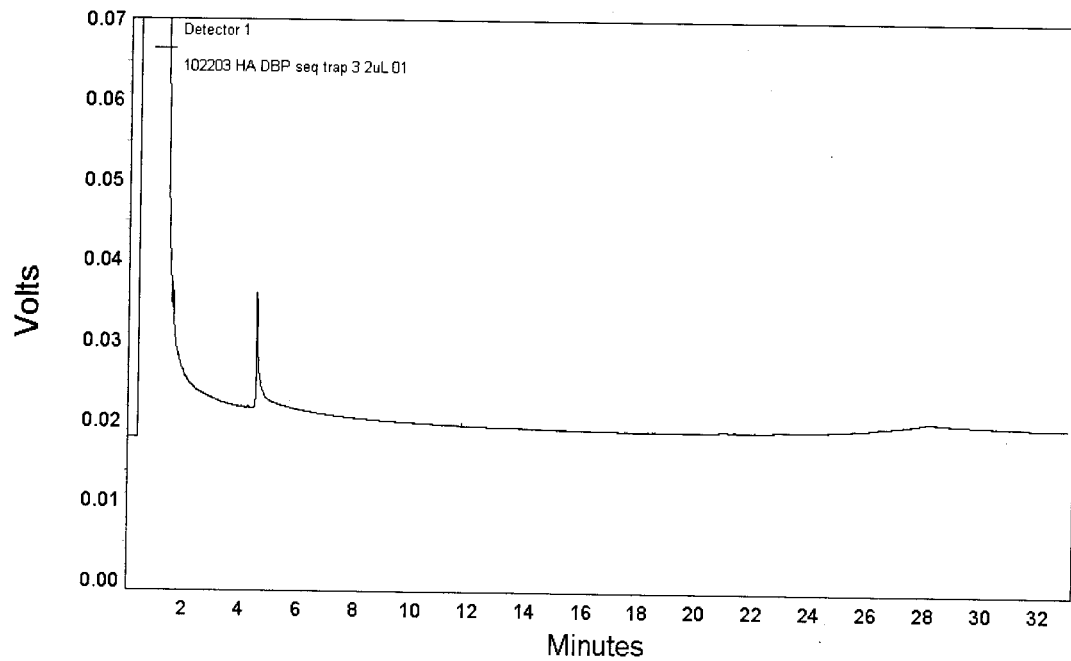
Figure 125: Gas Chromatograph of the fraction collected in Trap 3.

171
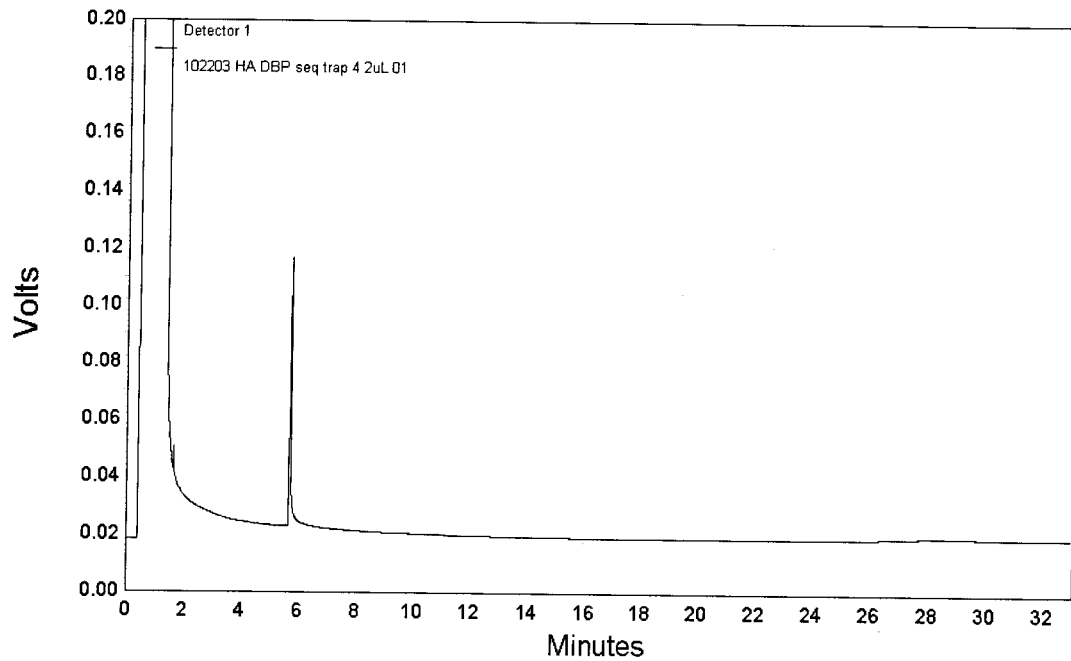
Figure 126: Gas Chromatograph of the fraction collected in Trap 4.
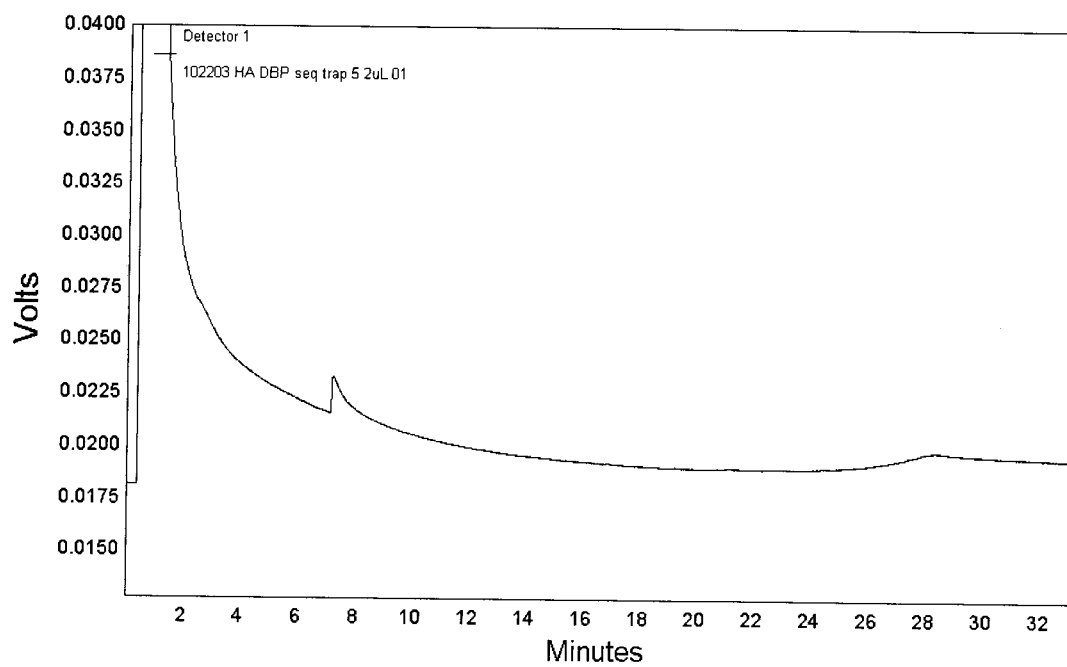
Figure 127: Gas Chromatograph of the fraction collected in Trap 5.

In addition to the work on DBPs, a few representative samples of water were collected

172

and the organics isolated for analysis using LC-TOF MS. These samples were merely practice runs to give the researchers a feel for the real samples arriving from the Reckhow group and to establish sample analysis protocols. The following figure represents a characteristic sample analyzed by LC-TOF MS.
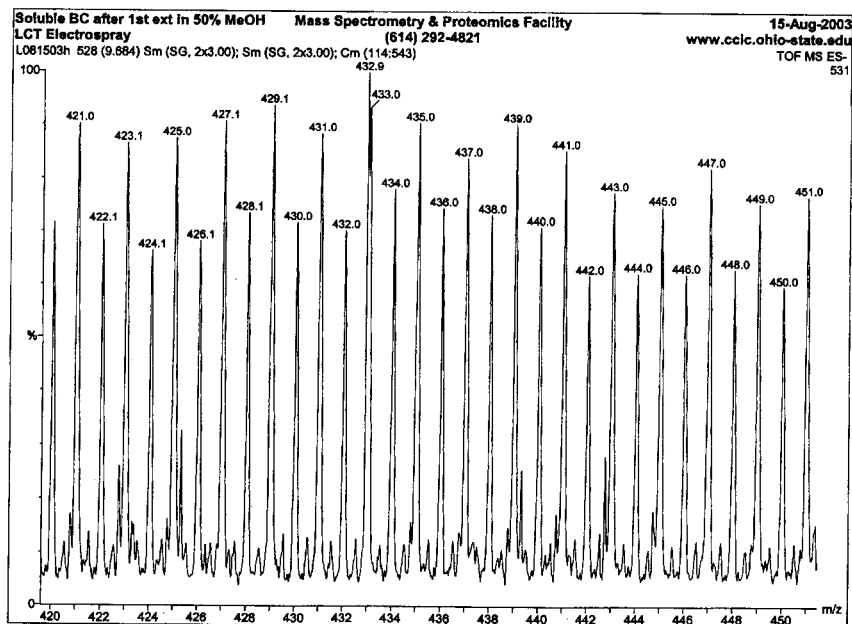
Figure 128: ESI Q-TOF mass spectrum of an aqueous sample obtained from extraction of exhaust pipe soot from an old car.

ADVANCED CHARACTERIZATION OF UNKNOWN TOX IN WINNIPEG SAMPLES

During the current period, research at OSU was focused on analyzing the Winnipeg samples received from UMass. The four samples were those of raw water, chlorinated water and two samples where chlorination was done in presence of bromide and iodide. The samples were analyzed by electrospray time of flight mass spectrometry (ESI-TOF-MS) using a Micromass LCT in both the +ve and -ve ion modes.

All glassware was washed, dried and baked in furnace at 400°C prior to use. The samples received on C18 extraction disks were washed with 80:20 methanol/water to elute out the organics from the disks. The samples were then analyzed by electrospray TOF-MS. A first look

173

at the data reveals that all the data intensity is concentrated in the small m/z region, below 1000 amu. Figure 129 below shows an overlay of the +ve and -ve ion data for raw water. Figure 130 to Figure 132 show the data for the chlorinated samples. The data above 650 amu was virtually noise.
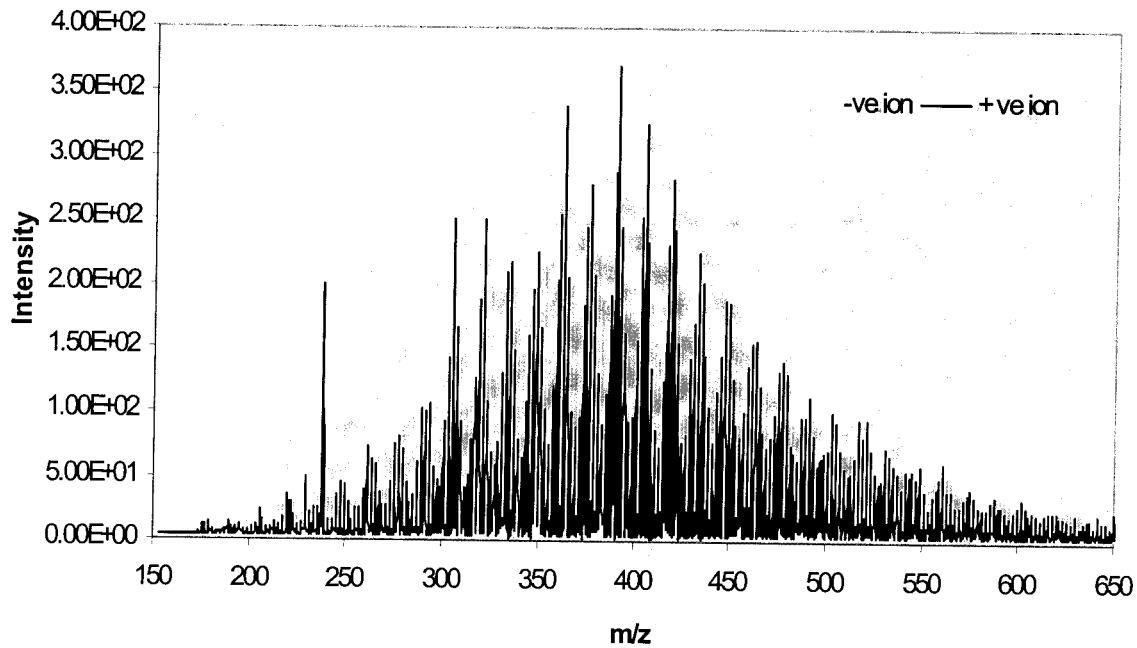
Figure 129. Electrospray TOF Spectra of Raw Water Sample from Winnipeg
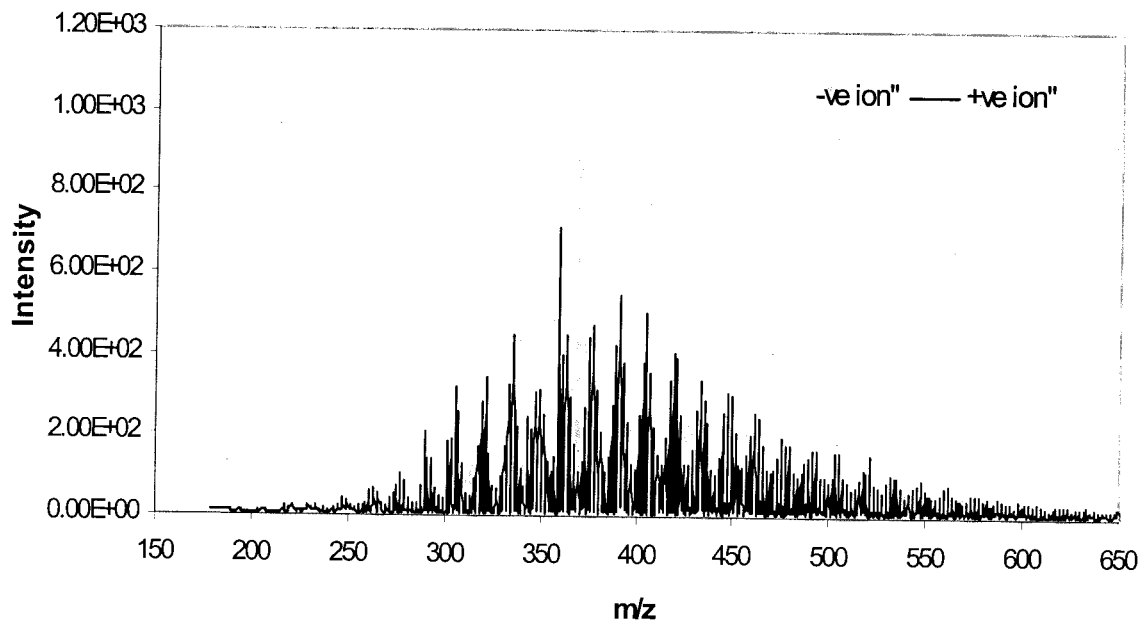
Figure 130. Electrospray TOF Spectra for Chlorinated Winnipeg Sample
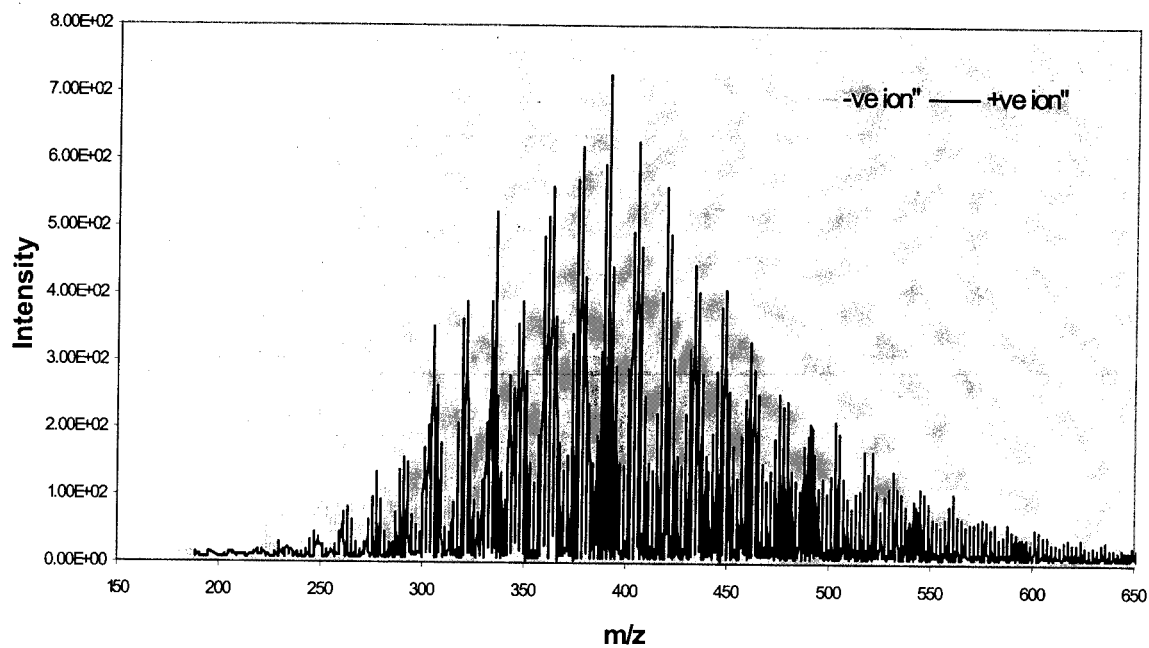
Figure 131. Electrospray TOF Spectra for Chlorinated Winnipeg Sample Fortified with Bromide
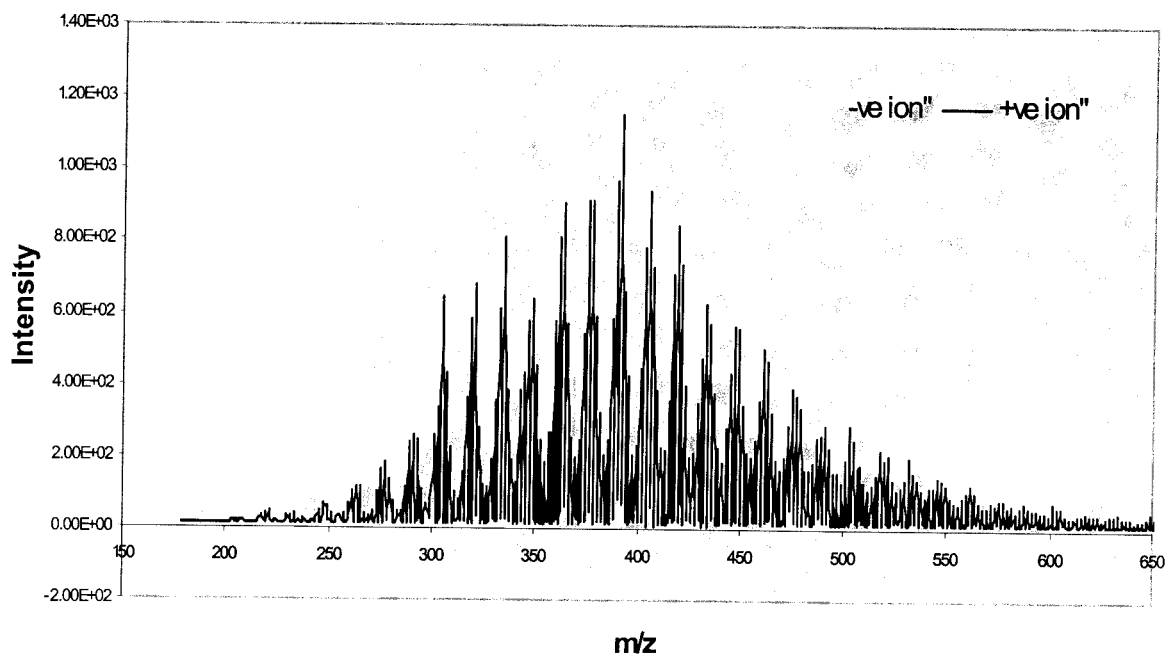
Figure 132. Electrospray TOF Spectra for Chlorinated Winnipeg Sample Fortified with Iodide
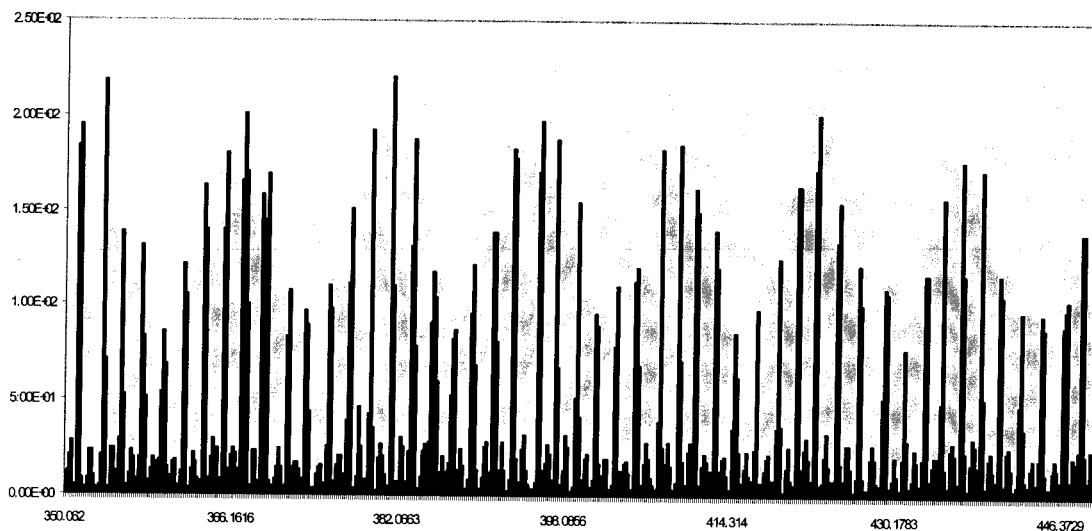
Figure 133. Fine Detail in Electrospray TOF Spectra of Raw Water Sample

The data is very characteristic of DOM samples (Stenson et al., 2002, 2003). A closer look at the spectrum, also reveals the complexity of the data. Figure 133 shows the most intense region of the spectrum for the raw water in negative ion mode and is a representative of the type of patterns observed in all the samples. The spectrum consists of sets of signals separated by 14Da. On closer observation, we see the repetitive pattern of alternating big and small signals every mass unit. This pattern of intense signals every other mass unit has been reported and is obtained in, both the positive and negative ion modes (Ikeda et al., 2000; Plancque et al., 2001; Kujawinski et a l., 2002). The spacing of signals 2Da apart has been attributed to differences in the degree of unsaturation (number of double bond equivalents) in neighboring signals (Brown & Rice, 2000; Leenheer et al., 2001). The separation of clusters of signals 14Da apart has been attributed to the presence of homologous series of compounds (Ikeda et al., 2000; Plancque et al., 2001; Kujawinski et a l., 2002). Figure 134 shows the overlay of the mass spectrum of raw water and chlorinated water in negative ion mode in the m/z range of 415 – 425. It is interesting to note that the complexity of the spectrum increases on chlorination and this pattern is repeated

176

throughout most of the spectrum.
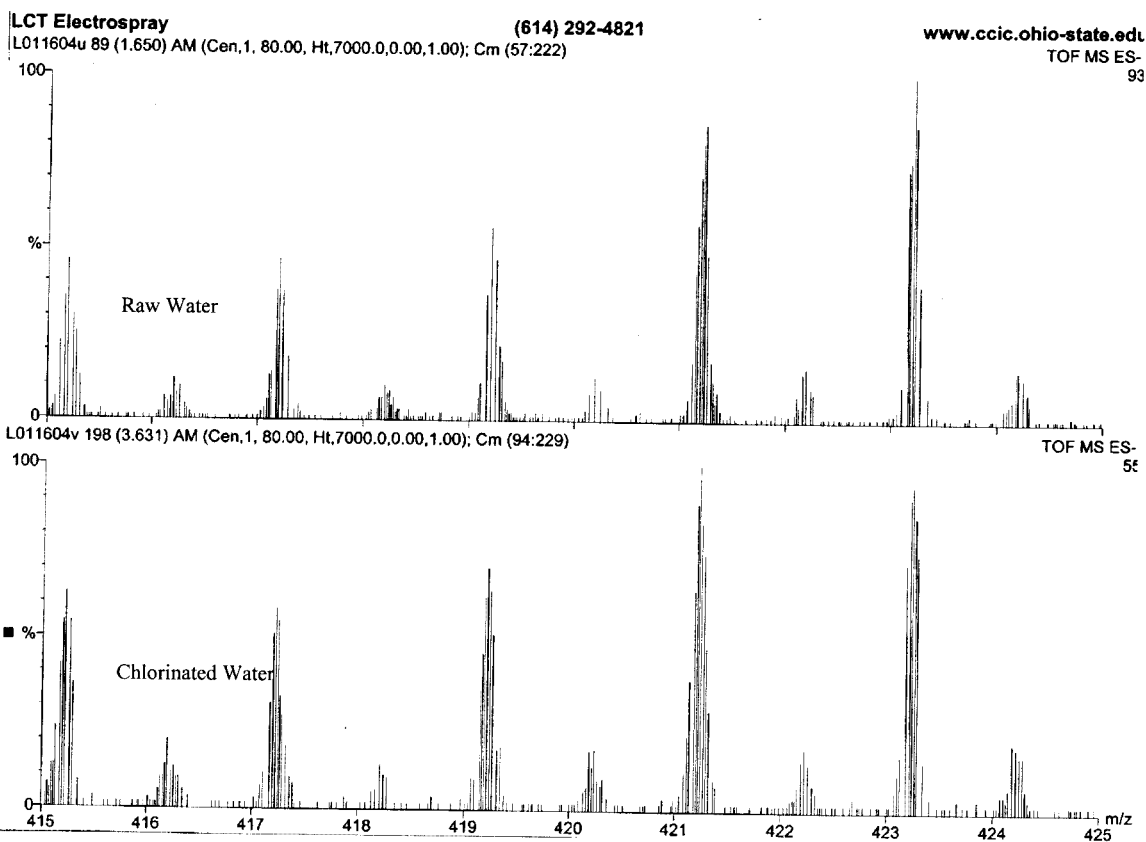
Figure 134. Comparison of Mass Spectral Complexity Before and After Chlorination: Winnipeg Sample

CONCLUSIONS

Our initial analysis of the four water samples by Electrospray Time of Flight Mass Spectroscopy shows spectral features characteristic of DOM samples. The mass spectral data are very complex and we also notice the increase in the complexity of data on chlorination. Since the DOM samples have spectral intensity every 2 Da apart, analysis of the isotope patterns due to presence of halogen atoms in the molecules is not feasible at the resolution of the instrument. In order to resolve the complexity of the data, to comment on the extent of chlorination and for determination of molecular formulae of the compounds present in the water samples, we will need high resolution mass spectral analysis of the samples using 9.4 T FTICR at the National High Magnetic Field Laboratory at Florida State University.

CHAPTER 8: CONCLUSIONS

Focused study of halogenated DBP recovery by the classic TOX procedure was a major early component of this research. Results showed that most of the common DBPs were completely recovered, except the monohaloacetic acids. These latter compounds appeared to be significantly washed out of the GAC columns during nitrate rinse. The use of lower volumes of nitrate rinse helped to minimize this problem. In addition, use of a non-standard coal-based carbon seem to result in less carryover. Nevertheless, the standard carbon showed complete recovery of at least one problematic compound (i.e., monobromoacetic acid) when used with the classical 2-column sequence. No significant differences were evident when comparing side-by-side performance of the two commercial TOX analyzers with laboratory-prepared standards. When investigating actual chlorinated raw waters, it was evident that the standard carbon resulted in slightly higher TOX values, suggesting better recovery. With the same samples, the two analyzers both performed equally well and gave nearly identical values by microcoulometric detection. However, combined use with IC proved to be more accurate with the Euroglas setup.

As mentioned above, details of the nitrate wash protocol were found to be important to halide rejection. Rinse volumes of 10 mL or less are likely to result in substantial positive bias in waters of elevated salinity. However, larger volumes of rinse will exaggerate washout of weekly-adsorbed compounds (e.g., the monohaloacetic acids). A compromise protocol of 15 mL of a 1000 mg/L nitrate rinse was adopted.

A completely new approach to TOCl, TOBr and TOI analysis involves peroxide-assisted UV oxidation followed by in-line analysis by IC. In-line sample pretreatment using commercially-available resin cartridges were also needed to eliminate inorganic interferences. This scheme (using a prototype instrument) gave complete recovery of many TOX standards, and proved to have MDLs in the range of classical TOX analysis.

Ion chromatographic analysis using commercial columns and the conventional detector (i.e., conductivity) failed to result in a single set of conditions that could adequately resolve all three halides from the background matrix. Only some of the anion exchange columns for IC are able to produce sharp, quantitative peaks for iodide ion. Among those phases, none was capable of resolving the other two halides (chloride and bromide) while avoiding interference from nitrate and bicarbonate. In particular, the resolution of bromide from nitrate was problematic. It

was also found that small, but significant amounts of nitrate from the rinse step were carried over into the pyrolysate trap. The tentative solution is to use two separate columns, one for chloride and bromide, and the second for iodide. Using this approach, we were able to achieve complete recovery for some simple model DBPs coupling the Euroglas adsorption and pyrolysis steps with chemical-suppression ion chromatography. Applying this halide-specific analysis to the chlorinated raw waters showed the method to be quite accurate as compared to conventional TOX. Differences between the two methods were in the range of 0-10% with no significant bias.

Study and analysis of ICR data revealed a wide range in TOX speciation across the US. The central tendency was in agreement with fundamental studies of aquatic fulvic acids, but the spread was greater, reflecting a substantial diversity in NOM types. Based on this analysis, a set of utilities was selected for possible inclusion in the phase 2 site list. Other criteria for selecting members of this list were presented for consideration.

A final set of conditions to be used in the CuO degradation studies was adopted. An extensive search of the literature and consultation with researchers applying these methods in other fields has clarified the options available to us. A draft SOP is nearly complete, along with a full set of QC protocols. The preferred method includes pre-extraction with C_{18} disks, thermal digestion, and concentration followed by LC/UV and LC/MS.

In the first year of this project, analytical methods to identify unknown TOX molecules have been developed. First, a technique to extract organic molecules from natural water samples was developed. By employing a C_{18} disk SPE, DOM in acidified natural water was isolated and desalted with a simple filtration setup in either a laboratory or at a field site. This protocol also efficiently removes inorganic materials that may be problematic for analysis by ESI-MS. The material obtained from C_{18} disk constituted the majority (over 60 %) of DOM and reflected the original functional group distribution. From the high resolution mass spectrum and elemental analysis of DOM, it was found that a series of molecules with a mass difference equivalent to -CH_2, -H_2 and -O and a low content of nitrogen contribute to the observed odd mass dominant peak pattern. In a followup study, the van Krevelen diagram was shown to be an effective and informative graphical method for displaying complex ultrahigh resolution mass spectrometric data of complex mixtures.

Secondly, an ultra-high resolution FT-IR technique was applied to the extracted samples to produce highly resolved mass spectra. As a result, elemental compositions of each peak

observed in the mass spectra could be calculated. This is a very important protocol for the identification of unknown TOX molecules in future experiments. By means of the analytical procedures developed in this study, elemental composition libraries can be constructed from water samples before and after they are subjected to halogenation processes. The libraries of elemental compositions can be compared to identify unknown TOX molecules. The van Krevelen analysis developed in this study can contribute when the two libraries are compared. As it was shown earlier in this report, each elemental composition library can be constructed as a van Krevenel diagram. van Krevelen analysis can contribute to this investigate and help to visually present plausible reaction pathways of molecules displaying resolved peaks in an ultra-high resolution mass spectrum. Additionally, qualitative analyses of the change in major classes of compounds after halogenation processes can be studied.

Finally, a preparative capillary GC protocol was developed and applied to chlorinated samples of natural aquatic organic matter. Also completed is the analysis by LC-TOF MS of extracts of the treated Winnipeg sample (Task 1b). These show some classic features of NOM (signs of homologous series' and various levels of unsaturation). Chlorination seemed to complicate the spectra.

Detailed laboratory treatment of two contrasting waters (Winnipeg and Tulsa) showed remarkable similarities. Both readily formed iodinated byproducts in the presence of elevated levels of iodide. There was a tendency for reduced iodine incorporation as compared to bromine incorporation, at equivalent inorganic halide levels. Higher levels of iodide seemed to result in lower levels of TOX, somewhat in contrast to the case of bromide. In conclusion, iodine seems to be much less prone toward incorporation into NOM molecules than either chlorine or bromine.

CHAPTER 9: LITERATURE CITED

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184

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CHAPTER 10: GENERAL PROGRESS AND PROJECT MANAGEMENT

This chapter is includes information unique to a progress report, and not likely to be found in a final project report.

PROJECT MANAGEMENT

As of the 12-month stage, tasks 1 and 4 were progressing well, but not as quickly as planned. In the past 6 months a concerted effort has been made to accelerate the pace (Table 35). As of the 18 month stage, we have essentially completed task 1, are well into tasks 2 and 4, and at the beginning stages of task 3.

The official start date for this project was established as September 15, 2002. This was shortly after formal notification of acceptance was received for the project QAPP. During the period leading up to September 15th we made every effort to find alternative employment for our previously-selected graduate research assistants and to avoid firm employment commitments for those not yet selected. In this way, we were able to keep Mr. Guanghui Hua (UMass PhD Student) on the project, but lost the second UMass RA.

To compensate for the loss of a key graduate student, we entered into a 1-year agreement with a sabbatical faculty from Nigeria. He came highly regarded as the Chemistry Department Chair at his home university, and his credentials indicated a high level knowledge of organic synthesis and characterization. This individual was initially given the tasks of method development for iodinated DBPs under task 1b and CuO degradation method refinement under task 4a. The intent was to have him move on to implementation of task 1b and 4a. Unfortunately he failed to make any real progress in the initial method development work. After much deliberation, he was issued a notice of termination effective March 28th due to unsatisfactory progress.

Table 35: Project Timeline

188
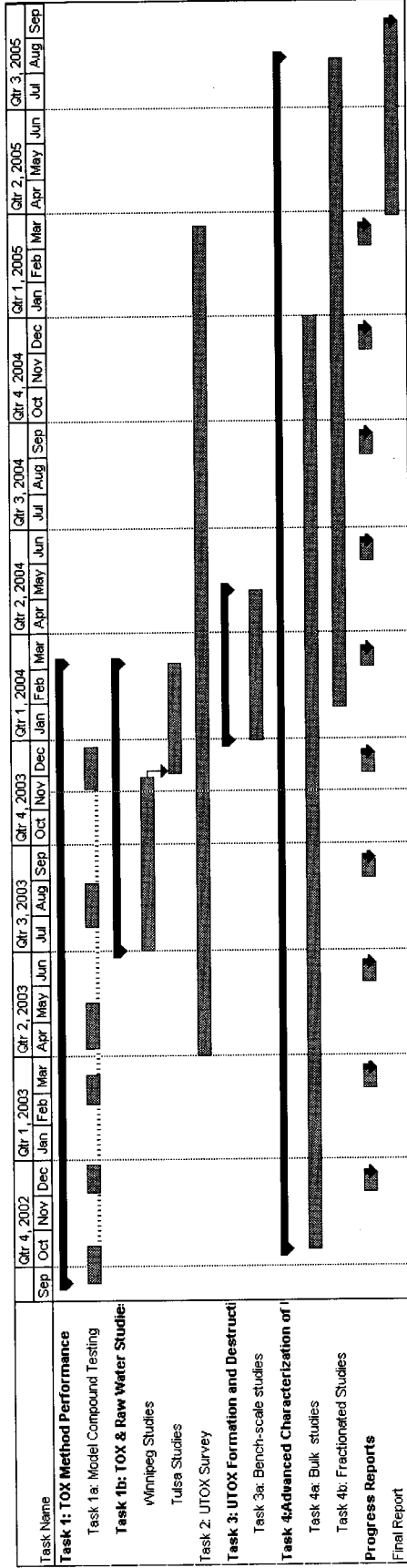
Our revised personnel plan was to bring on two additional long-term researchers so that progress during the third and fourth quarters might proceed at a faster rate than initially proposed. The first was Dr. Jun-sung Kim, a post-doctoral researcher from South Korea. The second was Mr. Chunshan Li, a graduate student previously assigned to a small utility project. Dr. Kim was to be given the CuO, GC and LC method development tasks that remained uncompleted. He was also assigned to pursue task 4a. Mr. Li was take primary responsibility for task 1b. All of this proceeded as planned during the 3rd quarter, except for Mr Li's involvement. He was forced to withdraw from UMass quite suddenly due to health problems.

We have also faced numerous equipment problems. Our Varian Saturn GC/MS was to be dedicated to this work. It was needed for identification of iodinated DBPs and for fragmentation products of CuO treatment. During the aforementioned methods development work, this instrument became damaged beyond repair. Because of the critical nature of this instrument, the UMass administration agreed to finance the purchase of a new Waters GC/TOF-MS for the duration of the current project. We received delivery of this instrument in early September, but Waters field engineers are still fine tuning it as of this writing. We also entered into a lease/purchase of a programmable microwave digestor for use with the CuO method. After 3 months of testing and use, it self ignited and was destroyed. Although the manufacturer offered to replace the unit, we decided to develop a new and safer protocol using a conventional oven.

OUTREACH

There following publications were submitted during this first 18 months of the project:

Sunghwan Kim, Andre J. Simpson, Elizabeth B. Kujawinski, Michael A. Freitas and Patrick. G. Hatcher "High resolution electrospray ionization mass spectrometry and 2D solution NMR for the analysis of DOM extracted by C_{18} solid phase disk." *Organic Geochemistry (In press).*

Sunghwan Kim, Robert W. Kramer and Patrick G. Hatcher "An informative graphical method for analysis of ultrahigh-resolution broadband mass spectra of natural organic matter – the van Krevelen diagram." *Submitted to Analytical Chemistry.*

189

BUDGET

TO: Djanette Khiari, Project Manager

Exhibit C2

PROJECT TITLE: Characterization of TOX Produced During Disinfection Processes

Project No. 2755

FROM: University of Massachusetts

REIMBURSEMENT REQUEST FOR PERIOD NO. 1
From September 15, 2002 - March 15, 2003

CONTRACTOR EXPENDITURES						
Category	Budget		Spent This Period		Spent To Date	
	%	$	%	$	%	$
Permanent Personnel	7.0%	$24,392	0.0		0.0	
Graduate/Undergrad Assistant	24.8%	$86,580	0.0		0.0	
Fringe Benefits	3.0%	$10,473	0.0		0.0	
Travel	0.5%	$1,900	0.0		0.0	
Materials and Rented Equipment	5.7%	$20,000	0.0		0.0	
Copying/Phone/Postage	0.5%	$1,750	0.0		0.0	
Graduate Health Insurance	2.5%	$8,705	0.0		0.0	
Consultant Services	0.0%	$0	0.0		0.0	
Subcontract	39.3%	$137,169	0.0		0.0	
Equipment Maintenance	0.0%	$0	0.0		0.0	
Conference Seminars	0.0%	$0	0.0		0.0	
Indirect costs	16.7%	$58,193	0.0		0.0	
CONTRACTOR SUBTOTAL	100%	$349,162	0.0	$0	0.0	$0

[A]

IN_KIND CONTRIBUTION RECORDS						
Category	Budget		Spent This Period		Spent To Date	
	%	$	%	$	%	$
Permanent Personnel	12.9%	$18,000	0.0		0.0	
Fringe Benefits	3.8%	$5,382	0.0		0.0	
Tuition and Fees	17.2%	$24,130	0.0		0.0	
Utility In-kind contributions	23.0%	$32,200	0.0		0.0	
Indirect on UMass In-kind	3.3%	$4,676	0.0		0.0	
Subcontractor In-kind	39.8%	$55,680	0.0		0.0	
IN-KIND SUBTOTAL	100%	$140,068	0.0	$0	0.0	$0

FOR AwwaRF STAFF ONLY		CONTRACT AMOUNT	$349,163.00
Approved:		Less 20% Retainage	$69,832.60
Approved Date:		Less project advance	$34,916.30
Payable #		Funds available [B]	$244,414.10
Vendor #			
Payable Date		Amount of [A] or [B] whichever is less	$0.00
G/L Date		Less previous payments including project advance	-$34,916.30
Account		Amount due this period	-$34,916.30
Account		Plus project advance	$34,916.30
Account		Total due	$0.00

190

APPENDICES

APPENDIX 1: TASK 1A DETAILED EXPERIMENTAL DESIGN

Prepared by:
Guanghui Hua

Task Description

Despite extensive efforts to identify unknown disinfection by-products (DBPs), a significant fraction of DBPs is still unidentified. However, halogenated DBPs have been considered to be major contributors to human health risk of drinking water. Total organic halide (TOX) measurements have played an important role in estimating the extent of total halogenated DBPs and their risks. By comparing the TOX values with the halides attributed to known identifiable byproduct, it is found that about 50% of TOX still remain unidentified.

The objectives of this research are: (1) to determine the nature and chemical characteristics of the unkown fraction of the total organic halogen (UTOX) produced during chlorination and alternative disinfection processes (i.e., chloramination, chlorine dioxide, ozone disinfection), (2) to assses the impact of treatment on removal of UTOX precursors; (3) to assess the stability of UTOX in a model distribution system and (4) to determine the best TOX protocol for use with IC analysis for the purpose of discriminating between TOCl, TOBr and TOI.

As the first part of this project, a preliminary assessment of TOX method performance will be made. This will assess the impact of different TOX analyzers (Euroglass and Dohrmann), different commercially available activated carbons on the recovery of the TOX. Ion Chromatography will be combined with TOX analyzers to determine amounts of TOCl, TOBr and TOI. The results of this task, as designated as task 1, will provide answer for the following questions: (1) How do the various commercial TOX analyzers compare with respect to TOX (TOCl, TOBr and TOI) recovery, and halide ion (Cl, Br and I) rejection? (2) How well do the

various analyzers/protocols work when combined with IC analysis?

Experimental Design

The first portion of task 1 involves the analysis of known solutions of chlorine, bromine and iodine containing HAAs, THMs and others (e.g., halogenated nitrogeneous compounds). Each will be run on two analyzers (Euroglass and Dohrmann) using the standard activated carbon and two other commercially available carbons. The description of these three activated carbons is shown in table 1.

Table 1: Three Commercially Available Activated Carbons for TOX Analysis

Carbon	1 (standard)	2	3
Company	CPI	CPI	Prepared in the lab using Calgon F-600 GAC
P/N	475-002	475-001	
Source	Coconut	Coconut	Coal
Particle Size	100-200 mesh	100-200 mesh	100-200 mesh
Background	0.4 μgCl/40mg	≤1.0 μgCl/40mg	

The standard compounds selected for the TOX recovery test include four chlorine and bromine containing THMs, nine chlorine and bromine containing HAAs, selected iodide containing THMs and HAAs, selected halogenated nitrogenous compounds and other compounds of interest. The results of this portion will clarify the influence of the two analyzers and three activated carbons on the TOX recovery of different compounds.

Final determination will be done by IC to differentiate the TOCl, TOBr and TOI. The off-gas from TOX combustion furnace will be collected in water instead of being titrated in a microcoulometric cell. Then the concentrations of chloride, bromide and iodide ions are determined by IC analysis.

The second group of Task 1 experiments will make use of two contrasting groups of precursors for production of unknown TOX that can be used to test the methodologies. The waters selected for this task are raw waters from Tulsa's Jewell plant and from the city of Winnipeg. The former is largely allochthonous and the latter is heavily autochthonous. The two

waters used in Task 1b will be treated with chlorine after being dosed with varying levels of bromide and iodide ion. The purpose is to form a range of unknown brominated and iodinated byproducts which can be tested for relative recovery by the various TOX protocols. Additional experiments will be run where the halide ions are added after quenching the chlorine. The purpose here is to see if bromide or iodide ions will interfere with TOX measurements using these protocols.

Analysis Procedures

TOX Standards Recovery Test

1. Prepare Primary Stock Solution

a) Place a 10 mL volumetric flask partially filled with acetone in an analytical balance and record the weight

b) Add standard compound about 50 mg equivalent chlorine and record the weight

c) Fill to the mark with acetone

d) The concentration of the TOX stock solution is determined by:

C_{stock} = (weight of equivalent chlorine (g)/10mL) × (1000mg/g)

The concentration should be around 5 mg Cl/mL.

Place the unused portion of this solution in autosampler vials, label them with name, concentration and date, and store them in refrigerator.

2. Prepare Intermediate Stock Solution

a) Fill a 50 mL volumetric flask to about 2/3 capacity with Super-Q water

b) Calculate the amount of the stock necessary to prepare a 5 mg Cl/L solution in 50 ml Super-Q water:

$$\frac{("x" \mu L / 1000 \mu L) \times (C_{stock} mgCl / mL)}{50mL \times (1L / 1000mL)} = 5mgCl / L$$

c) Add "x" μL of the stock solution to the volumetric flask.

d) Fill the volumetric flask with Super-Q water.

3. Prepare calibration standards

a) Add about 50 mL of Super-Q water to five 100 mL volumetric flasks.

b) Add a range of volumes of the intermediate stock solution to produce a standard curve.

> 0, 2, 6 and 10 mL of intermediate stock correspond to 0, 100, 300 and 500 µg Cl/L standards.

c) Add several drops of concentrated sulfuric acid, which make the pH equal 2 for final solution.

d) Fill the volumetric flaks with Super-Q water.

4. Pass about 50 mL of each standard for activated carbon adsorption and then follow the standard TOX analysis procedures.

TOCl, TOBr and TOI Detection by Ion Chromatograph

1. Prepare Calibration Standards

a) Weight out the following amounts of salts dried to a constant weight at 105°C

    (i)   0.2109 g KCl

    (ii)   0.1493 g KBr

    (iii)   0.1321 g KI

b) Dilute to each to separate volumes of 100mL. This leads to separate primary stock solution of 1000 mg/L of each as total anion

c) Add 1 mL of the primary ion stock solutions 100 ml volumetric flasks. Fill up with Super-Q water. This leads to separate intermediate stock solution of 10 mg/L of each as total anion.

d) Add a range of volumes of the intermediate stock solution to produce a standard curve.

> 0, 0.5, 1, 2, 3 and 5 mL of intermediate stock correspond to 0, 50, 100, 200 and 300 and 500 µg /L standards.

2. Follow the standard IC analysis procedures. Use 100 µL injection volume. Create standard curves for each anion based on the results from IC analysis.

3. Run activated carbon adsorption and pyrolysis for each TOX standard. Collect the off-gas from combustion furnace using 50 ml Super-Q water. Then, determine TOCl, TOBr and TOI of each sample by Ion Chromatograph. It is reported the use of carbon oxide in Dorhmann analyzer results in excessive interference in IC analysis of the halides. Preliminary experiment

will be taken to test this interference. If this is confirmed, a pre-IC nitrogen purge step will be applied to remove the dissolved CO_2 for samples from Dorhmann analyzer.

Raw Water Studies

1. Collect water samples from Tulsa's Jewell plant and from the city of Winnipeg.
2. Dose each with chlorine under the following conditions
   (1) bromide added prior to chlorination
   (2) iodie added prior to chlorination
   (3) bromide added after chlorine is quenched
   (4) iodide added after chlorine is quenched
   (5) no halide addition
3. Collect chlorinated waters, analyze for TOX, TOCl, TOBr and TOI.
4. Analyze for specific DBPs by GC.

APPENDIX 2: TASK 1B DETAILED EXPERIMENTAL DESIGN

Prepared by:
Guanghui Hua

Task 1b experiments will make use of two contrasting groups of precursors for production of unknown TOX that can be used to test the methodologies. The waters selected for this task are raw waters from Tulsa's Jewell plant and from the city of Winnipeg. The former is largely allochthonous and the latter is heavily autochthonous. The waters used in Task 1b will be treated with chlorine after being dosed with varying levels of bromide and iodide ion. The purpose is to form a range of unknown brominated and iodinated byproducts which can be tested for relative recovery by the various TOX protocols. Addition experiments will be run where the halide ions are added after quenching the chlorine. The purpose here is to see if bromide or iodide ions will interfere with TOX measurements using these protocols.

1. Collect initial raw water sample for general analysis
   A. Purpose: to establish raw water levels for key parameters
   B. Protocol: Collect 500 ml water sample, Analyze for:
      1. THMs
      2. HAAs
      3. <u>TOX</u>

4. TOC
5. UV absorbance
6. pH

2. Chlorine Dose Test

A. Purpose: to determine appropriate chlorine dose for subsequent tests

B. Protocol: collect 2 L water sample, add varying doses of chlorine to each 300 ml sample and test residual at a single characteristic contact time

- About 6 dose levels each (e.g., 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 mg Cl$_2$/L);

C. Criteria for selection of chlorine dose for subsequent tests

| 0.5 | mg/L residual |

at

48	hours contact time
20	°C
7	pH

3. Chlorination Treatment

A. Purpose:

- **to determine the DBPs produced by the chlorination**
- **to determine the possible interference with TOX measurements by adding halide ions after quenching the chlorine**

B. Protocol:

1) Chlorinate 5-L water at the dose determined in step 2.

48	hours contact time
20	°C
7	pH

2) Collect 1 L sample and analyze for chlorine residual, THMs, HAAs and TOX. The TOX analysis will be conducted with Euroglas and Dohrmann analyzer, both detector methods (microcoulometric detection and IC) and three carbons.

3) Collect 2 L sample after quenching, dose three levels of Br⁻ ion (2, 10 and 30 μmol/L) to each 600 ml sample and analyze for TOX. The TOX analysis will be conducted with Euroglas and Dohrmann analyzer, both detector methods (microcoulometric detection and IC) and three carbons.

4) Collect 2 L sample after quenching, dose three levels of I⁻ ion (2, 10 and 30 μmol/L) to each 600 ml sample and analyze for TOX. The TOX analysis will be conducted with Euroglas and Dohrmann analyzer, both detector methods (microcoulometric detection and IC) and three carbons.

4. Chlorination Treatment After Adding Br⁻

A. Purpose: to produce unknown brominated byproducts to test TOX protocols.

B. Protocol:

1) Add three levels bromide ions (2, 10 and 30 μmol/L) to each 1 L raw water sample.

2) Chlorinate each 1L water sample at the dose determined in step 2.

48	hours contact time
20	°C
7	pH

3) Analyze each sample for chlorine residual, THMs, HAAs and TOX. The TOX analysis will be conducted with Euroglas and Dohrmann analyzer, both detector methods (microcoulometric detection and IC) and three carbons.

5. Chlorination Treatment After Adding I⁻

A. Purpose: to produce unknown iodinated byproducts to test TOX protocols.

B. Protocol:

1) Add three levels iodide ions (2, 10 and 30 μmol/L) to each 1 L raw water sample.

2) Chlorinate each 1L water sample at the dose determined in step 2.

48	hours contact time
20	°C
7	pH

3) Analyze each sample for chlorine residual, THMs, HAAs and TOX. The TOX analysis will be conducted with Euroglas and Dohrmann analyzer, both detector methods (microcoulometric detection and IC) and three carbons.

APPENDIX 3: TASK 2 DETAILED EXPERIMENTAL DESIGN

Task 2 is intended to generate data on the range of UTOX values that may be observed in waters across North America. The first step will be to identify about two dozen waters of differing quality (considering various combinations of TOC, SUVA, bromide/iodide, alkalinity/hardness, and region) for study. This will be done using available data (ICR and other sources) and in consultation with the AWWARF project officer and the PAC. Once selected, raw waters and finished waters will be collected from each site at different points throughout the project period. These will be shipped to UMass[10] for treatment with disinfectants and chemical analysis. At UMass each will be treated with the five disinfection scenarios (chlorine, chloramine, both with an without preozonation, and chlorine dioxide). A standard set of protocols will be used for all samples (see Table below). All samples will then be quenched and analyzed for the full suite of DBPs (THM[11], HAAs, TOX, TOCl, TOBr and TOI).

Task 2 Test Conditions

	Standard conditions
Bromide/Iodide	Ambient
pH	Ambient
Pre-O_3 dose	1 mg-O_3/mg-C
Free Cl_2 target residual	1.5 mg/L
Chloramine target residual	2.5 mg/L
Cl_2/N ratio	4.5 g/g
ClO_2 dose	1.5 mg/L
Free Cl_2 Contact Time	12 hr
Disinfectant Contact Time	48 hr
Temp	20°C

At the same time, a characteristic distribution water sample will be collected from each of the Task 2 plants, quenched and shipped to UMass. This will be analyzed for the full suite of DBPs. In addition, a portion of this sample will be fractionated based on molecular size (ultrafiltration) and hydrophobicity (hydrophobic resin adsorption). The resulting fractions will be analyzed for the full set of DBPs as well. The intention is to develop a database on the general character (e.g., hydrophobicity and apparent molecular weight) of UTOX in North American waters.

Task 2 Summary: Survey of unknown TOX formation in disinfected waters
Group raw waters (across US, Canada) based on:
- TOC
- SUVA
- Bromide and Iodide

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[10] Many utilities have been contacted about potential collaboration for TOX/DBP study. To date, all have indicated that they are willing to conduct sampling and ship samples at their own cost, in return for learning more about their own water quality characteristics and DBP formation.

[11] Note that for the purpose of this research project, all THM analysis will be accompanied by determination of other neutral extracables (e.g., haloacetonitriles, haloketones, chloropicrin)

- Alkalinity and hardness
- Geographical Region/watershed ecosystem characteristics

Select one or several from each category and treat with each of the following disinfectants at a fixed dose, pH and reaction time

- Chlorine
- Chloramines
- Chlorine dioxide
- Ozone & chlorine
- Ozone and chloramines

Collect Distribution System sample from each of these systems for comparison, and fractionate (analytical scale) using the following techniques:

- Size (ultrafiltration)
- Hydrophobicity (hydrophobic resin adsorption)

For each of these tests, the following will be measured

- TOX (separating TOCl, TOBr, and TOI)
- THMs and other neutral extractables
- Haloacetic Acids
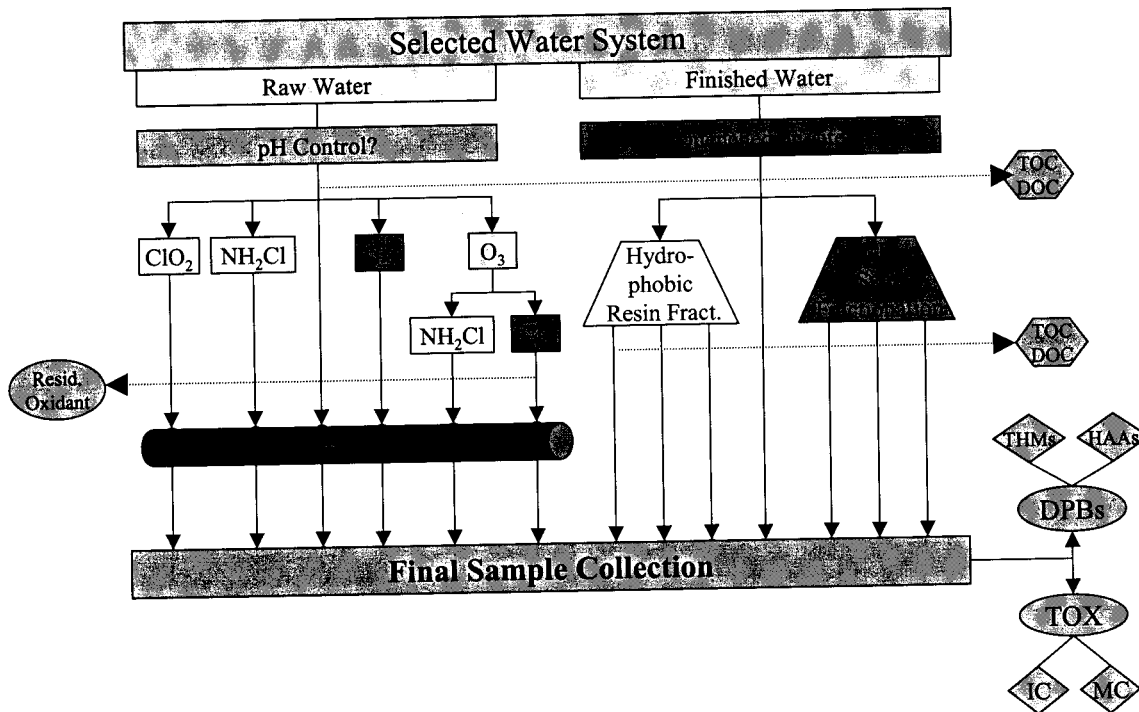
Based on this experimental design, for each sample there will be 26 TOX analyse24 (13 by IC, and 13 conventional) and 13 THM analyses and 13 HAA analyses. If we identify 24 test waters, we will then have 624 TOX measurements (312 by conventional method, 312 by IC), 312 THM analyses, and 312 HAA analyses.

In addition there will be 24 hydrophobic fractionations, 24 UF fractionations, 24 chlorine dioxide treatments, 24 ozone treatments, 48 chlorinations and 48 chloraminations.

APPENDIX 4: PARTIAL DRAFT OF TOX SUMMARY PAPER