CHEMICAL INDICATORS OF SURFACE WATER POLLUTION

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A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Masters of Science in the Department of Environmental Sciences and Engineering.

Chapel Hill
2006

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ABSTRACT

TALIA CHALEW: Chemical Indicators of Surface Water Pollution
(Under the direction of Dr. Howard Weinberg)

High quality surface water is critical for maintaining healthy ecosystems and ensuring safe drinking water, yet is often compromised by point and non-point contamination sources. Failed septic systems, an example of non-point source pollution, may generate pools of untreated or minimally treated wastewater that can runoff into nearby streams. There are currently no means of quickly determining the impact of this pollution on surface water.

Representative emerging contaminants (caffeine and triclosan) were targeted as indicators from failed septic systems and chlorination disinfection byproducts (haloacetic acids) for the effluent from conventional municipal wastewater treatment plants. Methods for the analysis of these compounds in various matrices were developed and applied to both effluent types and surrounding surface waters. Typical caffeine and triclosan concentrations in conventional municipal wastewater treatment plant effluents were 0.23µg/L and 0.3µg/L, respectively, as compared to 22µg/L and 7µg/L from septic tank effluents. Excitation-emission fluorescence spectrophotometry was also investigated as a tool for characterizing pollution from wastewater sources.

ACKNOWLEDGEMENTS

I would like to thank certain individuals who have contributed to and enriched my experience at University of North Carolina at Chapel Hill. I would first like to thank Dr. Weinberg for advising and guiding me through my research and graduate education. I would like to thank Dr. Ammozegar and Dr. Whalen for volunteering their time to read my thesis and serve on my committee. I would like to thank Jack Whaley and Katie Cretin for all their help over the past two years. Additionally, I would like to thank the other members of the Weinberg lab group for their help in and out of the laboratory, especially Ben Stanford and Katherine Stauffenberg.

I would like to thank the utililites, septic system operators and Orange County soil scientists who provided invaluable cooperation and assistance in information, sample sites and sampling. In addition, the funding for this research provided by the Water Resources Research Institute (# 70123) and the EPA STAR Fellowship (FP-91655001-0) is gratefully acknowledged.

Finally, I would like to thank my family and friends for all their support and encouragement through my time at UNC and always.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

BSTFA	Bis(trimethylsilyl)-trifluoroacetamide
℃	Degrees Celsius
Caffeine-d3	Deuterated Caffeine
ACN	Acetonitrile
CH_2Cl_2	Dichloromethane
CI	Chemical Ionization Mode
CV	Coefficient of Variation
DBP	Disinfection By-Product
DBPFP	Disinfection By- Product Formation Potential
DCAA	Dichloroacetic Acid
DCAA-d2	Deuterated Dichloroacetic Acid
DNR	Department of Natural Resources
E°	Standard Electrode Potential
ECD	Electron Capture Detector
EDC	Endocrine Disrupting Compound
EI	Electron Ionization Mode
EM	Electron Multiplier
ESI	Electrospray Ionization
Ft^3	Cubic Feet
G	Gram
GC	Gas Chromatograph
H_2SO_4	Sulfuric Acid
HAA	Haloacetic Acid
HCB	Hexachlorobenzene

HLB	Hydrophilic- Lipophilic Balanced
H^{-1}	Hour
L	Liter
LGW	Laboratory Grade Water
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
m	Meter
M	Molar
m/z	Mass to Charge Ratio (usually 1:1)
MBR	Membrane Bioreactors
MCAA	Monochloroacetic Acid
MDL	Method Detection Limit
MeOH	Methanol
mg	Milligrams
MGD	Million Gallons per Day
min	Minute
mL	Milliliter
MS	Mass Spectrometry
MSD	Mass Selective Detector
MSTFA	N-methyl-N-(trimethylsilyl)trifluoroacetamide
MtBE	Methyl *tert*-Butyl Ether
NCDENR	North Carolina Department of Natural Resources
ng	Nanogram
nm	Nanometer
NOM	Natural Organic Matter

NPS	Non- Point Source Pollution
PFTBA	Perfluorotributylamine
pH	Negative Log of the Hydrogen Ion Concentration
pKa	pH Value at which Acid is Half-Dissociated
PLRP-s	Polymeric Reversed Phase
PPCP	Pharmaceutical and Personal Care Products
PQL	Practical Quantification Limit
PRP-1	Poly(styrene-divinylbenzene)
PTFE	Polytetrafluoroethylene
RPD	Relative Percent Difference
s	Second
SAX	Strong Anionic Exchange
SIM	Single Ion Monitoring
SPE	Solid Phase Extraction
TCAA	Trichloroacetic Acid
Triclosan-d3	Deuterated Triclosan
µ	Micro
µg	Microgram
UHP	Ultra High Purity
µL	Microliter
USEPA	United States Environmental Protection Agency
V	Volts
WWTP	Wastewater Treatment Plant
WWTP DIS	Wastewater Treatment Plant Discharge Samples
WWTP DS	Wastewater Treatment Plant Downstream Samples

WWTP EFF	Wastewater Treatment Plant Effluent Samples
WWTP US	Wastewater Treatment Plant Upstream Samples

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

A common distinction between waste sources in the environment, in terms of qualification and regulation, is between point and non-point sources (NPS). Point sources are discharged from a specific location that can be identified, quantified, and regulated. In contrast, non-point sources are diffuse pollution sources that stem from many locations. For example, NPS is often caused by precipitation running over the ground and transporting natural and anthropogenic pollution into the waterways. Therefore, without one specific discharge location, it is difficult to identify, quantify or regulate NPS pollution. Lacking the ability to identify NPS specifically, monitoring programs use more general indicators of water quality and watershed health. Additionally, remediation efforts at water quality protection and clean up are limited by the inability to protect against diffuse NPS.

The objective of this study was to develop an indicator to differentiate between specific point source and NPS pollution in the environment. Knowledge of the specific pollution source would lead to more directed remediation and, therefore, protection of water quality. Because most surface waters in North Carolina become the drinking water source for a community downstream, a tool to identify the pollution source and facilitate remediation would benefit public health. Preventive protection of surface water quality might actually limit the amount of drinking water treatment necessary.

For this study, municipal wastewater treatment plant (WWTP) discharges were selected as a point source. WWTPs require regulated discharge permits from the U. S.

Environmental Protection Agency (USEPA). In contrast, septic systems are a decentralized form of wastewater treatment and were studied as NPS. Their failure can lead to diffuse sources of pollution throughout the watershed as contaminants from surfacing sewage that are dispersed with rain events or overland flow to surface or ground waters. In this study, there were several stages to identifying chemical indicators of wastewater contamination of surface waters. First, literature review was performed for an understanding of the treatment processes involved in municipal and on-site wastewater treatment. Indicators were proposed that survived one type of treatment more substantially than the other in order to differentiate between these types of wastes in the environment. Analytical methods were developed to determine concentrations of these indicators and then these methods were tested with environmental samples from municipal and on-site wastewater treatment and surrounding surface waters.

1.1 Water Quality Regulations

Water quality is regulated by several federal and State laws for the protection of the environment and public health. These laws create monitoring programs, control pollution discharges and allocate financial resources.

The first federal water pollution programs originated with the 1948 Water Pollution Control Act (USEPA 1999); this law was enacted to protect water quality. As water quality became an increasing concern, this law was amended several times. In 1956 and 1961, amendments allocated funding to municipalities to construct wastewater treatment works (USEPA 1999). The National Pollution Discharge Elimination System (NPDES) Program was established by the 1972 amendments (USEPA 2003). This

program required WWTPs to obtain a permit for effluent discharge into a receiving water and to use the most economical and best available treatment technology to meet the highest standards of effluent quality. The permits established both technology-based limits to pollution, based on industrial standards of treatment, and water-quality-based limits for the additional protection of water sources. Through NPDES permits, five conventional parameters: five-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), pH, fecal coliform, and oil and grease (USEPA 1999) and an additional 126 pollutants, are regulated. These additional pollutants are divided into two types: the priority pollutants, which include metals and man-made organics, and non-conventional pollutants, such as chlorine or ammonia. Amendments in 1982 required municipal WWTPs to reduce pollution to the "maximum extent practicable" (USEPA 1999).

The Water Quality Act of 1965 required states to develop and meet water quality standards, and to specifically quantify pollution discharges (USEPA 2003). To reach this goal, the North Carolina Division of Water Quality (DWQ) of the Department of Environment and Natural Resources (DENR) has established several monitoring programs. A series of 365 Ambient Monitoring Stations (AMS) were installed throughout the state, which have the ability to collect both grab and composite samples. Grab samples are collected at least once a month and analyzed for physical, chemical, and bacterial measurements (http://h2o.enr.state.nc.us/esb/ams.html). The results are stored in EPA's STORET database (http://www.epa.gov/storet/). Every five years, the data are summarized on the watershed level to provide Basin Assessment Reports (http://h2o.enr.state.nc.us/esb/bar.html).

Nationally, the U.S. EPA has many tools available to model and monitor water quality. For example, the Watershed Assessment, Tracking, and Environmental Results Program (WATERS), provides computer resources for understanding water quality. Examples of such tools are the EnviroMapper for water quality and the ability to query water quality parameters from the WATERS database (http://www.epa.gov/waters/). EnviroMapper is a Geographic Information Systems (GIS) type program with the ability to map areas with different pollution sources and landscape features.

The Source Water Assessment Program (SWAP) was created under the 1996 Safe Drinking Water Amendments (http://www.epa.gov/safewater/protect/swap.html). Each state is required to set up a program to delineate source water assessment areas, inventory potential contaminants, and determine the susceptibility of public water supplies to contamination. In North Carolina, the Public Water Supply (PWS) Section of the Department of Natural Resources (DENR) oversees NC SWAP (http://204.211.89.20/Swap/). Within this program is NC SWAPinfo, a computer application similar to ArcGIS for mapping watersheds, water supply sources and pollution discharges. This database contains watershed SWAP assessment reports, which classify watershed health upstream of drinking water intake locations. The reports also contain useful maps that provide information on land use, annual precipitation, slope, permitted discharge locations, and prior pollution incidents.

However, the usefulness of NC SWAPinfo in mapping NPS pollution is limited by the available data. The data originates from state agencies and since it does not include county level data, not all pollution sources are identified. For example, failed

septic systems are not considered in SWAPinfo, because county offices handle this information and do not communicate with state agencies.

1.2 Municipal Wastewater Treatment and On-Site Wastewater System Design

WWTPs and septic systems employ similar processes, but septic systems operate on a simpler scale. They also differ in the amount of regulation and investment in infrastructure.

1.2.1 Municipal Wastewater Treatment

The main treatment processes in municipal wastewater treatment are mechanical, biological, and chemical. Primary treatment uses screens, filtration or sedimentation basins to separate solid from liquid waste. This process can be facilitated by the use of chemicals, such as alum, to improve settling or flocculation. Secondary treatment is the biological (either aerobic or anaerobic) treatment of supernatant and settled solids using activated sludge or trickling filter oxidizing beds. Lastly, tertiary treatment involves a finishing stage, such as lagoon, micofiltration, sand-filtration, constructed wetlands or disinfection so that wastewater meets discharge standards. If effluent is disinfected with chlorine, it is dechlorinated before being discharged into receiving waters to protect water quality. However, there is the potential for disinfection-by-products (DBPs) to form as the chlorine reacts with organic constituents in the waste, but, the extent to which DBPs form depends on additional factors, such as the use of nitrification and/or denitrification to control ammonia content. Figure 1.1 shows an overview of WWTP design. As discussed above, WWTPs require NPDES permits from the USEPA, which are

administered by state and local governments. The discharges must meet national standards on contaminants.

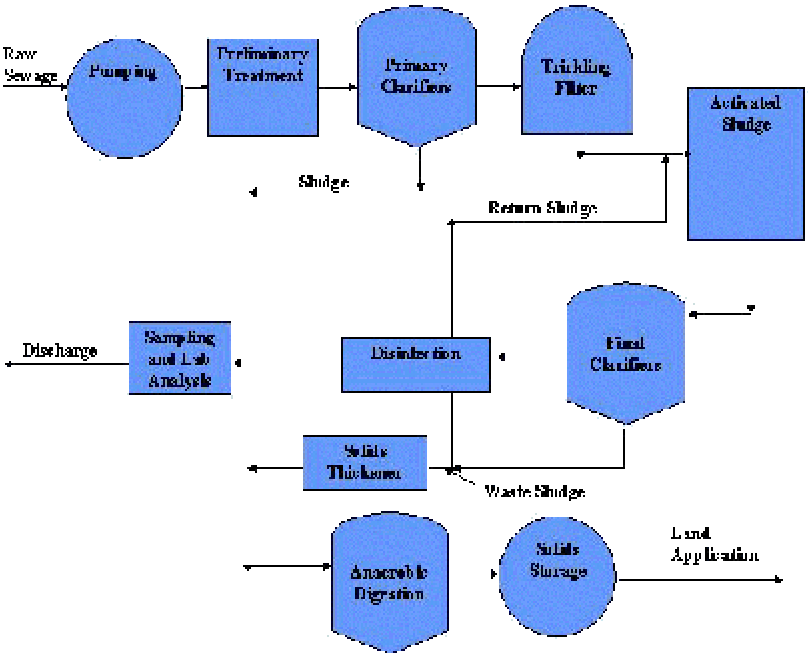

Figure 1.1 Overview of a WWTP design. (http://www.state.sd.us/denr/DES/Surfacewater/img001.gif)

The NPDES permits closely regulate nutrient removal in the effluent. The main nutrient removal process, which occurs in the secondary treatment stage is activated sludge (Mulder 2003). In conventional nitrification/denitrification processes, nitrogen as ammonium is converted via nitrite to nitrate (nitrification) and then denitrification occurs as the nitrate is transformed into nitrogen gas and released. Another means of nitrogen removal is nitrification/denitrification via nitrite. In this process, ammonia is oxidized into nitrite, and under anaerobic processes nitrite is converted into nitrogen gas and released. In alternative treatments, such as algal ponds or constructed wetlands, ammonia is incorporated into algal or plant biomass.

Despite complex treatment, there are many chemicals remaining in the effluent after discharge, especially pharmaceuticals and personal care products (PPCPs).

Conventional WWTPs are not designed to remove low concentrations of such compounds as PPCPs (Batt et al. 2006). During treatment, PPCPs conjugate and either are transformed completely to CO_2, transformed partially into metabolites, or remain unchanged (Jorgensen and Halling-Serenson 2000). Soprtion onto sewage sludge or biosolids may be the main removal mechanism for many of these compounds within the WWTP (Xia et al. 2005), but its efficiency is determined by the octanol-water coefficient (K_{ow}). If a compound is extremely polar, it is unlikely to sorb, and therefore, likely to be found in the effluent. PPCPs may re-enter the environment as runoff from land applied biosolids.

Microbial degradation may not be possible for all PPCPs, especially antibiotics (Kummerer 2003). Even if degradation is possible, it often requires a longer exposure time than the usual retention time within the WWTP (Batt et al. 2006). Removal rates vary significantly based on compound physical properties. For example, studies have shown more than 80% removal for triclosan, ibuprofen, and nonylphenols (Ternes 1998, Korner et al. 2000, Bester 2003). In contrast, diclofenac, fenofibric acid, phenazone, and carbamezapine have low removal rates (Heberer 2002a).

Additional wastewater treatment, such as UV disinfection and membrane bioreactors (MBRs), has the potential for further effluent purification. UV disinfection, an oxidative treatment, has the potential to degrade compounds, but may result in the formation of more toxic daughter metabolites (Daughton 2002). Adams et al. (2002) investigated removal of five sulfonamide antibiotics and trimethoprim using UV and chlorine disinfection from surface water, as a surrogate for wastewater. Ninety percent of sulfonamides were removed within 28 minutes and 90% of trimethoprim was removed

within 40 minutes with the use of chlorine. In contrast, UV disinfection at 254nm only removed 50-80% of the antibiotics. All of the antibiotics tested have been shown to absorb UV, so the lack of degradation may be due to competition with other natural organic matter (NOM) for the UV. Competition with NOM will be even greater in wastewaters.

MBRs have the ability to remove a higher percentage of suspended solids. In a comparison study of pharmaceutical and endocrine-disrupting compound (EDC) removal rates, Clara et al. (2005) evaluated influent and effluent of MBRs in conventional WWTP with nitrification and denitrification. Overall, the removal rates were similar, although slightly lower for MBRs. Wintgens et al. (2004) evaluated the use of nanofiltration and MBRs for removal of the EDCs nonylphenols and bisphenyl A. Results showed 70-100% retention of compounds in the filters due to the combination of size exclusion and sorption onto solids that were filtered out.

Ternes et al. (2002) conducted laboratory batch experiments to determine the effectiveness of different drinking water treatment technologies for the removal of pharmaceuticals, the results of which can be applied to wastewater treatment as well. Selected compounds were bezafibrate, clofibric acid, diclofenac, carbamazepine, and primidone, all of which can be used as indicators of a broader spectrum of PPCPs. After 28 days in a batch reactor, removal of compounds through sorption, aerobic, and anaerobic biodegradation ranged from 6 to 39%. These low removal rates verify that sorption and biodegradation are not effective removal mechanisms for these types of compounds. Membranes alone will not have a significant effect on removal because the compounds are not sorbed to particles. Therefore, chemicals of anthropogenic origin are

likely to be found in WWTP effluent even with the use of advanced technology, such as MBRs.

1.2.2 On-Site Wastewater Treatment Systems: Design and Regulations

Despite the move to centralized wastewater treatment, approximately 25% of the U.S. population and 37% of new developments use septic systems (van Cuyk et al. 2004). Compared to WWTPs, septic systems have a much simpler design, consisting of a septic tank and drainfield (see Figure 1.2 for a diagram of septic system design). Overall treatment relies on infiltration and percolation of liquid effluent through the soil with a sufficient depth and width to prevent chemicals or pathogens from reaching the groundwater. Septic tank designs vary in such elements as the number of chambers, construction, material, and number of baffles, but the underlying principles are the same in all systems. The tank is designed to retard wastewater flow sufficiently to allow a sludge layer to form as solids settle to the bottom and lighter compounds, such as grease, float to the surface and form a scum layer. Only the middle liquid layer can pass through the chamber out of the tank. As fresh influent enters the tank, this middle layer of wastewater is forced out to the drainfield (CMHC 2004).

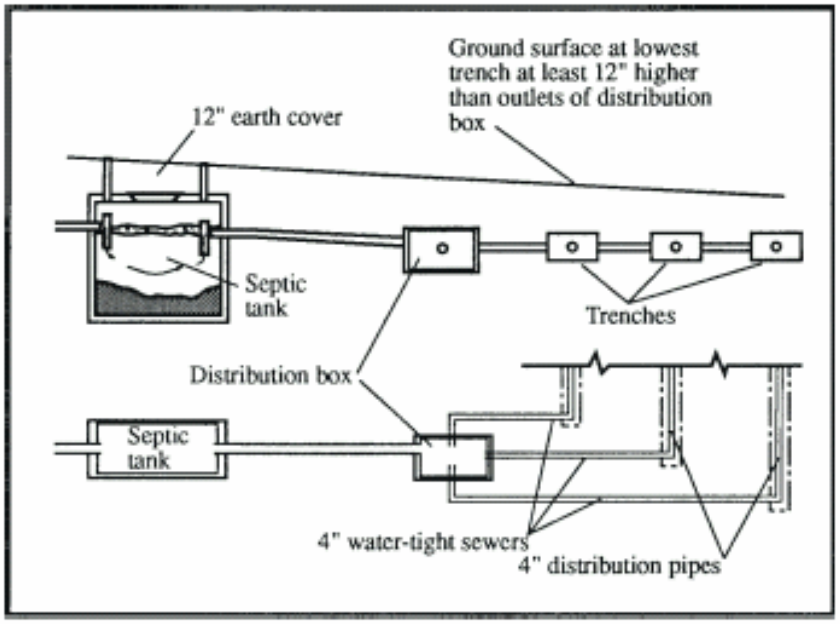

Figure 1.2 Overview of a simple on-site wastewater treatment system design (Hammond and Tyson 1999)

Septic tanks are designed for a minimum retention time of 24 hours, with the assumption that ½ to ¾ of the tank volume is filled with scum and sludge. Retention time is defined as the time that wastewater spends in the tank and is calculated by:

Retention Time (days) = Effective Volume (gallons)/Flow Rate (gallons/ day) (1.1)

Effective volume is the liquid volume between the scum and sludge layers, during which time active solids separation and anaerobic degradation occur. If the tank is not pumped frequently enough, the scum and sludge layers will build up and reduce both the effective volume and retention time. The amount of treatment, such as settling and digestion, that waste undergoes in the tank is a factor of retention time. Anaerobic degradation within the septic tank is responsible for a 40% reduction of sludge volume, 60% reduction of BOD, and 70% reduction of TSS (Reneau et al. 1989).

Under optimal conditions, wastewater should remain in the tank two to three days for sufficient treatment. However, several factors limit retention time and degree of treatment. If a significant amount of water is flushed into the septic tank at one time, the

retention time decreases (Lawrence 1973). If cleaning the tank is very infrequent, the amount of scum and solids will accumulate and lower the retention time. To prevent these conditions, tanks should be pumped out (cleaned) every three to five years.

Clarified wastewater effluent leaves the tank into the drainfield, where it is pumped or gravity-fed below the soil surface. The effluent flows through perforated pipes, built into gravel-lined drainfield trenches, and seeps into the gravel soil interface until it infiltrates through the soil. Drainfield size is estimated from the expected flow and loading rate, which are calculated by the number of bedrooms (Charles et al. 2005). Purification occurs as wastewater travels through the biological mat that accumulates on the sidewalls and bottom of the drainfield trenches. This biomat is composed mainly of anaerobic microbes that can slow infiltration to the soil, creating ponds of wastewater before it seeps into the soil.

Below the biomat, the wastewater flows into the unsaturated soil and eventually to the water table. Infiltration of wastes into the soil is based on soil pore geometry, which influences the hydraulic conductivity (Reneau et al. 1989). The effluent load to the drain field is typically 0.4 to 5 cm/day over the absorption area of the field (Wilhelm et al. 1994). Effluent loading rates influence infiltration rates. During this time, aerobic degradation occurs, which is controlled by the amount of oxygen available. Most aerobic degradation occurs immediately below the biomat layer and unsaturated conditions facilitate the volatilization and diffusion of organic compounds. If the soil under the drain field becomes saturated, anaerobic conditions will persist and impede the natural degradation processes, which can cause system failure. As organic matter from waste

accumulates in the septic tank and drainfield, sorption conditions may become a more significant limitation of degradation.

The main variables that influence biogeochemical reactions are the redox level and pH of the waste (Wilhelm et al. 1994). There are two major redox zones in the septic system: the anaerobic zone in the septic tank, which has low dissolved oxygen and high organic matter content and the unsaturated soil of the drainfield, where aerobic degradation occurs. After passing through into the saturated zone, the waste does not undergo much further oxidation because oxygen supply is limited. Oxidation of ammonia from the microbial degradation of effluent in the aerobic zone releases H^+, which alters the pH. The pH controls many factors of degradation, such as hydrolysis, sorption and enzymatic degradation (Alhajjar et al. 1990). The sand type and clay content of the drainfield soil also influence the effluent pH and biological activity (Willman et al. 1981).

There are many potential causes of septic system failure. High levels of suspended solids (SS) and BOD in the tank effluent, which occur from insufficient treatment within the septic tank, may affect infiltration capacity in subsurface areas, resulting in hydraulic failure and effluent surfacing (Charles et al. 2005). The effectiveness of the biomat as a filter is diminished if the septic tank is not properly pumped because accumulation of solids reduces effluent treatment time and leads to an excess of organic material that significantly reduces permeability. Over time, the hydraulic conductivity of the biomat, and not the soil, determines the effectiveness of the septic system in treating wastes (Wilhelm et al. 1994).

Uneven distribution of effluent into the drainfield leads to elevated loading rates. High loading can cause saturated flow conditions, which decrease infiltration (Reneau et al. 1989). Therefore, uniform distribution is important to prevent soil clogging and for the septic system to treat wastes effectively. Additionally, a change in the infiltration rate can lead to a decreased efficiency of treatment. If the infiltration rate is too slow, wastes can pond and clog the system. If the infiltration rate is too fast, the soil does not have sufficient time to treat the wastes. Precipitation events can change infiltration rates and lead to contamination of the groundwater (Cogger et al. 1988). Another source of system failure is soil clogging, which can develop with improperly treated septic tank effluent. More concentrated effluents lead to a greater likelihood of soil clogging because of the accumulation of organic matter (Siegrist and Boyle 1987).

In contrast to WWTPs which are federally regulated by the U.S. EPA, states set septic system regulations, although in North Carolina, individual counties have the ability to set more stringent standards. The vertical distance between the bottom of the absorption trenches and the seasonal high water table is regulated differently by each state. In North Carolina, the distance for individual single family systems is 30cm. Cogger et al. (1988) found that this distance was too small to consistently enable adequate waste treatment and that a larger soil buffer was necessary to protect groundwater sources. Incorrect trench installation and seasonal changes further reduced the adequacy of waste treatment with a 30cm distance (Cogger et al. 1988).

According to North Carolina law, local health departments are authorized by the State to regulate septic system installation and management. They evaluate sites for proposed systems and issue permits for system installation and design. The regulations

require, at a minimum, that systems be set back 100 feet from a public or private water source, including streams, and 50 feet from lakes. Additionally, the long-term acceptance rate (LTAR), which determines the required length of drainfield trenches, is based on the most hydraulically limiting naturally occurring soil horizon within 3 feet of the ground surface or to the depth of 1 foot below trench bottom, whichever is deeper. Finally, the bottom of any drainfield trench has to be at least 1 foot above any wetness condition. State regulations also require routine health department inspections for non-conventional septic systems, the frequency of which is dependent on the size and complexity of the septic system. During inspections, the health department checks the frequency of pumping, the alarm and electrical systems, the functionality of the dosing pumps, piping failure, and adherence to other regulations. If a system is not in compliance, the owner has a set period of time to fix the problem and have the system re-inspected. Overall, the septic tank owner is responsible for its maintenance.

Along with inspections, the local health departments educate homeowners about functions and maintenance of the septic systems to preserve their longevity and protect water quality. However, in general, homeowner knowledge of septic system functioning and maintenance is limited. Schwartz et al. (1998) conducted a survey to assess homeowner understanding of their septic system design and maintenance requirements in three counties in upstate New York. In two counties, less than 50% of homeowners had pumped their septic tank within the past five years and one third had never pumped their tank. Respondents could not accurately describe the components of their septic system or identify which authorities to contact for help with water quality or septic system pollution issues. Their study also found that socio-demographic factors influenced whether

homeowners contacted local authorities for help with their system. A similar study in North Carolina showed that 43% of homeowners incorrectly believed that a septic system did not need pumping until it failed (Halvorsen and Gorman 2006).

Septic system failure is often not reported to the health department because the homeowner wants to avoid the expense of repairs. If a homeowner is unaware of the warning signs of septic system failure, a system may be causing pollution for an extended period of time before it is repaired. In this regard, an indicator of NPS pollution from septic systems could identify failing systems before they fail beyond repair.

1.2.3 Septic System Pollution Research

Past research into septic system pollution has focused on nutrients, bacteria and viruses, not on chemical degradation. Van Cuyk et al. (2004) tested virus removal in lab and field septic systems and found 98-99.9% removal of surrogate bacteriophages through a simulated drainfield before reaching groundwater. Harrison et al. (2000) measured fecal coli bacteria in septic tank effluent and in the drainfield through suction lysimeters. Their study found 91.1% removal of bacteria from the effluent to the drain field. With additional sand-filtration pre-treatment, reduction of bacteria rose to 99.8%. Scandura and Sobsey (1997) showed that even in sandy soils extensive reduction of viruses, bacteria and nutrients can be achieved if the clay content is over 15%.

Geary (2005) found that the majority of nitrogen entered the septic tank as organic nitrogen degraded to ammonium. As effluent reached the aerobic drainfield, the ammonia was quickly oxidized. Harrison et al. (2000) found a 47% decrease in total nitrogen between septic tank effluent and drain field lysimeters: 79% of nitrogen

collected in lysimeters was present as organic-N and NH_4-N, with the remainder as NO_2-N or NO_3-N. In contrast, a system employing sand-filtration pre-treatment resulted in a 74% decrease of nitrogen from the effluent to the drainfield, with lysimeters containing over 91% of nitrogen as NO_3-N. A visualization of nitrogen removal processes within the drainfield is shown in Figure 1.3.

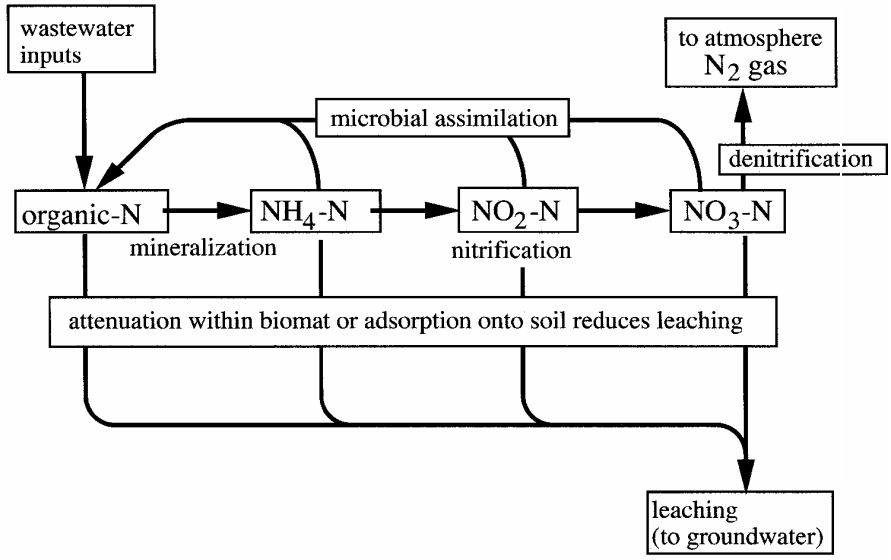

Figure 1.3 Potential sources, transformations, and losses of nitrogen in an on-site treatment system (Harrison et al. 2000).

1.3 Indicators of Pollution

There are several uses and benefits to an indicator of pollution which can be physical, chemical or biological. An indicator can track a type of pollution through the environment, indicate the presence of more harmful compounds or act as a surrogate for the behavior of a wide range of possible compounds. Additionally, a chemical indicator, that is representative of a variety of compounds, can be targeted for monitoring, because, it is time consuming and expensive to search for all possible compounds.

The objective of this study was to identify chemical indicators that could track and perhaps distinguish point and NPS pollution through the environment, which would

be a useful tool for directed cleanup of an impaired waterway. Targeting the known pollution source would be an effective use of limited resources available for remediation of contaminated natural waters.

1.3.1 Microbial Indicators and Disadvantages

Many researchers have studied microbial indicators to determine whether water bodies and drinking water sources have been impaired (Scott et al. 2002, Glassmeyer et al. 2005). A common indicator is fecal coliform, which is found in human and animal wastes. It is easy to monitor, indicates the presence of more harmful pathogens found in fecal matter, and can provide an indication of waterways that have been polluted by waste. However, there are many disadvantages to bacterial indicators. Glassmeyer et al. (2005) explain that biological techniques can be time consuming (e.g. taking at least 18 hours to complete the assay). For determining dangerous pathogens in water, this time lag could have a detrimental impact on public health. Barrett et al. (1999) found, in their review of marker species for urban groundwater recharge, that microbial indicators may be effective only when used in combination. Individually, however, they are not effective indicators because of misdetection, die-off, or the presence of competitive organisms.

The main drawback to microbial indicators is their lack of specificity. Sometimes the presence of high concentration of an indicator cannot be attributed to fecal contamination (Glassmeyer et al. 2005). Even if fecal pollution is determined, microbial indicators often cannot differentiate between human and animal sources, which limits their usefulness for guiding remediation.

Ahmed et al. (2005) studied fecal indicator bacteria for tracking septic system pollution. Samples were collected from septic tanks, and both up- and downstream in a watershed containing septic systems. Significantly higher levels of fecal bacteria were found downstream than upstream; however, there was greater bacterial diversity in the stream than in the septic tank. This implies other sources contributed fecal bacteria to the stream and that the presence of fecal bacteria could not pinpoint septic tanks as the pollution source.

1.3.2 Benefits of Chemical Indicators

Compared to microbial assays, chemical indicators have many advantages. In many cases, the processing time of chemical indicators is much shorter than microbial methods. Chemical indicators can target categories of chemicals, measure their usage, and transformation by humans, and thereby identify the pollution source and differentiate between human and animal pollution sources.

Various studies have proposed and rejected different chemical constituents as potential indicators, either individually or in combination. Barrett et al. (1999) found that nitrogen isotopes in combination with microbial methods were effective indicators of waste. Verstraeten et al. (2005) also proposed nitrogen species, specifically ammonia, as indicators to identify septic contamination of drinking water wells. However, as nitrogen species move through the aquifer, they are biologically mediated and can no longer be used for source tracking. Additionally, the variety of other potential nitrogen sources, such as fertilizer, animal waste and geological sources, limits its usefulness as an indicator of specific waste source.

Several pharmaceutical products used only by humans, such as clofibric acid, carbamazepine or diclofenac, have been suggested as potential indicators of WWTP pollution. Heberer (2002a) detected these compounds in an average of six German WWTP effluents at 0.48µg/L, 1.63µg/L, and 2.51µg/L, respectively. However, other studies have not found comparable levels of these compounds. Weigel et al. (2002) found watershed levels of clofibric acid ranging from 0.01–1.35ng/L. Carbamazepine was detected in 70% of low-flow samples of stream water downstream of urban areas, in the range of 0.14ng/L-0.263µg/L (Kolpin et al. 2004). Stackelberg et al. (2004) detected carbamazepine in 100% of stream samples collected below two WWTPs, all at levels above the reporting limit of 0.01µg/L. Thomas and Foster (2004) tested for diclofenac in WWTP effluent treated with biological nutrient-removal processes and UV disinfection, but did not detect it. Ibuprofen, another potential indicator, has been detected in WWTP effluents in concentrations ranging from 0.01-0.1µg/L (Heberer 2002ab, Thomas and Foster 2004). Other possible chemical indicators include musk compounds, fluorescent whiting agents or DEET (Heberer 2002a, Buerge et al. 2003a, Xia et al. 2005), but their domestic usage is low and too inconsistent to enable them to be effective measures, especially of septic system pollution.

1.4 Chosen compounds: Caffeine, Triclosan, HAAs

For this study, caffeine and triclosan were chosen as potential indicators of septic system pollution and haloacetic acids (HAAs) were chosen as potential indicators of WWTP pollution. Their high usage in the United States, detection in the respective domestic waste stream, and the fact that homeowners would feel comfortable discussing

their use make them effective indicators. For example, pharmaceutically active agents (PhACs), such as anti-epileptics (e.g., carbamazepine), which have been proposed as indicators (Heberer 2002a), may not have high enough usage levels to be detected in septic systems, and homeowners may be unwilling to discuss prescription medications if a usage study were undertaken to estimate analyte concentrations in septic influent. To select a pollution indicator distinguishing point from non-point sources, compounds chosen as potential indicators from septic systems need to have high removal rates during municipal wastewater treatment, whereas indicators of municipal treatment effluent should not be found in the effluent from on-site treatment.

1.4.1 Caffeine

1.4.1.1 Chemical Parameters

The structure of caffeine is shown in Figure 1.4. Caffeine is unlikely to degrade in surface waters based on its low K_{ow} and high solubility. See Appendix 1 for further chemical properties.

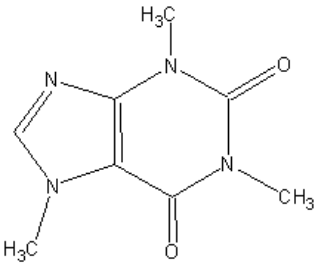

Figure 1.4 Caffeine structure.

1.4.1.2 Usage

Caffeine usage is abundant in modern society. Worldwide, the largest consumers of caffeine are Europeans, who consume an average of 4.6kg/person/year. North and Central America consumers ingest 3.6kg/person/year, whereas the rate in Oceania and South America is 2.3kg/person/year (Carrillo and Benitez 2000).

Caffeine is an ingredient in food, beverages and medications. Caffeine content in chocolate ranges from 5 to 35mg/oz (Ferreira et al. 2005). In drinks, caffeine averages about 40 mg in a 12 oz. can of soda, with a range of 30-60mg (Carrillo and Benitez 2000, Ferreira et al. 2005). Caffeine in coffee varies with brewing method, type of coffee bean, quantity brewed, serving size and portion of drink consumed. It can be as high as 92-120mg caffeine/serving (Grosso and Bracken 2005). In the United States, average consumption of coffee is over 150mg/day (Grosso and Bracken 2005). Additionally, caffeine is found in over-the-counter medications, such as Anacin and Excedrin at concentrations ranging from 30-200mg per tablet (Ferreira et al. 2005). It is estimated that over 80% of adults in North America are caffeine consumers, with an estimated average intake of 200-250mg/day (Carrillo and Benitez 2000). When this intake is corrected for an average bodyweight, average intake is 2.5mg/kg/day for adults and 1mg/kg/day for children under 18 (Carrillo and Benitez 2000).

Caffeine is efficiently metabolized by the human body with only 0.5-10% unmetabolized caffeine excreted (Buerge et al. 2003b) and an average half-life in the body of 3-6 hours (Carrillo and Benitez 2000). After oral ingestion, caffeine reaches its maximum concentration in the blood after approximately one hour. Because of its hydrophobic nature, caffeine crosses all membranes and is found in blood, saliva, human

milk and feces. Bonati et al. (1982) tested dosing and elimination rates of caffeine exposure in four men and determined an absorption rate constant of $6.3\pm1.9h^{-1}$ and elimination rate constant of $0.11\pm0.02h^{-1}$. The absorption rate was found to increase with increasing dose of caffeine. Standley et al. (2000) found that after drinking coffee containing 170mg/L caffeine, 3mg/L was excreted in urine.

Despite its efficient human metabolism, caffeine is detectable in wastewaters. Its presence in wastewaters is mainly due to the washing of residual coffee granules, rinsing of pots, or pouring liquid coffee or tea down the drain (Gardinali and Zhao 2002). The abundance of caffeine and its high consistent consumption rates make it a good potential indicator of wastewater contamination.

1.4.1.3 Occurrence

Caffeine has been detected in many occurrence studies, including in samples collected upstream of WWTP discharges (see Table 1.1). In a recent study, Glassmeyer et al. (2005) detected caffeine in 70% of samples, down to a reporting limit of 0.016µg/L. In their study, caffeine was detected in 63% of upstream samples, 73% of wastewater treatment plant effluents, 80% of downstream samples close to WWTPs, and 60% of sampling locations farther downstream. However, there was insufficient information about the proximity of upstream sampling to WWTPs to determine whether upstream locations were pristine. Bendz et al. (2005) detected caffeine upstream of a WWTP but at levels below 5ng/L. In their study of a Brazilian watershed, Ferreira et al. (2005) detected caffeine in all river samples ranging from 130-350µg/L, although they do not indicate proximity of sampling locations to wastewater treatment plant discharges.

Conventional wastewater treatment results in a high removal rate of caffeine. Bendz et al. (2005) calculated the overall removal efficiency for caffeine at 89%. In a survey of sixteen WWTPs in Switzerland, Buerge et al. (2003a) found an average caffeine removal efficiency of 99.6±0.2%. Thomas and Foster (2005) found that aerobic and anaerobic degradation was responsible for the majority (51-99%) of caffeine degradation. Up to 44% of the compound removal was found to occur in primary treatment, when suspended solids settle out. However, even with these high removal rates, caffeine has been detected in WWTP effluent. Weigel et al. (2004) detected caffeine in effluents from four WWTPs that used manual filtration without biological treatment: levels ranged from 30-120µg/L. Stackelberg et al. (2004) detected caffeine in 100% of samples collected downstream of WWTP discharge, all at levels above the reporting limit of 0.014µg/L.

Once in the environment, photolysis and photo-degradation are the main degradation mechanisms by which caffeine is degraded. In batch experiments where samples were exposed to natural sunlight, caffeine was degraded following first-order decay with a half-life of about 12 days: sorption to particles in the surface water was found to be an insignificant removal factor (Buerge et al. 2003a). Ogunseitan (2002) suggested that it is rare to find microorganisms capable of degrading caffeine in freshwater systems.

Godfrey and Woessner (2004) identified caffeine in community and individual household septic tanks. Caffeine concentrations in community systems were detected at levels as high as 500µg/L and in individual household systems in concentrations as high as 400µg/L. Wren (2001) collected septic tank effluent grab samples from a multi-

housing unit and a condominium complex and detected caffeine in the tank effluents at 137 and 95mg/L, respectively. However, these values represent the results of isolated individual samplings and may not represent average septic tank effluent concentrations.

Seiler et al. (1999) detected caffeine in groundwater wells in combination with nitrate, which they concluded stemmed from septic system pollution. In another study of groundwater contamination, Verstraeten et al. (2005) detected caffeine in 9 out of 19 groundwater wells tested, but the concentrations were not specified.

Table 1.1 Occurrence data for caffeine and triclosan from the literature

Location	Caffeine Concentration Range	References	Triclosan Concentration Range	References
WWTPs, influent	20µg/L 7-50µg/L	(Siegener and Chen 2002, Buerge et al. 2003a)	500-1300ng/L 3.8-16.6µg/L 70-650ng/L	(Lindstrom et al. 2002, Sabaliunas et al. 2003)
WWTPs, effluent	0.06-0.08 µg/L 6.7µg/L 28-9480ng/L	(Seiler et al. 1999, Siegener and Chen 2002)	42-213ng/L 0.18µg/L	(Singer et al. 2002, Paxeus 2004)
Rivers and streams	<0.014-6µg/L 160-357µg/L <0.014-115 ng/L 13+/- 28 ng/L	(Standley et al. 2000, Kolpin et al. 2002, Ferreira et al. 2005)	1.4-74ng/L	(Lindstrom et al. 2002)
Septic tanks	95-137mg/L 0.0014-1.008mg/L	(Wren 2001, Godfrey and Woessner 2004)	3.4-6.4mg/L	(Wren 2001)

1.4.1.4 Caffeine's Potential as a Tracer

Although common in the environment, caffeine is considered an effective indicator of urban pollution for several reasons. Because it is not naturally present in the environment, the presence of any caffeine indicates anthropogenic influences. In addition, its low sorption potential to solids (Seiler et al. 1999) and low degradation under anaerobic conditions suggest that caffeine is more likely to survive septic tank treatment than conventional WWTP processes.

Caffeine has been used as an indicator of pollution from various sources. Standley et al. (2000) proposed its use in combination with fragrances (HHCB and AHTN) as an indicator of WWTP effluent, because the detection of these compounds in series correlated with WWTP discharges in the watershed. In contrast, Buerge et al. (2003a) concluded that caffeine was source-specific from domestic wastewater. Because of WWTP's high removal efficiency of caffeine, they determined that caffeine concentrations in the environment were linked to high rainfall events when storm water flooded WWTPs and untreated effluent was discharged in the environment. Seiler et al. (1999) proposed that the presence of caffeine in groundwater samples indicated septic system pollution.

Although caffeine has been linked to domestic wastewater, identification of its specific source is difficult. Occurrence data on caffeine concentrations in WWTP effluents and surface water are variable, depending on the type of WWTP sampled and the time and location of sampling. Caffeine, in this study, is proposed as an indicator of septic system pollution because of its high removal rates from WWTP, its low sorption rates, and the fact that aerobic degradation as the primary removal mechanism. Because of these properties, it is hypothesized that minimal caffeine degradation will occur within the septic tank and that caffeine concentration from the effluent of a failed septic system will be detectable downstream.

1.4.1.5 Toxicology/ Public Health Significance

Little research has been conducted into the environmental impact of caffeine at sub-therapeutic levels, such as those found in the environment, except for a few studies

on amphibians. Smith and Burgett (2005) tested the effect of high concentrations of caffeine on American Toad tadpoles and found no significant change in tadpole activity with exposure to caffeine. However, tadpoles exposed to caffeine had higher levels of developmental anomalies, and they hypothesized that exposure to sub-lethal doses could affect predation, survivorship, and reproduction. In another study, Fraker and Smith (2004) found that caffeine, even at low environmental doses, affected tadpole activity and the startle response, which could affect their survivor.

1.4.1.6 Analytical Methods

There are several methods for the analysis of caffeine in surface and wastewaters described in the literature. Most researchers use some form of extraction, either solid phase (SPE) with differing cartridges or liquid-liquid (LLE) combined with liquid chromatography-mass spectrometry (LC-MS) or gas chromatography-mass spectrometry (GC-MS) analysis. Standley et al. (2000) extracted drinking water influent samples with C-18 cartridges eluted with CH_2Cl_2 and CH_3OH. Extracts were derivatized with N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) and analyzed by GC-MS for a recovery rate of 82%. Ferreira et al. (2005) used CH_2Cl_2 with LLE analysis by high performance liquid chromatography (HPLC) with a variable ultraviolet detector for extraction of surface water samples. Buerge et al. (2003a) extracted wastewater and river samples with Bio-Beads eluted with CH_2Cl_2, passed over silica mini-columns for clean-up, eluted with 95:5 ethyl acetate (EtOAc):methanol (MeOH) and analyzed using gas chromatograph-mass spectrometry-single ion monitoring (GC-MS-SIM) for a limit of

detection (LOD) of approximately 10ng/L. The elution solvents for the above methods are toxic and are, therefore, not considered as a reasonable option for this current study.

Eaton et al. (2004) used hydrophilic-lipophilic balanced (HLB) cartridges eluted with acidic methanol to separate an extract, which was injected onto an HPLC-UV for detection of caffeine from wastewater samples but found that this method was not sensitive enough because of the complexity of the matrix. Chen et al. (2002) used on-line SPE extraction of surface water samples followed by analysis with HPLC using individual test columns: C-18, poly(styrene-divinylbenzene) (PRP-1), polymeric reversed phase (PLRP-s) or Bond-Elut Env. Recovery rates ranged from 84 to 98%, with PLRP-s yielding the highest recovery. Positive electrospray ionization (ESI+) mode of LC-MS was used for analyte detection with a LOD of 0.1µg/L. Gardinali and Zhao (2002) used LLE with CH_2Cl_2 to extract caffeine from 1L surface water samples: they analyzed these samples using LC-MS, yielding 89% recovery and LOD of 4ng/L. Thomas and Foster (2004) used Oasis HLB cartridges eluted with ETOAc to extract wastewater. The extracts were derivatized with N,N-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) in an oven at 60°C and analyzed using gas chromatograph-mass selective detector (GC-MSD) yielding only 34% recovery for caffeine with a method detection limit (MDL) of 19ng/L.

1.4.2 Triclosan

1.4.2.1 Physical Properties

Triclosan (Figure 1.5) has a relatively high K_{ow}, indicating that it is likely to sorb to particles once in the environment. Because its pK_a of 8.1 is in the pH range of natural water (7-9), the specific pH of the water will influence speciation and less sorption will occur at higher pH values. Triclosan is stable against hydrolysis. For further properties, see Appendix 1.

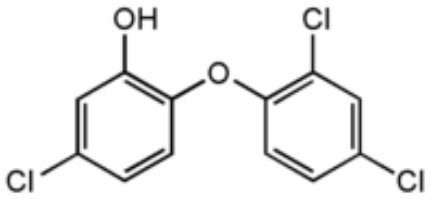

Figure 1. 5 Triclosan structure.

1.4.2.2 Usage

Triclosan is present as an anti-microbial agent in many personal care products; such as toothpaste, shampoo, mouthwash, deodorants, skin care products, lotions, and hand soaps; at weight-based concentrations ranging from 0.1-0.3% (Sabaliunas et al. 2003). Despite the low concentration, these products are washed down the drain and enter the waste stream. In addition, triclosan is found in plastic cutting boards and sports apparel, such as underwear, socks and shoes (Bester 2003).

1.4.2.3 Occurrence

Triclosan has been detected in a wide range of environmental samples (see Table 1.1). Glassmeyer et al. (2005) detected triclosan in 62.5% of samples collected around WWTPs: 11% of upstream samples, 100% of WWTP effluents, 70% of initial downstream

samples, and 60% of samples found farther downstream. However, there was no indication whether the upstream sampling sites were themselves downstream of another WWTP.

Lindstrom et al. (2002) detected triclosan, ranging from 0.5-1.3µg/L, in the influents of all WWTPs sampled during research in Switzerland. Normalizing these concentrations for the populations served by these plants, they determined an average of 0.5mg/person/day, which is equivalent to approximately 0.2g/person/year.

Triclosan has been found to be well removed during conventional wastewater treatment. In general, triclosan removal is over 90%. McAvoy et al. (2002b) found 96% removal from activated sludge plants, 71% for trickling filter plants, and 32% for primary treatment plants. Sabaliunas et al. (2003) measured over 95% removal of triclosan through both activated sludge and advanced trickling filter WWTPs.

Bester et al. (2003) looked at the removal processes of triclosan within the WWTP. They found that 65% of triclosan in the influent "disappeared" (i.e., was not detected in the sludge or effluent water). About 5% of triclosan was found to be released through hydrolysis processes within the WWTP, 30% of triclosan was sorbed to sludge, and 50% of triclosan was found to be transformed into unknown metabolites or bound to residues. Singer et al. (2002) determined that a large proportion of the triclosan degradation was due to aerobic degradation and sorption to sludges. Federle et al. (2002) found triclosan to be mineralized in activated sludge.

Even though triclosan is an antibiotic, at low levels of concentration small microbial communities degrade this chemical. With a gradual increase in exposures to triclsoan at higher concentrations, the ability of the microbial community to degrade triclosan also

increases. This hypothesis was proven with the detection of triclosan resistant bacteria in the environment that were able to co-metabolize triclosan (Meade et al. 2001).

Despite high levels of removal from WWTP, triclosan has been detected in WWTP effluents in concentrations ranging from 42-213ng/L (Singer et al. 2002). Once in the environment, there are many removal processes for triclosan, such as sorption or transport into sediments, chemical or biological degradation, volatilization or photolysis (Lindstrom et al. 2002). Lindstrom et al. (2002) used laboratory data to model triclosan degradration in the environment and determined there would be high seasonal variability in rates of photolysis. Sabaliunas et al. (2003) measured triclosan at several points downstream of WWTP effluents and found a die-away rate of 0.21±0.08ng/L. Triclosan was still detected 3.5km downstream, with an estimated travel time of 5.2 hours. Using a computer model, they found average removal rates of triclosan were 0.06-0.33h^{-1}.

Morrall et al. (2004) studied triclosan degradation in rivers. They found first-order loss rate of triclosan to be 0.06h^{-1}, corresponding to a half-life of 11 hours. In an 8km reach of stream, they measured a 19% loss of triclosan to sorption and settling. This study showed a possible half-life of triclosan in surface waters of 15 minutes due to photolysis from direct sunlight. However, rates of photolysis are dependent on many variables, such as pH, suspended solids, turbulence, river depth and intensity of sunlight, which is affected by season and weather (Sabaliunas et al. 2003).

McAvoy et al. (2002b) developed a mathematical model to predict the fate of analytes found in products disposed of down the drain into a septic system. The study assumed that the compounds were water soluble at concentrations used by consumers. The model predicted that under unsaturated conditions, analytes would sorb or biodegrade. Sorption

was the main removal mechanism in the near field, but biodegradation became more important in the far field (McAvoy 2002a). However, under saturated conditions, analytes were expected to reach the groundwater because of the shift in the soil environment to oxygen-limited and anoxic conditions, thereby, limiting degradation. The implications are that most of the sorption and degradation occur in the drainfield, under aerobic conditions. If a septic system fails, triclosan emerging from the tank is expected to be at similar concentrations to those found in influent.

1.4.2.4 Toxicology/ Public Health Significance

The effects of triclosan in the environment are only beginning to be understood. Triclosan has been found in human breast milk, up to levels of 300µg/L, although the effects of this concentration have not been studied (Adolfsson-Erici et al. 2002). Triclosan is acutely toxic to many aquatic organisms. For example, the concentration where 50% of the effects are observed (EC_{50}) for rainbow trout is 350µg/L (Lindstrom et al. 2002). Triclosan in the environment has been shown to be toxic to certain algal species, such as *Scenedesmus subspicatus*, which has a no-observed effects level (NOEL) of 500ng/L (Singer et al. 2002). Adolfsson-Erici et al. (2002) measured levels of triclosan in the bile of fish exposed to WWTP effluent and found concentrations of triclosan up to 120mg/kg of bile.

Triclosan has the potential to cause microbial resistance to antibiotics. It blocks lipid biosynthesis by inhibiting the enzyme enoyl-acyl carrier protein reductase (Singer et al. 2002). With larger exposure and concentrations of triclosan in the environment, microbial resistance can build up and reduce efficacy of antibiotic soaps and drugs in killing disease organisms (Rooklidge 2004).

1.4.2.5 Analytical Methods

Most methods in the literature use SPE to concentrate triclosan from aqueous samples with analysis by GC-MS. Thomas and Foster (2004) used Oasis HLB cartridges eluted with ETOAc for extraction of wastewater samples, with the extracts being derivatized with BSTFA in a heated oven and analyzed by GC-MS with 89% recovery. Lindstrom et al. (2002) extracted surface water samples with bio-beads to concentrate triclosan, which was then eluted with MeOH/CH_2Cl_2 and subsequently analyzed by GC-MS with LOD of 0.4ng/L. Wastewater samples extracted with bio-beads were eluted with 50:50 MeOH:acetone onto silica gel cartridges for clean up. They eluted silica gel cartridges with 95:5 EtOAc:MeOH and analyzed extracts using LC-ESI-MS with LOD of <2ng/L. Boyd et al. (2004) used SDB-XC Empore disks (Varian, Palo Alto CA) to extract surface water and eluted the extract with MeOH and CH_2Cl_2 followed by silica gel clean up and GC-MS analysis. The MDL without the silica gel cleanup was 0.2ng/L. McAvoy et al. (2002b) developed a method, which was later used by other researchers, of extracting surface and wastewater with C18 cartridges eluted with ETOAc, toluene, and hexane; these extracts were subsequently concentrated and analyzed by GC-MS, yielding 79% recovery from WWTP influent (Sabaliunas et al. 2003, Morrall et al. 2004). Paxeus (2004) developed a method for extraction of surface and wastewater that used C18 SPE cartridges eluted with acetone and methylated with methyl chloroformate before GC-MS analysis. In contrast, Bester (2003) extracted wastewater using LLE with toluene, which was analyzed directly using GC-MS for 88% recovery with a limit of quantification (LOQ), the lowest level quantifiable from the method, of 3ng/L.

1.4.3 Haloacetic Acids

1.4.3.1 Chemical Parameters

Haloacetic acids (HAAs) are strongly acidic and hydrophilic with pKa values ranging from 0.63-2.9 (Loos and Barcelo 2001). For this study, only two HAAs, dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA), as depicted in Figures 1.6 and 1.7, were targeted as by-products of WWTP chlorination because of the higher likelihood of their formation. Low values of K_{ow} indicate that these compounds are unlikely to sorb to particles, and therefore, would be detected downstream. For additional chemical parameters, see Appendix 1.

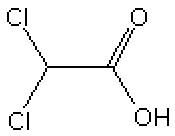

Figure 1.0.6 Dichloroacetic acid structure.

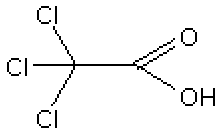

Figure 1.0.7 Trichloroacetic acid structure.

1.4.3.2 Occurrence

DBPs are formed by the interaction of chlorine and organic matter in waters. Hypochlorous acid is a common disinfectant in water and wastewater treatment plants and is formed after the addition of sodium or calcium hypochlorite or gaseous chlorine to the water. During chlorination, natural humic and fulvic compounds, as well as wastewater-derived compounds, are transformed into DBPs (Westerhoff et al. 2004).

Krasner et al. (2005) detected DBPs formed from chlorination of wastewater effluent. The level of ammonia in the effluent has a significant influence on the formation of DBPs. Chlorine first interacts with ammonia to create combined chlorines (chloramines) and then with natural organic matter to form DBPs. High ammonia levels result in lower concentrations of some halogenated DBPs, but can generate higher levels of nitrosamines. Low levels of HAAs were detected in the effluents examined, but all the treatment plants had high levels of ammonia, which likely limited their formation.

Most research on DBP formation and biodegradation has been performed in drinking water; however, these results can be extrapolated to surface water. Sirivedhin et al. (2005) studied the disinfection by-product formation potential (DBPFP) of various waters. Their study found that formation of DBPs was more likely in waters with an anthropogenic influence, caused by the high levels of dissolved organic carbon (DOC). The DCAA concentrations, for example, were higher in anthropogenically influenced waters than in pristine waters. This implies that the high levels of DOC in wastewater effluent (relative to drinking water) are likely to contribute to a high DBPFP.

In studies of drinking water treatment plants, HAAs formed more quickly than trihalomethanes (THMs) upon reaction with chlorine, and therefore are more likely to form during water treatment (Speight and Singer 2005). Given the relatively short time between chlorine addition and dechlorination in wastewater treatment, HAAs are also likely to be found in the treated water, provided there is a free chlorine residual. Hence, HAAs may be potential indicators of WWTP effluent.

HAAs have been detected in environmental samples. Scott et al. (2000) detected HAAs in lake waters in North America. Concentrations of DCAA ranged from <1-1500ng/L

and TCAA levels ranged from <1-37ng/L, with higher concentrations in lakes in closer proximity to urban populations. Their study also found HAAs in precipitation samples in Canada; DCAA was detected in the range of <0.4-7300ng/L and TCAA was detected in the range of <0.4-870ng/L.

Calculations were performed using EPISuite software for an estimation of biodegradation and environmental persistence. SMILES notation, generated from the chemical structure created in ChemSketch was imported, along with water solubility and melting point (www.chemfinder.com). DCAA biodegradation was estimated to occur on the order of weeks, whereas TCAA was estimated to biodegrade over weeks to months. Total estimated removal from WWTP for DCAA was 1.9%. Of this amount, biodegradation accounts for 0.09%, sludge adsorption for 1.79%, and volatilization for 0.02%. It was also estimated that the half-life of DCAA in a model river was 79 days. Total removal for TCAA was 1.93%, of which 0.09% comes from biodegradation, 1.84% from sludge adsorption, and there was no volatization. The estimated half life of TCAA in the river was 2310 days.

1.4.3.3 Toxicology/ Public Health Significance

DBPs in drinking water are considered carcinogenic, mutagenic, and teratogenic to both humans and animals (Sirivedhin and Gray 2005). The USEPA has set a maximum contaminant level for the sum of the five regulated HAAs at 60µg/L. Because HAAs are unlikely to sorb to particles and have a low biodegradation rate, it is likely that DBPs in WWTP effluent will be found downstream, possibly in drinking water treatment plant influents. Hanson and Solomon (2004) determined that HAAs at current environmental concentrations do not pose a threat to aquatic vegetation using both probabilistic and

deterministic methods. Investigating toxicity to phytoplankton found that monochloroacetic acid (MCAA) was the most toxic of the HAAs, but that other species had the potential for toxicity.

1.4.3.4 Analytical Methods

The most common method for the detection of HAAs in water is EPA Method 552.2 (Urbansky 2000), which uses liquid-liquid extraction (LLE), with methyl-tert-butyl ether (MtBE) as the organic phase, from an acidified sample. Diazomethane, which is able to convert acids to esters, is often used as the methylating agent (Urbansky 2000). The methylation reaction is shown in Figure 1.8. This method may be less effective for more complex matrices.

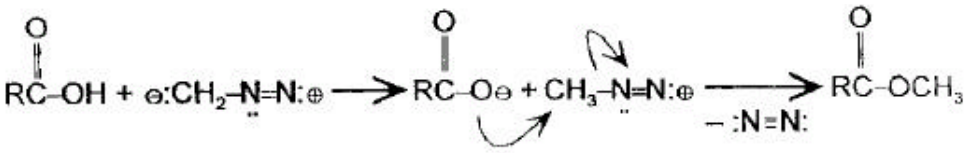

Figure 1.8 Mechanism for methylation of a carboxylic acid with diazomethane (Urbansky 2000).

Additionally, there are several methods used to concentrate HAAs using SPE. Martinez et al. (1998) compared the effectiveness of four different SPE cartridges for concentration of HAAs with a range of sample volumes. They acidified samples to below pH 0.5 and conditioned cartridges with 5mL MeOH then 5mL LGW at pH 0.5. After samples were passed, the cartridges were washed with LGW at pH 0.5 and eluted with 2mL 50:50 v:v MeOH:LGW. Researchers compared SAX-LiChrolut EN, LiChrolut EN, Envi-Carb, Oasis-HLB solid phase with LLE. LiChrolut EN was found to be the most effective at recovering analytes, especially DCAA and TCAA. For 100mL sample, recovery of DCAA

and TCAA was 94 and 95% from LiChrolut EN, 65 and 78% from HLB, and 70 and 80% from LLE.

Loos and Barcelo (2001) also compared the efficiency of SPE cartridges in concentrating HAAs. Samples were acidified to pH 1.8 and cartridges were conditioned with 5mL MeOH then 3mL LGW at pH 2.5. After samples were extracted the cartridges were washed with 1mL LGW at pH 2.5 and eluted with a 4mL mixture of 0.5mL LGW + 3.5mL MeOH:acetone (1:1, v:v). Fifty mL samples were tested using LiChrolut EN, Isolute ENV+, and Oasis HLB cartridges. Recovery for DCAA and TCAA were 55 and 75%, respectively, from LiChrolut EN and 42 and 51% from HLB.

Yoo et al. (1992) used C18 disks for the concentration of HAAs, and showed 71% recovery of DCAA from 200mL samples when two disks were used in series. From 100mL of sample using two C18 disks in series, DCAA recovery was 83% and TCAA recovery was 95%. Sample pH was adjusted below 1 and cartridges were washed with 10mL MtBE, 10mL MeOH, and two rinses of 10mL LGW. After passing the sample, the cartridges were individually eluted with 10mL of MtBE.

1.5 Fluorescence spectroscopy

Fluorescence spectroscopy is an emerging tool in water quality management, particularly because of its potential for real-time analysis. Water samples exhibit unique fluorescence patterns; therefore, excitation and emission spectral data can be used to provide both intensity data and an indication of the water source. Samples can be analyzed on-line, yielding results in minutes.

Approximately 40-60% of natural organic matter is fluorescent mainly because of the presence of proteins and organic acids (Baker 2001). When these molecules are excited, such as with a high energy light source, they release energy in the form of light, which can be measured to provide a fluorescence excitation-emission matrix (EEM). Common zones are characterized by general types of fluorescent material: aromatic compounds, described as humic and fulvic substances, and aromatic proteins (mainly tryptophan and tyrosine). An explanation of general fluorescence regions and wavelengths is shown in Table 1.2 and in Figure 1.9 and 1.10. Tryptophan shows maximum fluorescence around an excitation wavelength of 280nm and an emission wavelength of 360nm. Tyrosine shows a maximum at 275nm excitation, and 305nm emission. Fulvic-like molecules fluoresce with peak maximum at 320-340nm excitation and 410-430nm emission, whereas humic-like molecules fluoresce at 370-390nm excitation and 460-480nm emission.

Water types are distinguishable by the intensity of various types of fluorescence. Sewage samples show high intensity around the tryptophan area and to a lesser extent, the fulvic-like area, expressed as a broad fluorescence band at 350nm and two less intense bands at 390 and 430nm (Reynolds 2003). In comparison, samples collected after aerobic digestion have a reduced signal around 350nm. Comparing water samples collected throughout a catchment area, samples with high tryptophan-like intensity correlated with poor chemical water quality parameters and urban streams polluted by wastewater (Baker and Inverarity 2004).

Baker et al. (2003) collected monthly river samples for a reconnaissance study of water quality using fluorescence spectroscopy. They found that incidents of above- average tryptophan intensity correlated with sewerage overflow and the release of untreated sewerage

into the stream. In a separate study, Baker (2001) collected water samples up- and downstream of WWTPs. There was a significant difference in the intensity and variability of tryptophan fluorescence between these locations, characterized by tryptophan intensity and the tryptophan/fulvic ratio. Fluorescence, therefore, has potential as an indicator and monitoring tool to differentiate between point and NPS pollution.

Table 1.2 Description and location of fluorescence regional integration areas (Chen et al. 2003)

	Organic matter type	excitation wavelength (nm)	emission wavelength (nm)
Region I	Aromatic proteins	230-260	300-340
Region II	Aromatic proteins	230-260	340-380
Region III	Fulvic acids	230-260	380-500
Region IV	Soluble microbial products	260-400	300-380
Region V	Humic acids	260-400	380-500

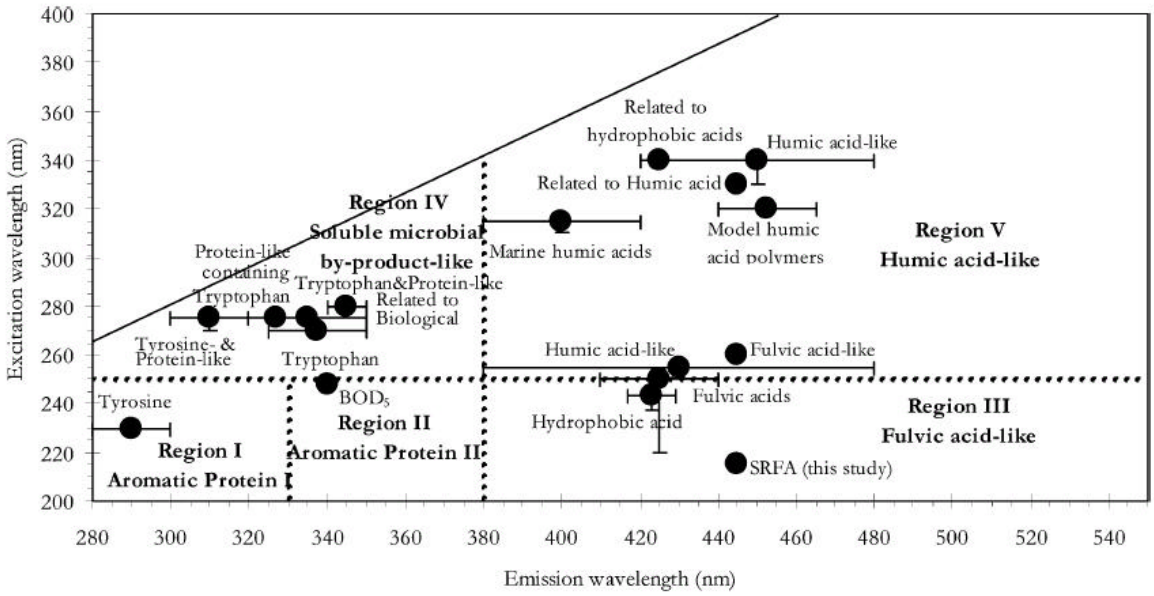

Figure 1.9 Location of EEM peaks (circles) based on literature reports and excitation- emission wavelength boundaries (dotted lines) for five EEM regions (Chen et al. 2003).

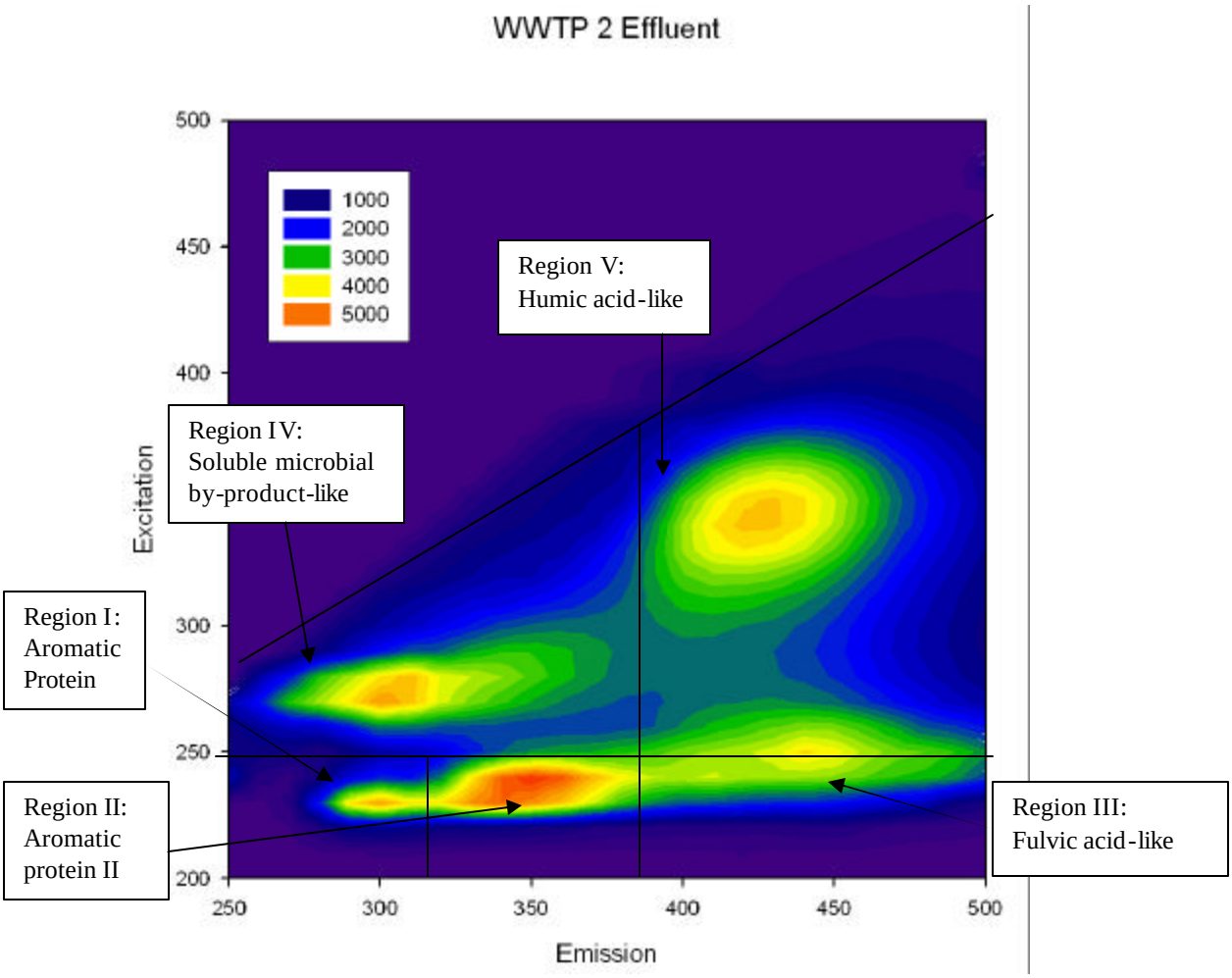

Figure 1.10 Example EEM from WWTP2 effluent showing fluorescence regions.

1.6 Objectives of Research

Currently, state and national monitoring programs can identify impaired waterways, but cannot specifically determine pollution sources, which limits these programs' usefulness in remediation and improving water quality. This deficiency is particularly apparent with septic system pollution. Homeowners are responsible for maintenance of their septic systems, but often do not understand their functioning and are unable to perform proper upkeep. The high percentage of septic systems in North Carolina and throughout the United States and their high failure rate combine to create a great pollution potential. A rapid

indicator of such pollution would have the ability to monitor changes in water quality, prevent long-term septic system impairment, and potentially help identify the source of pollution.

Additionally, little research has been conducted on concentrations of analytes in septic systems. Use of an indicator as a tracer of pollution through the system will give an indication of the potential ability of septic systems to degrade these compounds.

The objectives of this study were to identify potential chemical indicators to differentiate between point and non-point source pollution in the environment. The tools will facilitate for more efficient remediation and protection of water quality, with all its ramifications. This work is the beginning of a larger study to differentiate between point and non-point sources of pollution in the aquatic environment.

CHAPTER 2: MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals and SPE Resins

Acetonitrile (ACN, Optima HPLC grade), ethyl acetate (ETOAc, HPLC grade), methanol (MeOH, HPLC grade), sulfuric acid (ACS grade), and potassium hydroxide were purchased from Fisher Scientific (Pittsburgh, PA). Acetone (HPLC grade) was procured from Burdick and Jackson (Morristown, New Jersey), while methyl tert-butyl ether (MtBE, Ultra-Resi Analyzed grade), silicic acid, sodium acetate, and sodium azide were from J.T. Baker (Phillipsburg, NJ). Hexane (95%+, HPLC grade), diazald, anhydrous magnesium sulfate, 1,2-dibromopropane neat standard, citric acid monohydrate, perfluorotributylamine (PFTBA), and carbitol (diethylene glycol monethyl ether) were obtained from Sigma-Aldrich (St. Louis, MO). N,N-Bis(trimethylsilyl)trifluoroacetamide (BSTFA + 1% TMCS), hexachlorobenzene (HCB) neat standard, haloacetic acids 9 (HAA9) Mix 552, and the haloester EPA 552.2 ester calibration mix were purchased from Supelco (Bellefonte, PA). Sodium hydroxide pellets and pyridine were from Mallinckrodt (Paris, KT). Caffeine neat standard, triclosan neat standard, quinine hemisulfate salt monohydrate purum (98+%), and sulfuric acid (95+%) were from Fluka (St. Louis, MO). Triclosan deuterated standard (triclosan-d_3) was purchased from Toronto Research Chemicals (North York, Canada) while deuterated dichloroacetic acid-d_2 (DCAA-d2) and deuterated caffeine (caffeine-d_3; 1-methyl-d_3) were obtained from CDN Isotopes (Pointe-Claire, Canada). Laboratory grade water (LGW) was prepared in-house from a Pure Water Solutions (Hillsborough, NC) system,

which pre-filters chloraminated tap water to 1µm, removes residual disinfectant, reduces total organic carbon to less than 0.2 ppm with an activated carbon resin, and removes ions to 18 MO with mixed bed ion-exchange resins.

Stock solutions were prepared for caffeine, caffeine-d_3, and triclosan at 1g/L by weighing the standards into Fisher brand plastic weigh boats (1.5" by 1") or directly into a volumetric flask using Fisher Scientific Balance (accu124D dual range). The weigh boat was rinsed into the volumetric flask with solvent and the flask was then filled to the mark with the same high purity solvent. Stock solutions were stored in amber vials for several months in a freezer at -15°C. HAAs, triclosan-d_3 and dichloroacetic acid-d_2 stock solutions were prepared by dilution from neat standards and were stored in amber vials in the freezer. Primary dilutions and working standards were prepared by dilution of stock standards and were stored in amber vials in the freezer for up to four months. A working internal standard of 12.8mg/L HCB was prepared in 5mL pyridine and stored for up to four weeks in an amber vial in the freezer.

Several types of SPE cartridges were utilized; 200mg/6cc Strata X cartridges (Phenomenex, Torrence, CA), Oasis HLB cartridges, both 200mg/6cc and 60mg/3cc sizes (Waters, Milford, MA), C-18 cartridges 500mg/6cc (Alltech, Deerfield, IL), SAX Bond Elute cartridges 500mg/6cc (Varian, Palo Alto, CA), silica gel cartridges SupelClean Si 200mg/3mL (Supelco, Bellefonte, PA) and Strata Si 200mg/3mL (Phenomenex, Torrence, CA).

All glassware was detergent washed, followed by 3x LGW rinses, soaked in a 10% nitric acid bath for >4 hours, final LGW rinse (3x), then a 3x wash with MeOH for

volumetric glassware or drying in an oven at 110°C for non-volumetic glassware. PFTE tubing was washed with MeOH and LGW.

2.1.2 Instrumentation

Gas chromatographic analysis was performed on a Varian 3800 GC with Saturn 2000 ion trap MS, a Hewlett Packard (Palo Alto, CA) HP5890 GC Series II with HP5972 quadrupole mass selective detector (MSD), or HP5890 GC Series II with an electron capture detector (ECD). The GC columns were either Varian (Palo Alto, CA) Factor-Four, VF-5ms/DB5-ms, 30m x 0.25mm with 0.25µm film thickness or J & W Scientific (Folsom, CA) DB1 30m x 0.30mm with 1.0µm film thickness.

2.1.2.1 Ion Trap MS

A Varian 8200 autosampler injected 1µL of sample extract through a Varian 1079 injection port fitted with a deactivated glass SPI liner (Restek, Bellefonte, PA). The carrier gas was UHP Helium set at a constant flow rate of 1.5mL/min. The injection port temperature was held at 60°C for 0.1 minutes and then was ramped at 200°C/minute to 250°C for analysis by an ion trap mass spectrometer in electron ionization (EI) mode.

After a 5 minute solvent delay, the trap was set to run in EI mode with axial modulation voltage set at 4.0v, emission current at 10µamps, the scan range from mass-to-charge ratio (m/z) of 65 to 550, and automatic gain control (AGC) on. The oven program for caffeine and triclosan was adapted from the method of Ollers et al. (2001), which started at 90°C, held for 1 minute, then ramped at 15°C/min until 150°C, and held at 150°C for 15 minutes. The temperature was then ramped at 5°C/min until 200°C, held at 200°C for 5 minutes and then ramped at 15°C/min to 290°C where it was held for 6 minutes. An

additional GC method utilized was adapted from a method by Thomas and Foster (2004), with initial temperature at 60°C held for 2 minutes and then ramped 5°C/min to 290°C, where it was held for 6 minutes.

The instrument settings remained the same as above when using chemical ionization (CI) mode to analyze for HAAs. Injection port temperature started at 60°C for 0.1 minute, was increased to 150°C at a rate of 100°C/min and held at 150°C for the duration of the analysis. Initial oven temperature was 37°C held for 21 minutes, ramped to 136°C at 20°C/min and held at 136°C for 1 minute before ramping to 250°C at 25°C/min where the temperature was held for 4 minutes. Data were analyzed using the Varian MS Workstation software v. 6.41. Ion chromatograms were extracted for targeted ions and used for quantification with a minimum chromatographic peak signal to noise of 10 and a minimum peak area of 1000 counts.

2.1.2.2 GC- ECD

A Hewlett Packard 6890 autosampler injected 1µL of sample extract through an injector port fitted with split/splitless injector sleeve, containing deactivated glass wool of 4mm inner diameter (Supelco, Bellefonte, PA). The carrier gas was UHP Helium set at a flow rate of 1.0-1.5mL/min. The make-up gas was UHP Nitrogen set at a flow rate of 50mL/min. The injector port temperature was held constant at 180°C.

The oven program for detection of HAAs used an initial temperature of 37°C which was held for 21 minutes before ramping to 136°C at a rate of 5°C/min, this temperature was held at 136°C for 3 minutes, before a final ramp to 250°C at a rate of 20°C/min when the temperature was held for 3 minutes.

2.1.2.3 GC- MSD

A Hewlett Packard 7673 autosampler injected 1µL of sample extract through an injector port fitted with split/splitless injector sleeve containing deactivated glass wool of 4mm inner diameter (Supelco, Bellefonte, PA). The carrier gas was UHP Helium set at a constant flow rate of 1.0-1.5mL/min. The injector port was held constant at either 180° (for HAAs) or 250°C (for caffeine and triclosan).

After a 4 (for caffeine and triclosan) or 6.5 (for HAAs) minute solvent delay, single ions were targeted for monitoring. Electron multiplier (EM) voltages ranged from 1940 to 2025V during this study. The quadrupole temperature was set at 165°C. Perfluorotributylamine (PFTBA) was used for internal calibration during daily tuning methods. The data were processed using an older version of Hewlett Packard (HP) Chemstation (B.02.02) with data analysis G1034C Version C.01.05.

Oven temperature for caffeine and triclosan was based on the method of Thomas and Foster (2004). Inlet temperature was 250°C and detector at 300°C. Initial oven temperature was 60°C held for 2 minutes, ramped to 230°C at a rate of 5°C/min, ramped to 290°C at a rate of 15°C/min and held for 6 minutes. Hexachlorobenzene (HCB) was targeted by selecting the fragment ion at m/z 184, immediately after the 4 minute solvent delay. Concurrently, caffeine was targeted through its fragment ion at m/z 194 and deuterated caffeine at m/z 197, beginning at 26 minutes into the run. Triclosan at m/z 347, and deuterated triclosan at m/z 352 were simultaneously targeted from 32 minutes into the run.

The HAA program has a 6.5 minute solvent delay. Injector temperature was held constant at 180°C. Initial oven temperature was 37°C held for 3 minutes, raised to 112°C at a

rate of 5°C/min, and held at 112°C for 2 minutes. The temperature was then ramped to 250°C at a rate of 20°C/min and held for 3 minutes. During the same time segments, dichloroacetic acid (DCAA), with fragment ions at m/z 83 and 85, and DCAA-d2, with fragment ions at m/z 84 and 86, were targeted from 6 minutes. TCAA, with fragment ions at m/z 117 and 119, was targeted from 8.5 minutes and internal standard, 1,2-dibromopropane, with fragment ions 121 and 123, was targeted from 7.6 minutes.

2.1.2.4 Fluorescence

A Hitachi F-4500 Fluorescence Spectrophotometer (Parsippany, NJ) with a xenon lamp was used to generate excitation-emission matrices (EEMs) for filtered aqueous samples. A 5cm slit was opened as the excitation scanned from 200-900nm and emission scanned from 250-900nm. Daily standards were prepared at 100, 250, and 500µg/L from a stock solution of 7426ppb quinine hemisulfate (in 0.05M H_2SO_4) in 25mL volumetric flasks of LGW containing 69µL concentrated H_2SO_4. Hitachi FL solutions software and SigmaPlot (Systat, Point Richmond, CA) were used for data analysis and presentation.

2.1.3 Additional Software

U.S. EPA EPI Suite software was utilized to estimate K*oc* values, overall biodegradation potential and estimated removal through WWTPs (http://www.epa.gov/opptintr/exposure/docs/episuite.htm). The EPI Suite SMILES notations were generated from the chemical structure created in ChemSketch 8.0 FreeWare (http://www.acdlabs.com/download/chemsk.html). Additional properties of the compounds

necessary from calculations, such as water solubility and melting point, were imported from www.chemfinder.com (CambridgeSoft Corporation, Cambridge, MA).

ArcGIS 9.1 (ESRI, Redlands, CA) was utilized to create maps of sampling areas. A Garmin Geographic Positioning System (GPS) unit (Olathe, KS) was used to collect latitude and longitude locations during sampling trips. Accuracy was within 7-10 feet. Discrepancies are visible between data points and geographical data layers as stream sampling locations and WWTPs do not exactly align with streams. The Minnesota Department of Natural Resources (DNR) extension was used to import GPS data points into ArcGIS. Geographical data layers, such as North Carolina streams, roads, and county information, were obtained from UNC GIS Libraries. ArcGIS was used to calculate distances between sampling locations. ArcGIS, with appropriate data, could be used to calculate additional information about runoff potential and overland flow, using contour data and stream distances.

2.2 Site Descriptions

All sampling sites were within the Piedmont region of North Carolina.

2.2.1 On-Site Wastewater Systems

2.2.1.1 Advanced treatment systems

Two advanced treatment septic systems were chosen for sampling due to ease of sampling location and as a surrogate for individual household systems. Both systems, a high school and an office building, were designed by Dr. Halford House and Integrated Water Systems (IWS, Pittsboro, NC). The high school advanced septic treatment system (HS) was

upgraded from the original septic system in 1997 by IWS. Figure 2.1 shows the original IWS engineering designs for the system upgrade. However, neither the recirculation of effluent from the first pass vegetated sand filter to the septic tank nor primary dosing tank was built. Estimated population of the school was 405 students and staff. Facilities included a gymnasium, cafeteria, and field house with washing machines that also enter the septic tank. The treatment system was comprised of the following components: grease trap, septic tank, dosing tank, equalization/pump tank, dual recirculating sand filters, programmable controller system, and ultraviolet disinfection system discharging into a creek. The system was designed and permitted for processing 10,000gpd during the school week, but actual capacity averages about 50% of this capacity, at about 35,000gal/week.

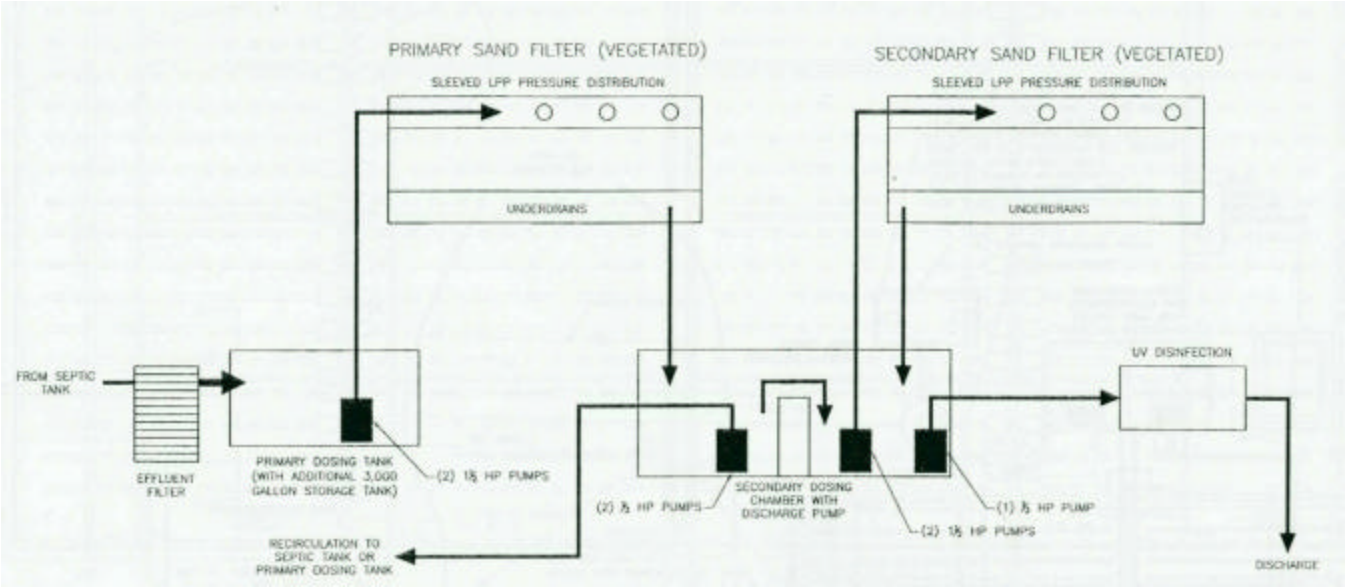

Figure 2.1 Engineering diagram of the high school advanced treatment system.

Two vegetated sand filters, approximately 42 by 42 by 46 feet, were dosed on an hourly cycle with effluent. The system was designed for an average dosing volume of 474 gallons/hour cycle, with the pump running for 5.7 minutes so the filter floods 3.2 inches. There was a 50% target recirculation of effluent to the second vegetated sand filter. The

second sand filter was dosed 400 gallons on a 45 minute cycle to target at 50% recirculation ratio. Schematic diagrams of the high school system are shown in Figure 2.2, where sampling locations are shown with an asterisk.

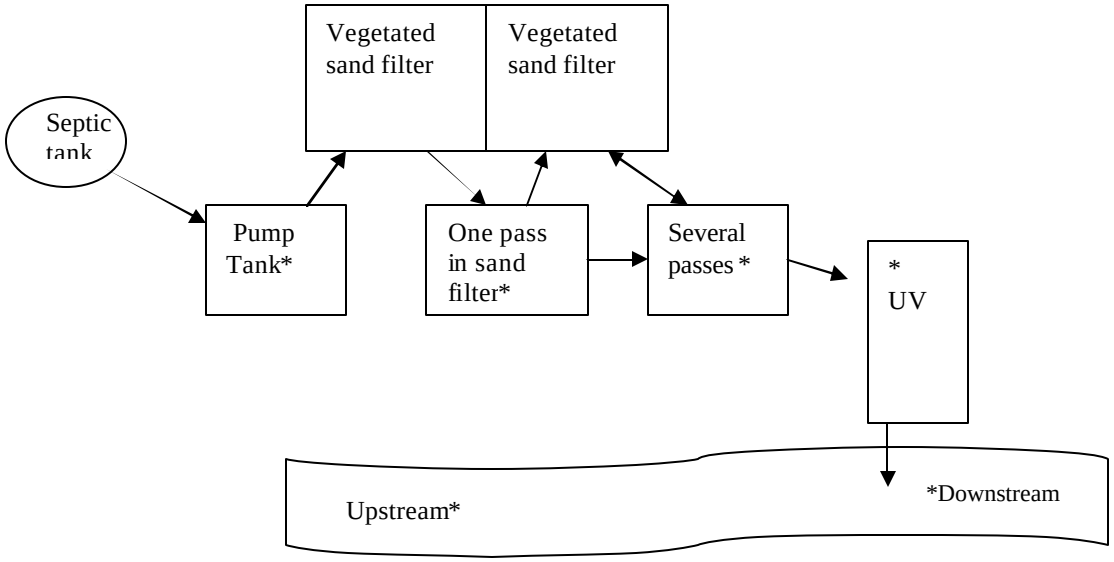

Figure 2.2 Overview of high school on-site wastewater treatment system. * indicates sampling locations.

The second advanced treatment system is at the IWS office at the Old Triangle School in Pittsboro, NC. A schematic of the IWS system is shown in Figure 2.3, where sampling locations are highlighted with an asterisk. Waste from the building entered a septic tank, for settling and anaerobic degradation. Effluent passed through an aerobic wetland, an anaerobic wetland, and another aerobic vegetated filter in the greenhouse. Sampling locations were after the aerobic wetland (AW*), after the anaerobic wetland (ANW*), after the greenhouse treatment (GH*), and in the retention tank after chlorination (RT*). The effluent was chlorinated and split approximately in half between landscape irrigation and reuse in office toilets. The system was designed for a maximum of 60 people and 900 gpd, however, average daily usage ranged between 500-700 gallons.

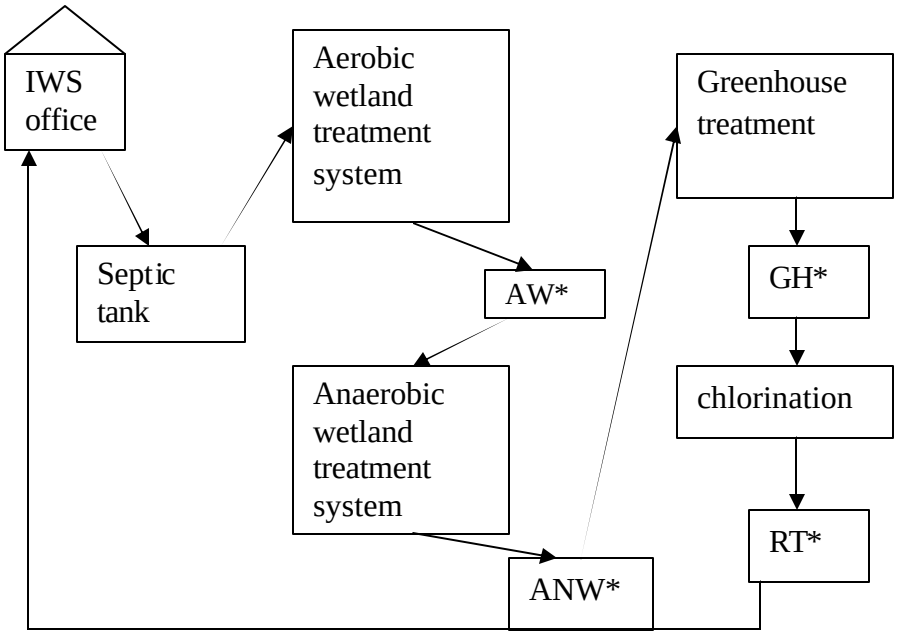

Figure 2.3 Overview of design of IWS Triangle School on-site wastewater treatment system. Sampling locations are indicated by *.

2.2.1.2 Individual Household Systems

Several individual households, mobile home parks, and a hotel were sampled with the help of the Orange County Health Department and North Carolina Department of Natural Resources (NCDENR) during inspections. Every five years, Orange County Health Department conducts inspections of household systems and NCDENR inspects systems installed with additional treatment, such as sand filter pre-treatment. If a system requires any advanced pre- or post treatment, such as sand filtration or land application of sewerage, NCDENR, and not the county health department, is responsible for permiting installation and inspecting system maintenance. Failed on-site wastewater systems were also identified through the Orange County Health Department. During routine inspections or investigations of failed septic systems, grab samples were collected from the septic tank, pump tank, surfacing sewage, and nearby surface waters.

After establishing an initial telephone conversation, Tom Konsler, from the Orange County Health Department, explained septic system installation, maintenance, local and state regulations and offered permission to attend septic system inspections, installations, and investigations into failed septic systems. After this initial contact, relationships were established with soil scientists. In Orange County, soil scientists are responsible for approving installations and repairs. They would call if a failed system was reported and allow me to sample the septic tank during repairs. After witnessing several installation and repair inspections, connections were established with local septic system operators who would provide information on additional failed systems.

2.2.2 Municipal Wastewater Treatment Plants (WWTPs)

WWTP 1 served a population of approximately 70,000, with a treatment capacity of 12 MGD. Until February 2006, effluent was disinfected with chlorine in the form of 15% sodium hypochlorite solution at an average dose rate of 2.5mg/L. Effluent was dechlorinated with 38% sodium bisulfite solution with an average dose of 3.5mg/L. The average contact time for chlorine contact was calculated based on a 24 hour flow rate of 8 MGD, for approximately 1 hour/gallon. The WWTP had two chlorine contact chambers each with a 0.183MG capacity. The contact time increased during lower flows and decreased during higher flows. In February 2006, the plant switched from chlorine to UV disinfection, using low pressure, high output lamps which maximized the "UV-C" wavelengths around 254nm. Average exposure of wastewater to the UV rays was 5-8 seconds.

Nitrification occurred in the aeration basins and in the clear water at the top end of the chlorine contact chambers. The NH_4^+-N levels in the effluent averaged below 0.5mg/L.

Figure 2.4 shows the locations of sample collection, which were 0.27 miles upstream of the point of discharge from the WWTP into the receiving creek (WWTP1 US), effluent collected straight from the discharge pipe (WWTP1 EFF), from the creek as the effluent was discharged (WWTP1 DIS), and 0.51 miles downstream of the point of discharge from the WWTP (WWTP1 DS). The WWTP1 DIS sampling location became inaccessible over the course of this study and sampling was discontinued from this location only. Upstream and downstream flow data were obtained from the U. S. Geological Survey (USGS) stream gages, available on-line at http://nc.water.usgs.gov/triangle/stations/. Monthly discharges were averaged from 2003-2004, for an average upstream discharge as 7.6ft^3/s, with a range of 0.43-32.2ft^3/s. Average downstream monthly discharge from 2003-2004 was 50ft^3/s, with a range of 13.3-194ft^3/s. Effluent discharge was estimated by subtracting upstream flow from downstream flow resulting in an average for 2003-2004 of 42.4ft^3/s with a range of 11.3-156ft^3/s.

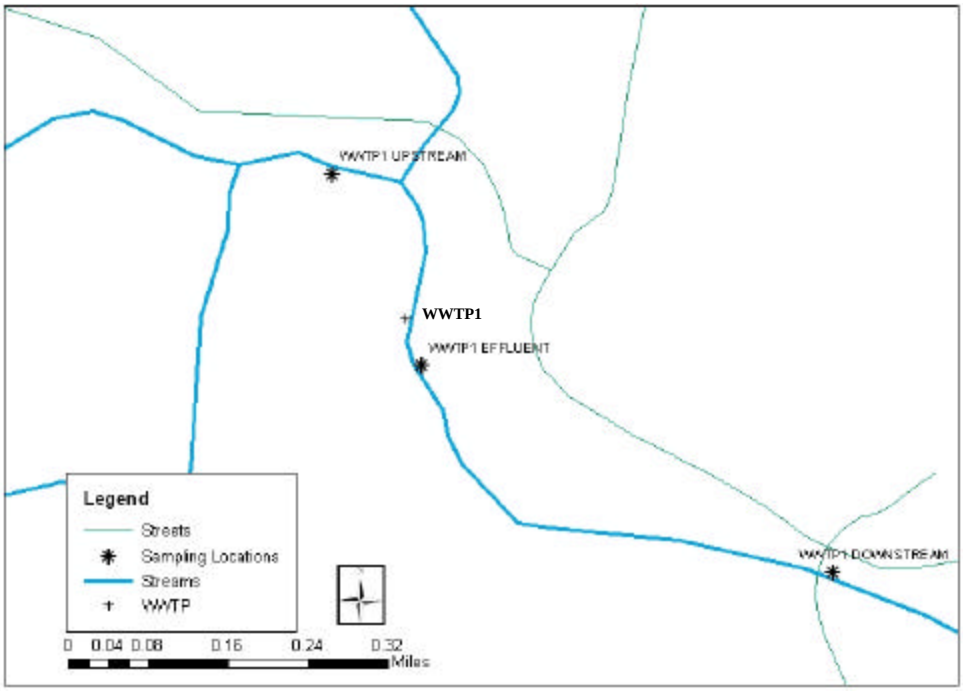

Figure 2.4 Map of sampling locations around WWTP1.

WWTP 2 had a treatment capacity of 12 MGD and an average treatment of 7-8 MGD of domestic and industrial sewage. In 1996, the population served by this WWTP was 26,000. WWTP discharges contributed 86% of stream flow during low flow conditions. Gaseous chlorine was injected directly using a jet chlorination system to disinfect the wastewater effluent, which was then dechlorinated with sulfur dioxide. Most of the time, the plant nitrified the waste, so NH_4^+-N was usually below analytical detection limit. Some denitrification occurred in the anoxic zone in the aeration basins, resulting in average effluent concentrations of 6-9mg/L total N, with 3-5mg/L NO_3-N and NO_2-N. Sampling locations, shown in Figure 2.5, were 1.5 miles upstream of the point of discharge from the plant into the receiving creek (WWTP2 US), an effluent sampling point within the plant immediately before stream discharge (WWTP2 EFF), at the point of discharge into the receiving creek (WWTP2 DIS), and 0.39 miles downstream of point of discharge (WWTP2 DS).

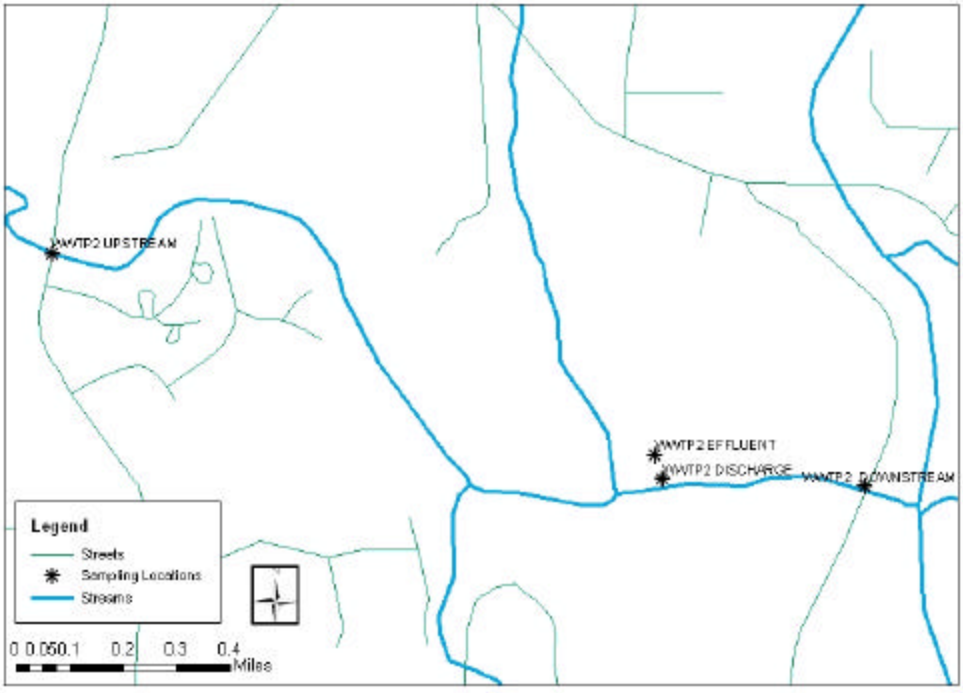

Figure 2.5 Map of sampling locations around WWTP2.

WWTP 3 served a population of almost 9,000, with a treatment capacity of 2.5 MGD and average treatment of 1.1 MGD. WWTP discharges contributed over 90% of stream flow during low-flow conditions. Wastewater effluent was chlorinated then dechlorinated with sulfur dioxide. The plant was not designed to denitrify, but typical effluent contained about 4.0mg/L total N. Samples were collected 0.2 miles upstream of the point of WWTP discharge into the receiving creek (WWTP3 US), within plant effluent from a sampling location following dechlorination immediately before discharge (WWTP3 EFF), 1.51 miles downstream of point of discharge (WWTP3 DS1), and from 3.6 miles downstream of point of discharge (WWTP3 DS2) (see Figure 2.6).

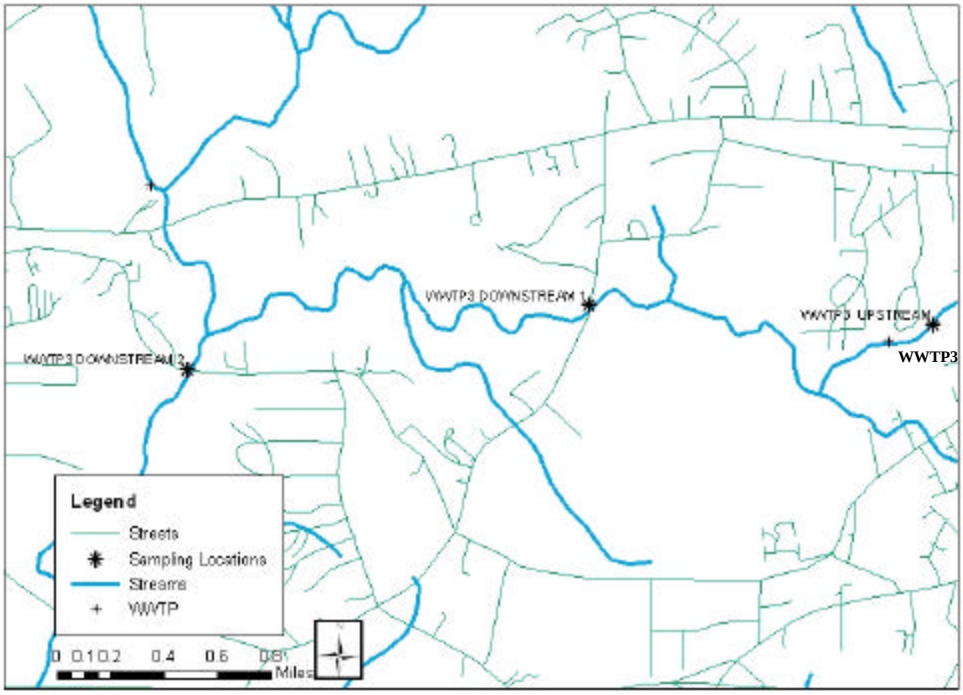

Figure 2.6 Map of sampling locations around WWTP 3.

2.3 Methods

2.3.1 Sample Collection Methods

Sample collection and storage bottles were detergent- and acid-washed amber glass bottles, except for high-density polypropylene plastic nalgene detergent-washed bottles used in the river sampler. River samples were collected either by submerging the sample bottle directly into the stream or by using a 1L bridge sampler (see Figure 2.7). The bridge sampler was lowered from a bridge above the river to collect up to 1L of sample, which was then transferred to an amber glass bottle at the sampling site. The bridge sampler was rinsed with sample before sample collection and with LGW and MeOH between samples. Septic tank grab samples were collected using a plastic dipping cup on a pole, the contents of which were then transferred into an amber glass bottle. The dipping cup was rinsed with sample before collection and then rinsed with LGW and 70% ethanol between samples. WWTP effluent samples were collected either directly into the amber bottle from the effluent flow or by using a plastic dipping cup for transfer. All field samples were stored in a cooler filled with ice or ice packs during sampling trips. Upon returning to the UNC laboratory, samples were stored in a walk-in cooler at 4°C until processing time. A HACH (Loveland, CO) chlorine test kit pocket colorimeter was used to measure free and total chlorine in samples.

Figure 2.7 Bridge sampler with high density polypropylene nalgene bottle.

Samples for SPE analysis of caffeine and triclosan were measured in 250mL aliquots using a volumetric flask and then transferred into 250mL amber bottles before spiking with 1.8µg/L deuterated standards when these became available. Suspended solids were removed from septic samples by successive filtration through Whatman (Florham Park, NJ) glass fiber filters GF/D (2.7µm) and 934-AH (1.5µm) filters. Wastewater effluent and river samples were only filtered with 1.5µm filters. Caffeine and triclosan samples were processed within a few hours of filtration.

Samples for HAA analysis were transferred without head space to 40mL clear glass vials containing 80µg/L sodium azide and approximately 20mg of ammonium sulfate. These samples were held at 4°C for up to two weeks prior to analysis. When HAAs were extracted from samples using SPE or larger volume LLE, 100mL aliquots were measured with a volumetric flask into 125mL amber bottles and the samples were spiked with 4µg/L DCAA-d2, once it was available for the project. Samples collected from septic tanks were successively filtered with 2.7 and 1.5µm glass fiber filters, while surface and WWTP effluent was filtered only with 1.5µm filters. After filtration, samples were returned to 125mL amber bottles, preservatives were added (125µL of 80mg/L sodium azide and approximately 50g ammonium sulfate) and samples were stored at 4°C for no more than two weeks before analysis.

2.3.2 Use of Deuterated Standards

Isotopically labeled compounds were evaluated for potential use in quantifying recovery of target analytes from wastewaters. These were caffeine-d3, triclosan-d3, and DCAA-d2, which are distinguishable from their non-deuterated counterparts using targeted

ion analysis in mass spectrometry. Since the only differences between deuterated and non-deuterated compounds are two or three mass units, the deuterated compounds should behave identically to the non-deuterated analytes in terms of partitioning and solubility. Deuterated compounds added to the samples at known concentrations can be used as surrogates to account for losses in non-deuterated analyte through the extraction of matrix samples. Additionally, the deuterated surrogate allows for comparison of extraction between matrices. The deuterated compounds were spiked into all measured sample aliquots before filtration. For each non-deuterated analyte, environmental concentrations were first determined approximately and then deuterated standards were spiked into samples at an equivalent concentration. DCAA-d2 was also evaluated as a surrogate for TCAA since labeled TCAA was not available.

Estimated concentrations of the analytes in wastewaters were determined using the approach described in EPA method 1668 for deuterated standards (EPA 1999). To determine concentrations of non-deuterated standards, a calibration curve was prepared in solvent containing deuterated standards at the concentration selected by the method just described. Non-deuterated standards were added at increasing concentrations that include the expected concentration range of analytes extracted from the environmental sample. For each point in the calibration curve, a relative response ratio (RR) was calculated using Equation 2.1, which compares the areas of the deuterated and non-deuterated compounds:

$$RR = \frac{(\text{area of non-deuterated} \times \text{concentration of deuterated})}{(\text{area of deuterated} \times \text{concentration of non-deuterated})} \quad (2.1)$$

In this equation, "area of non-deuterated" is the area of the non-deuterated analyte for a specific calibration point. The concentration of the deuterated standard is constant for each point, since the deuterated compound was always spiked at the same level. The "area of the

deuterated" is the area (detector response) of the deuterated compound in each calibration sample. The "concentration of the non-deuterated" is the calibration spike concentration. The relative response ratios for all calibration points are averaged, and the coefficient of variation (CV) should be less than 20% to ensure the ratio remains similar throughout the entire concentration range. The average RR is then utilized to calculate the non-deuterated analyte concentration in environmental samples according to Equation 2.2:

$$\text{Concentration} = \frac{(\text{area of non-deuterated} \times \text{concentration of deuterated})}{(\text{area of deuterated} \times \text{average RR})} \qquad (2.2)$$

In this equation, the "area of the deuterated" and "non-deuterated" are from the analysis of environmental samples, the "concentrated deuterated" is the concentration of the spike into the samples and calibration curve and the average RR is calculated from the solvent calibration curve. The ratio of the deuterated and non-deuterated should remain constant assuming that the analytes behave similarly through all matrices and processing. The RR is determined separately for each batch of samples and each calibration curve. This is the so-called deuterated standard method.

For one batch of samples, the method of standard addition and deuterated standard methods for concentration estimation were compared for caffeine. Effluent from the sand filter at the high school advanced treatment system was selected as the matrix. Duplicate sets of 250mL sample aliquots were spiked with 0.1, 0.2, and 0.4µg/L caffeine (standard addition). Triplicate unspiked sample aliquots (A,B,C) were prepared simultaneously. All aliquots were spiked with deuterated standards, filtered with 1.5µm filter, extracted using the caffeine and triclosan SPE method described in section 2.3.4, derivatized and analyzed by GC-MSD. The deuterated standard method for concentration estimation is described above.

The trendline equation from the standard addition curve, shown in Figure 2.8, was used to calculate concentrations using analyte areas from the unspiked extracts.

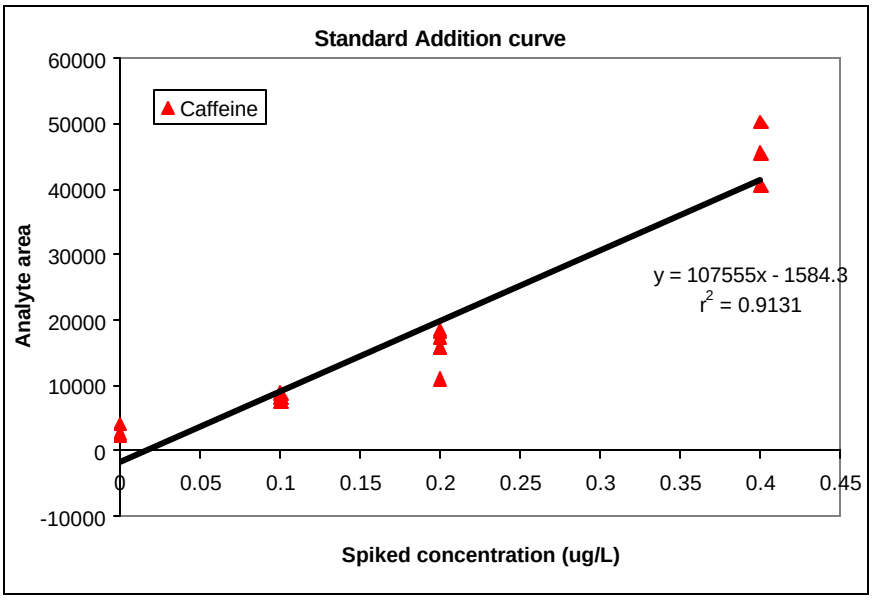

Figure 2.8 Standard addition trendline and equation for calculation of caffeine concentration in septic tank samples.

Overall, the concentration calculated from deuterated standards was 44% higher (as measured by relative percent difference (RPD)) than as calculated from standard addition, as shown in Table 2.3. In this table, areas are the absolute areas under the analyte peak on the chromatogram, which was integrated using the automatic integration with Varian MS WorkStation software. However, if sample A is considered an outlier, the difference drops to 32%, which is an acceptable level. Discrepancy between the estimated concentrations could be due to difficulties differentiating deuterated and non-deuterated caffeine by resolution of ions during analysis, especially in samples with high caffeine concentration. There was overlap of ions between these two compounds, which lead to high variability in the average RR calculation and therefore the concentration calculations. Despite this uncertainty, all environmental samples analyzed for caffeine and triclosan used the deuterated standard

method for determining analyte concentration and therefore, the comparisons between surface and wastewaters remains valid.

Table 2.3 Comparison of deuterated standard and standard addition methods for calculation of caffeine concentration in septic tank samples.

		Concentration (ng/L)		
Sample	Area	Deuterated Standard	Standard Addition	RPD
A	4133	107	53	68
B	2665	53	40	30
C	2225	50	35	33
Avg	3008	70	43	44
Avg without A	2445	52	37	32

2.3.3 Caffeine and Triclosan SPE Extraction Methods

The Thomas and Foster (2004) method was selected as the basis of the method for this current project due to demonstrated high recoveries of triclosan, use of smaller sample volumes, and practicality of analyzing for both caffeine and triclosan simultaneously. All materials used by Thomas and Foster were available at UNC laboratories and the same model GC-MSD (HP 5971) was utilized. Since parameters were similar, it was possible to contact the authors for help with the method, which was done when the caffeine peaks became broad and difficult to integrate.

The SPE methods were adjusted from the method of Thomas and Foster (2004) to increase caffeine recovery. The method development experiments and results are described in Results section 3.1. The final SPE Method was as follows: Strata X SPE cartridges were set up using a Supelco 24-port vacuum manifold. Cartridges were conditioned at a rate of 15mL/min using, in sequence, 3mL hexane, 3mL MtBE, 3mL MeOH and 5mL 10mM pH 7 phosphate buffer (diluted from 0.5M buffer prepared by measuring 16g of KH_2PO_4 into 500mL LGW). Filtered aqueous samples were loaded onto the cartridges using PTFE tubing

at a rate of 15mL/min and subsequently, the cartridges were rinsed with 5mL phosphate buffer to waste and allowed to dry under vacuum for an hour and then under UHP nitrogen at 20psi for 20 minutes. Two syringe volumes of air were forced over the cartridges to ensure removal of any last drops of sample. Cartridges were washed with 2mL hexane to waste and then eluted with 2mL 3:7 MtBE:Acetonitrile over silica gel cartridges (Bond Elute, Varian, Palo Alto, CA), which was captured in a test tube. Silica gel cartridges were conditioned with 4mL 3:7 MtBE:ACN, and after extraction were rinsed with 2mL 3:7 MtBE:ACN, which was captured along with the extracts into 10mL conical test tubes. The collected extracts were blown to dryness using either a Zymark (Hopkinton, MA) Turbocap-LV or Pierce (Rockford, IL) Reacti-Vap Model 18780 under a gentle flow of UHP nitrogen at 40° C.

Extracts were reconstituted in 200µL of ACN, 50µL of BSTFA, and 50µL of pyridine containing 12mg/L HCB. BSTFA was used to replace a hydroxyl group with a trimethylsilyl group on the triclosan molecule, the result of which is that these molecules interact less with the chromatographic column during subsequent analysis and increase sensitivity of detection. Extracts were vortexed three times, derivatized in a heated water bath at 65° C for 35 minutes, transferred to inserts inside 2mL autosampler vials, and analyzed with either GC-MS or GC-MSD. For this project, gas chromatography was chosen to analyze caffeine and triclosan since analysis is more sensitive than LC-MS and therefore requires smaller environmental sample volumes.

Average percent recovery for extraction of analytes from spiked LGW through the entire method compared to a solvent standard was 90% for caffeine and 79% for triclosan, as shown in section 3.2.10 of the Results chapter. The practical quantitation limit (PQL) was determined by the lowest calibration point area with a signal to noise ratio of three and

considering the concentration factor through the entire method for an estimated value of 60ng/L for both compounds.

Analyte concentration in environmental samples was calculated in two ways. Before acquisition of deuterated standards, sample concentration was extrapolated from the trendline equation of the standard addition curve. After deuterated standard acquisition, concentrations were calculated using the approach described in section 2.3.4.

The main reasons how this final method differed from the original Thomas and Foster method are the utilization of Strata X cartridges instead of HLB. Three:seven MtBE:ACN instead of EtOAc as an elution solvent, and silica gel cartridges were used for sample clean-up.

2.3.3.1 Detection of Triclosan with GC-ion trap-MS

A 0.5mg/L working solution of triclosan was prepared in ACN, derivatized as described in section 2.3.4, and analyzed on the GC-MS according to the method of Ollers et al. (2001), described in section 2.1.2.1. Figure 2.9A shows the total ion chromatogram and Figure 2.9B indicates the extracted ions at the retention time of 32.5 minutes for triclosan at m/z 347. Numbers underneath ion masses indicate abundance. Spectra have been background corrected. Figure 2.10 shows the structure of derivatized triclosan and MS breakdown products visible in Figure 2.9B. The mass of the derivatized triclosan is 362. The presence of three chlorines is visible by the chlorine isotope pattern surrounding ions m/z 362 and 347, which represents the loss of one methyl group. The relative ratio for 345:347:349 is 3:3:1.

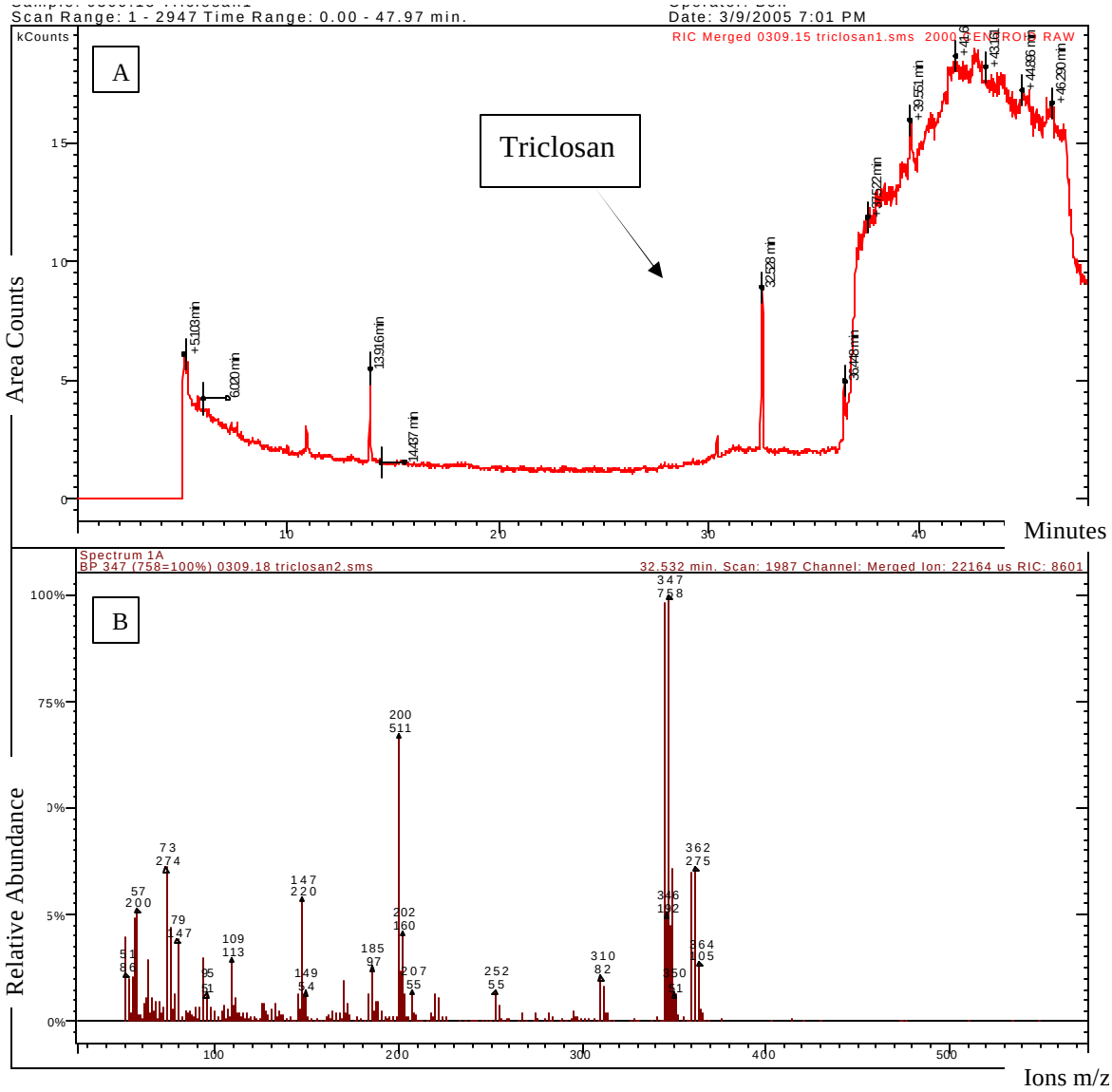

Figure 2.9 0.5mg/L derivatized triclosan in ACN analyzed by GC-MS, EI mode, using the GC temperature method of Ollers et al. (2001): (A) total ion chromatogram, (B) triclosan extracted with ion m/z 347.

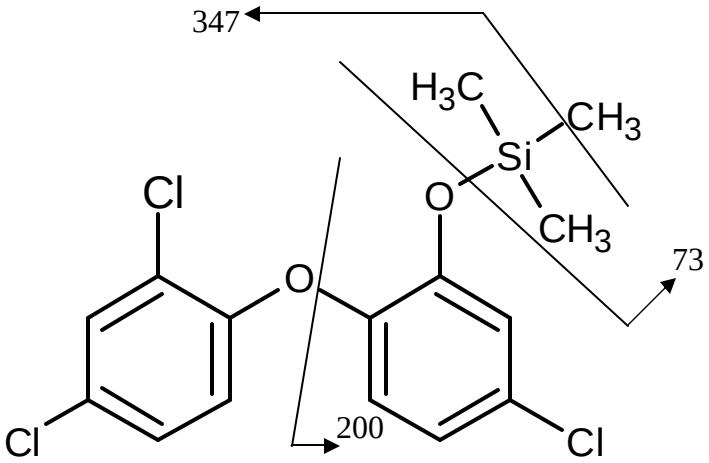

Figure 2.10 Structure of derivatized triclosan. Possible MS breakdown products are highlighted. The arrow indicates direction of break and the mass of the product.

Triclosan-d3 has an additional three mass units compared to triclosan, which is evident by the comparison of the spectra of the same concentration of derivatized standards of triclosan and triclosan-d3 in ACN, shown in Figure 2.11. Table 2.4 shows the abundance of ions for both analytes. Ion 352 was selected as the targeted ion for triclosan-d3 during SIM. Although ion 350 has a higher abundance in triclosan-d3, 352 is not present in triclosan. The relative ratio for triclosan ions m/z 345:347 is 1:1, as is the relative ratio of 348:350.

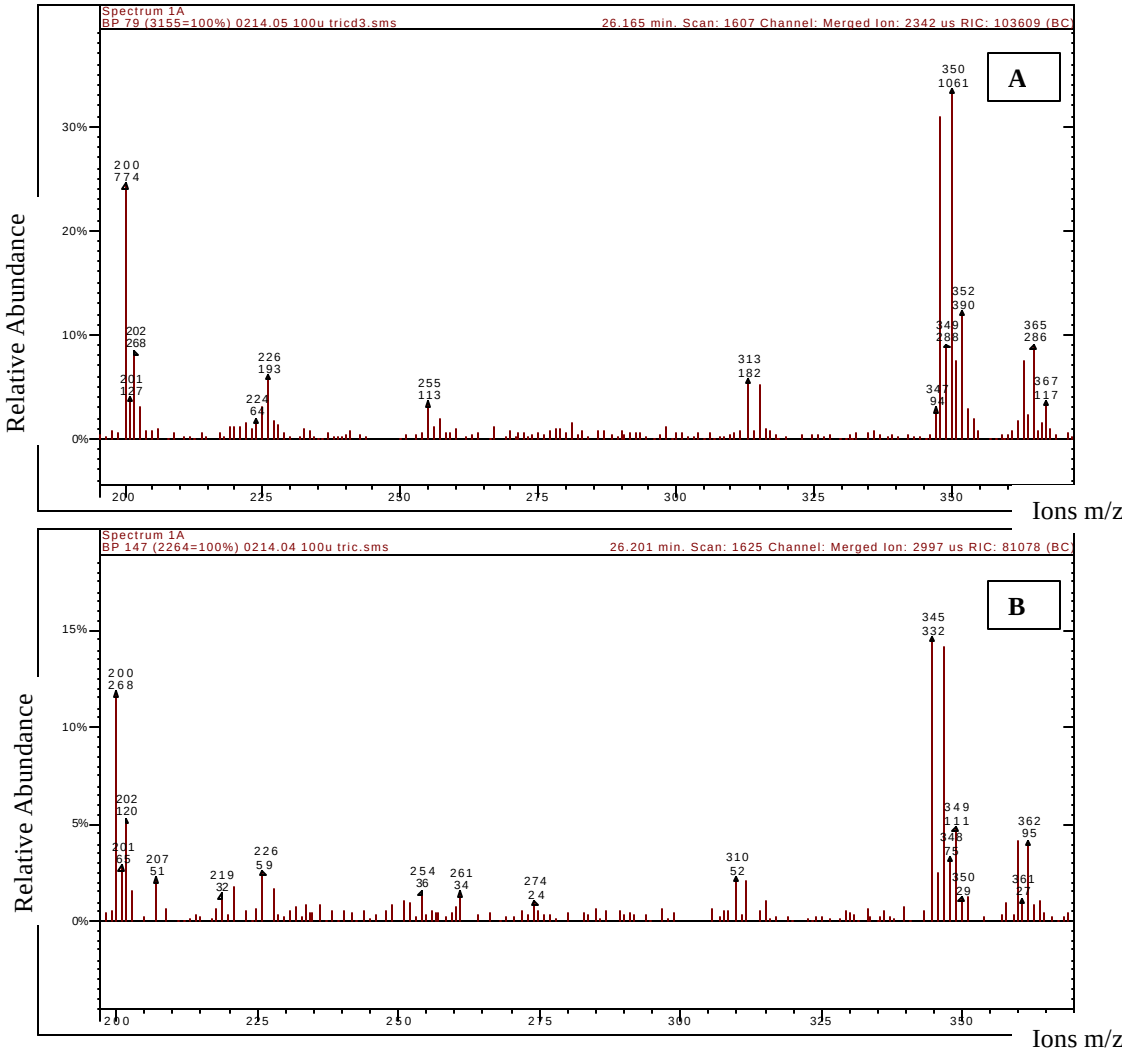

Figure 2.11 Spectra for derivatized working standards of (A) deuterated and (B) non-deuterated triclosan in ACN, analyzed by GC-MS in EI mode. The spectra represent the relative abundance of each ion m/z.

Table 2.4 Ions and abundance in spectra of triclosan and deuterated triclosan.

	Response in analyte (Intensity)	
Ion	Triclosan	Triclosan- d3
345	344	10
346	59	17
347	331	102
348	79	977
349	114	291
350	37	1064
351	31	236
352	-	396
355	38	69
360	102	24
362	101	13

2.3.3.2 Detection of Caffeine with GC-ion trap--MS

A 0.5mg/L working standard of caffeine was prepared in ACN and analyzed on the GC-MS according to the GC method by Ollers et al. (2001), described in section 2.1.2.1. Figure 2.9 shows the total ion chromatogram and Figure 2.12B indicates the extracted ions at a retention time of 23.8 minutes for caffeine at m/z 194. Numbers underneath ion masses indicate abundance. Spectra have been background corrected. Figure 2.13 shows the structure of caffeine and possible MS breakdown products to obtain ions m/z 56 and 109, which are visible in Figure 2.9.

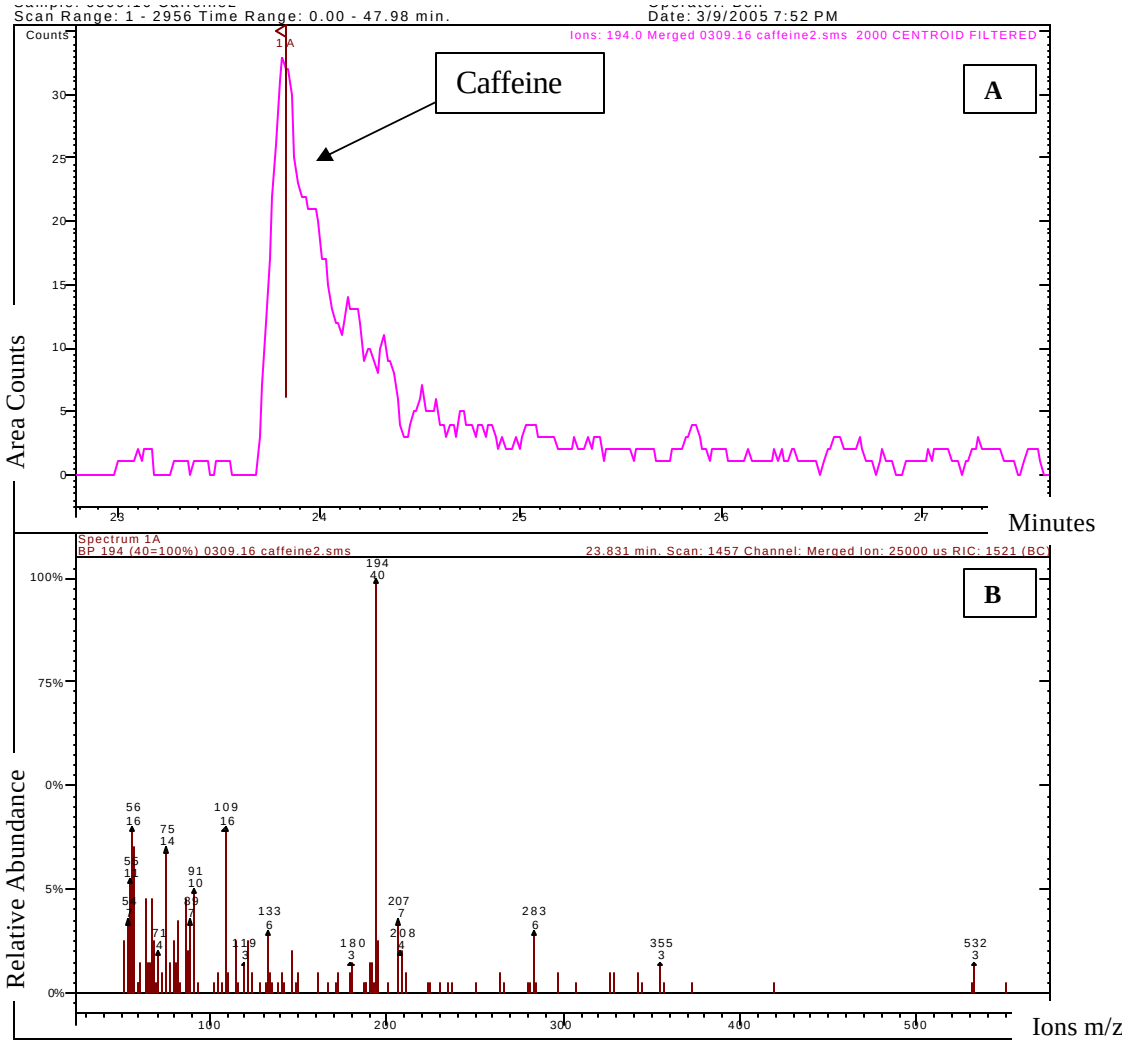

Figure 2.12 (A) Total ion chromatogram and (B) spectra of 0.5mg/L caffeine in ACN, extracted for ion m/z 194 using MS WorkStation software. Major ions are: 194, 109, and 56.

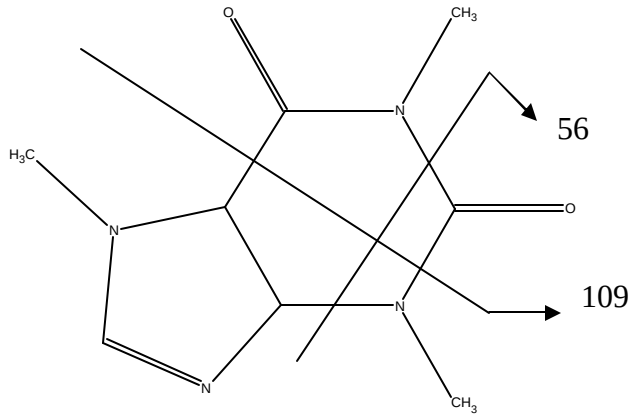

Figure 2.13 Structure of caffeine. Possible MS breakdown products are highlighted. Arrows indicate the direction of breaks and the masses of the products.

2.3.4 HAA Methods

For sample processing, three different HAA methods were utilized. The first method was based on EPA method 552 for detection of HAAs in finished drinking water (Brophy et al. 2000). This method was used to process all types of matrix samples, including surface and wastewaters, without any method adjustments. Twenty mL aliquots of sample were measured into 40mL glass vials and acidified with 1.5mL concentrated sulfuric acid. Four mL of MtBE containing 50µg/L internal standard, 1-2, dibromopropane, was added to extract the HAAs out of the water, with the addition of 6g baked sodium sulfate. The vials were vortexed for one minute and allowed to sit for five minutes. Two mL of the MtBE extract transferred into a 2mL volumetric flask were derivatized with 220µL of a diazomethane/MtBE solution in the refrigerator at 4°C for 15 minutes. For the diazomethane/MtBE solution, two reagents were prepared. Reagent 1 combined 3.3g diazald, 5mL carbitol, and 5mL MtBE mixed with a magnetic stir bar on a stir plate. Reagent 2 combined 6mL LGW, 10mL MeOH, and 4mL KOH and then gently swirled to mix. Six mL of each reagent were combined into a 40mL glass vial, which was connected with a

diazomethane generator to a 40mL glass collection vial containing 10mL MtBE. The diazomethane generator used PFTE tubing, which was submerged into the MtBE of the collection vial. The cap on the collection vial had a glass capillary tube to release pressure. Derivatization converts the HAAs into esters that can be analyzed using gas chromatography. Excess diazomethane remaining after derivatization reaction was quenched with silicic acid and the extracts were transferred to 2mL autosampler vials for analysis using GC-ECD. DCAA and TCAA concentrations in environmental samples were extrapolated from the trendline of the calibration curve prepared simultaneously with sample extraction of spiked LGW. No adjustments were made to account for method differences across matrices.

A second method for matrix samples, such as surface and wastewaters, used two 60mg/3cc Oasis HLB SPE cartridges in-series to concentrate DCAA and TCAA based on the method of Loos and Barcelo (2001). Several methods were tested and compared (described in Results section 3.3) before selection of the final SPE concentration method, which is as follows: the SPE cartridges were conditioned using 5mL MeOH and 3mL LGW adjusted to pH 2.5 with concentrated H_2SO_4. The two cartridges were then connected in series and 100mL filtered and then acidified (7.5mL concentrated H_2SO_4) aliquots were loaded at a rate of 5mL/min. Cartridges were subsequently rinsed with 1mL LGW at pH 2.5, dried under vacuum for one hour, dried under UHP nitrogen for 20 minutes, and finally air was forced through with a plastic syringe to remove any last drops of water. Each cartridge was separately eluted into the same test tube with 2mL MtBE each. Extracts were blown down to dryness at 30°C under a gentle stream of UHP nitrogen, reconstituted in 250µL MtBE, spiked with 50µg/L 1,2-dibromopropane internal standard, and derivatized with 25µL diazomethane for 15 minutes in 4°C. After the derivatization reaction, excess diazomethane was quenched

with silicic acid and the extract was transferred to inserts inside 2mL auto-sampler vials. These extracts were analyzed using single ion monitoring (SIM) on the GC-MSD or in CI mode on the GC-ion trap-MS. Average recoveries were calculated from extraction of spiked LGW through the entire method compared to a solvent standard, at 100% theoretical recovery. Recoveries were DCAA 98%, TCAA 91%, and DCAA-d2 84%, as shown in section 3.1.3.8 of the results chapter. DCAA and TCAA concentrations in environmental samples were calculated from the deuterated standard, DCAA-d2, as described in section 2.3.2.

The third method was a larger volume LLE, based on the first method. One hundred mL filtered sample aliquots, such as surface and wastewater samples, were acidified with 7.5mL concentrated H_2SO_4 and placed into a 250mL separatory funnel. Twenty mL MtBE was added to extract the HAAs with the addition of 6mg baked sodium sulfate. After several complete inversions of the flask, phases were allowed, for approximately five minutes, to separate. The bottom aqueous layer was drained off and the MtBE layer was collected into 40mL glass vials. These were blown down to dryness using the Turbovap in a 30°C water bath under a gentle stream of nitrogen. Extracts were reconstituted in 2mL MtBE containing 50µg/L 1,2-dibromopropane internal standard (IS) and transferred to 2mL volumetric flasks. The extract volume was adjusted to exactly 2mL with MtBE + IS, derivatized as described in the first method and analyzed using SIM on the GC-MSD or CI mode on the GC-ion trap-MS. Average recoveries were calculated from extraction of spiked LGW, compared to a solvent standard at 100% theoretical recovery. Recoveries were DCAA: 83- 75% and 85- 58%, TCAA: 117- 58% and 119- 42%, and DCAA-d2: 84- 62% and 86- 7.5%, as shown in section 3.1.2 of the results chapter.

2.3.4.1 Analysis of DCAA, TCAA and DCAA-d2 by GC-ion trap-MS

One mg/L HAA6 ester mix in MtBE was analyzed in CI mode on the ion trap GC-MS using the instrument settings described in section 2.1.2.1. During derivatization, the acids are methylated and the addition of the CH_3 group results in a mass increase of 14. Therefore, the masses of the ester forms of DCAA, TCAA and DCAA-d2 are 143, 177, and 144, respectively. CI, with softer ionization, reduces background noise and results in clearer peaks, although reduced sensitivity may result. Figures 2.14A and 2.15A show the total ion chromatograph for this standard mix and Figures 2.14B and 2.15B indicate extracted ions for DCAA (m/z:143, 145, 147) and TCAA (m/z: 177, 179, 181) respectively. Numbers underneath ion masses indicate abundance. Spectra have been background corrected. According to the identification of the targeted ions, retention time is 9.23 minutes for DCAA and 16.85 minutes for TCAA.

Chlorine has two natural isotopes at m/z 35 and 37, which appear in the DCAA, TCAA, and DCAA-d2 mass spectra in the classic pattern for chlorine. DCAA chlorine peak relative ratios, as seen in Figure 2.14, at m/z 143:145:147 were 10:7:1. TCAA chlorine peak relative ratios, as seen in Figure 2.15, at m/z 177:179:181 were 4:4:1.

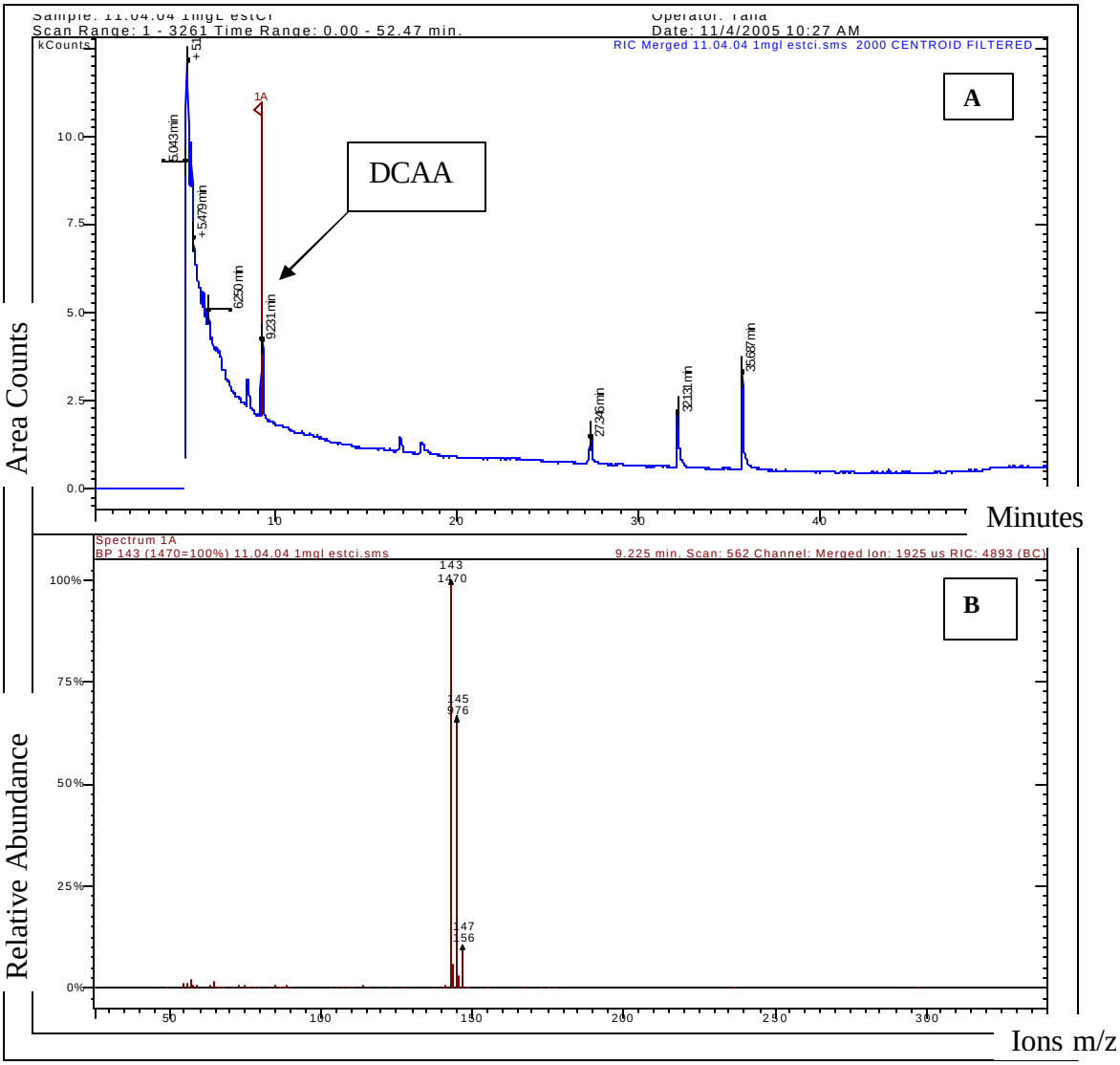

Figure 2.14 (A) Total ion chromatogram and (B) spectra of 1mg/L esters in CI mode, GC-MS, extracted for ions m/z 143. Spectra shows DCAA at retention time 9.23 minutes, identified by ions at m/z: 143, 145 and 147.

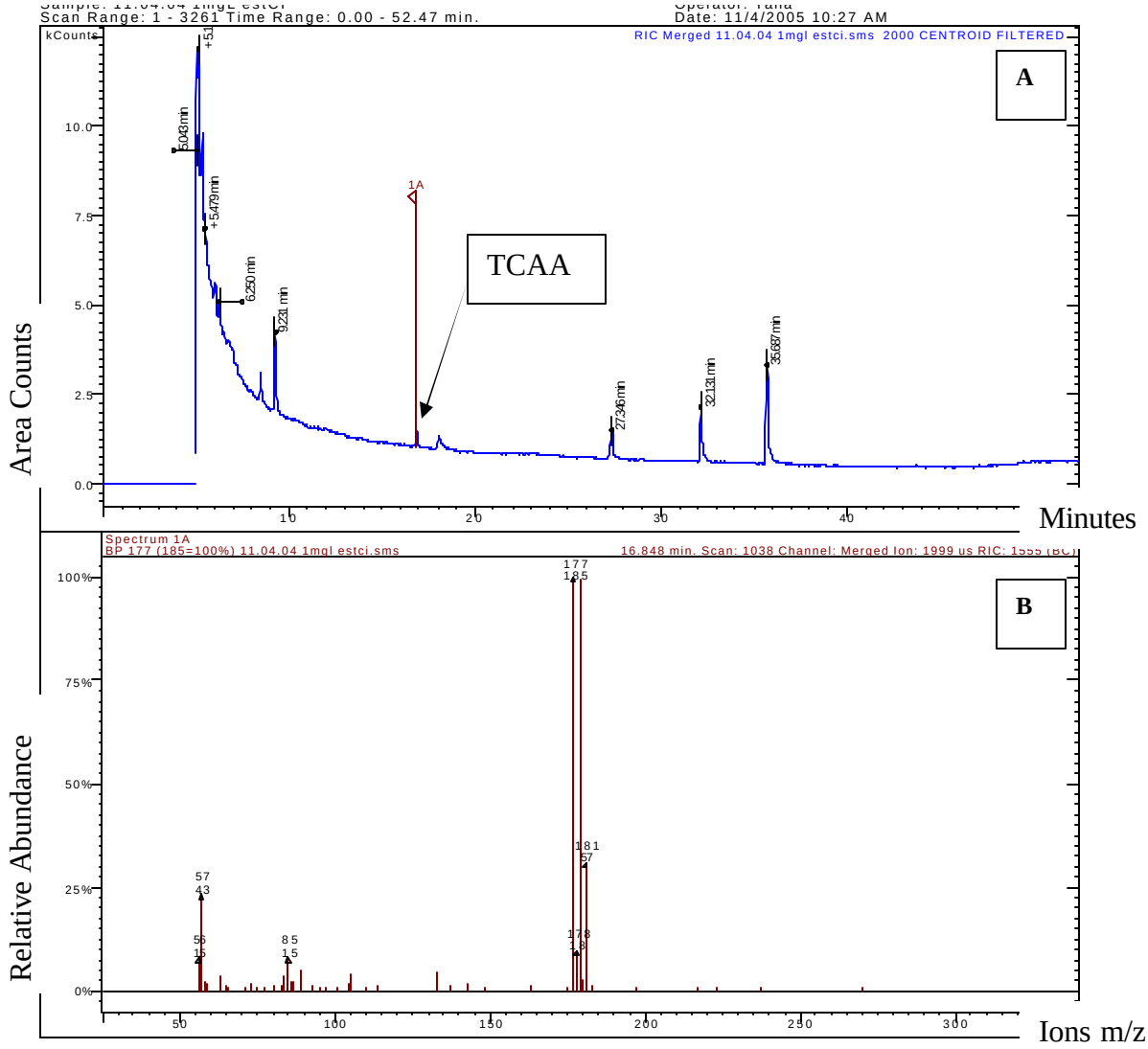

Figure 2.15 (A) Total ion chromatogram and (B) spectra of 1mg/L esters in CI mode, GC-MS, extracted for ion m/z 177. Spectra shows TCAA at retention time 16.85, identified by ions m/z: 177, 179 and 181.

DCAA-d2 was prepared at 1mg/L in MtBE, derivatized according to method 1, section 2.3.5, and analyzed by GC-ion trap-MS, CI mode. Figure 2.16A shows the total ion chromatogram and Figure 2.16B indicates the extracted ions for DCAA-d2 (m/z= 144,146,148) from which the retention time of 9.09 minutes is confirmed. Although the deuterated DCAA had two deuterated ions, one was lost in derivatization and, therefore, the methylated deuterated DCAA was only one mass unit higher than DCAA. The relative ratio of chlorine peaks as seen in Figure 2.16B for m/z 144:146:148 was 10:6:1. After retention

times and ions were determined, chromatographs were extracted using these targeted ions for each analyte.

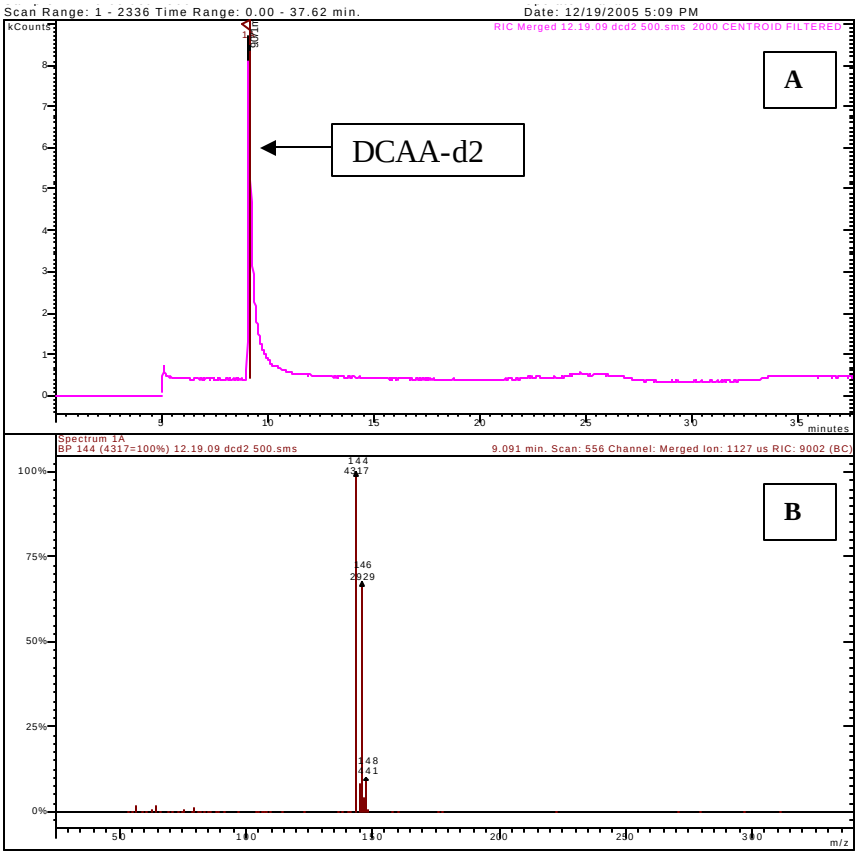

Figure 2.16 Methylated DCAA-d2 analyzed by GC-MS, CI mode. Spectra shows DCAA-d2 retention time at 9.09 minutes, identified by ions at m/z: 144, 146 and 148.

2.3.4.2 Analysis of DCAA, TCAA and DCAA-d2 on GC-MSD

A 1mg/L HAA6 ester mix was run in full scan on the GC-MSD, in EI mode, to determine retention time and targeted ions for SIM. Instrument conditions are described in section 2.1.2.3. Figure 2.17 shows a highlight of the chromatographic peaks from the full scan run, extracted for ion m/z 59, which represents the ion fragment of the methylated part of the ester, and therefore, should be present for all esters. Figure 2.18 shows the spectra for DCAA, with retention time around 7.2 minutes. Figure 2.19 shows the DCAA ester structure

with indications of where the molecule fragments in the mass spectrometer. The most common fragments occur due to the removal of the methylated half of the molecule or the chlorine half of the molecule, which distinguishes DCAA from the other HAAs. This unique half was targeted with ions m/z 83 and 85, with their characteristic ratio of 3:2, which was representative of the two chlorine atoms.

Figure 2.20 shows the spectra for TCAA, with retention time around 9.5 minutes. Figure 2.21 shows the TCAA ester structure with indications of where possible MS fragments occur. The expected fragments result from removal of the methylated half of the molecule, which was common to all esters. The other half, which includes the three chlorine atoms that distinguish TCAA from the other HAAs, was targeted with ions m/z 117 and 119 at a relative ratio of 1:1 characteristic of a molecule with three chlorine atoms. For compound confirmation, two ions were targeted for each compound.

DCAA and DCAA-d2 retention times were 7.2 minutes, TCAA was 9.6 minutes, and internal standard, 1,2- dibromopropane, was 7.7 minutes. To analyze the data with HP ChemStation software, targeted ions were extracted and each extracted chromatogram was integrated separately, setting integration parameters at a threshold height of 3 and width of 0.08.

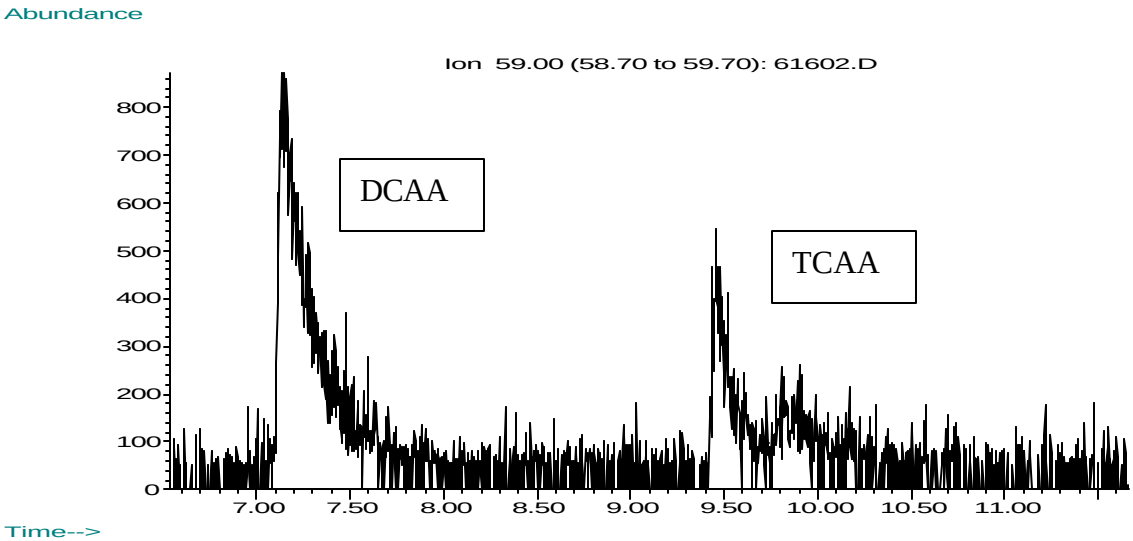

Figure 2.17 Highlight of full scan chromatogram of HAA6 ester mix by GC-MSD, extracted with ion m/z 59 to identify esters. Axis represent time and abundance.

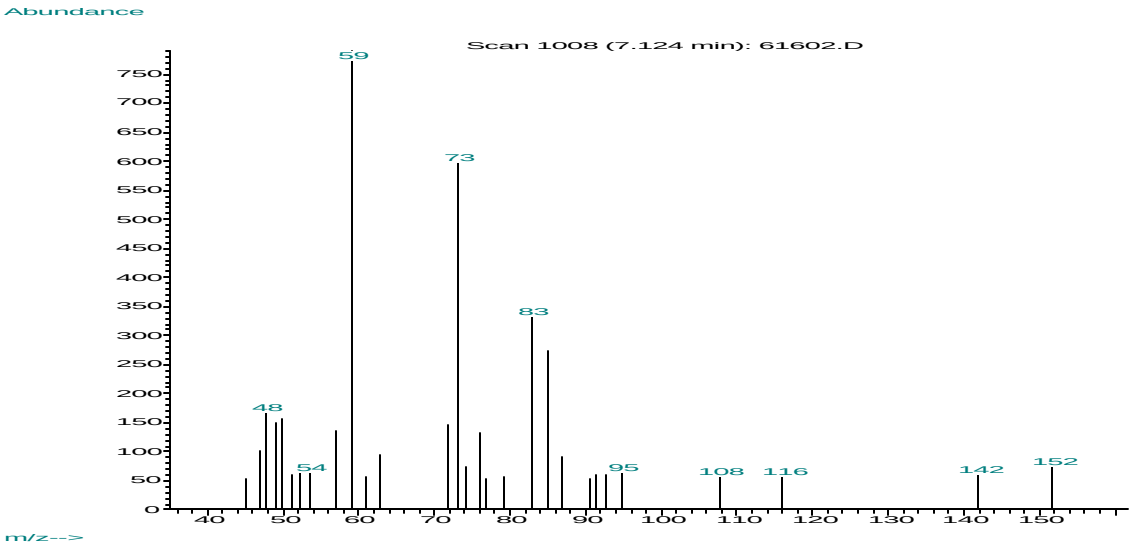

Figure 2.18 Spectra of methyl DCAA on GC-MSD, EI mode.

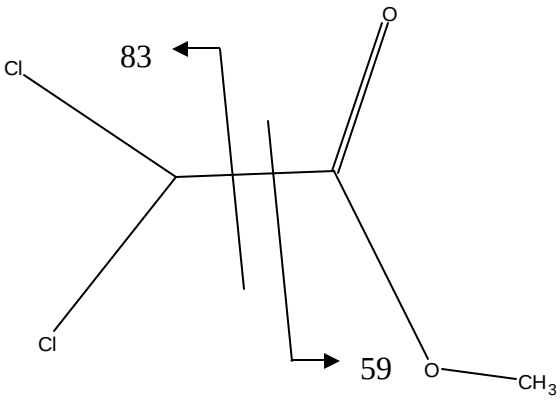

Figure 2.19 Structure of DCAA ester, showing possible MS fragments with arrow directions and accompanying masses.

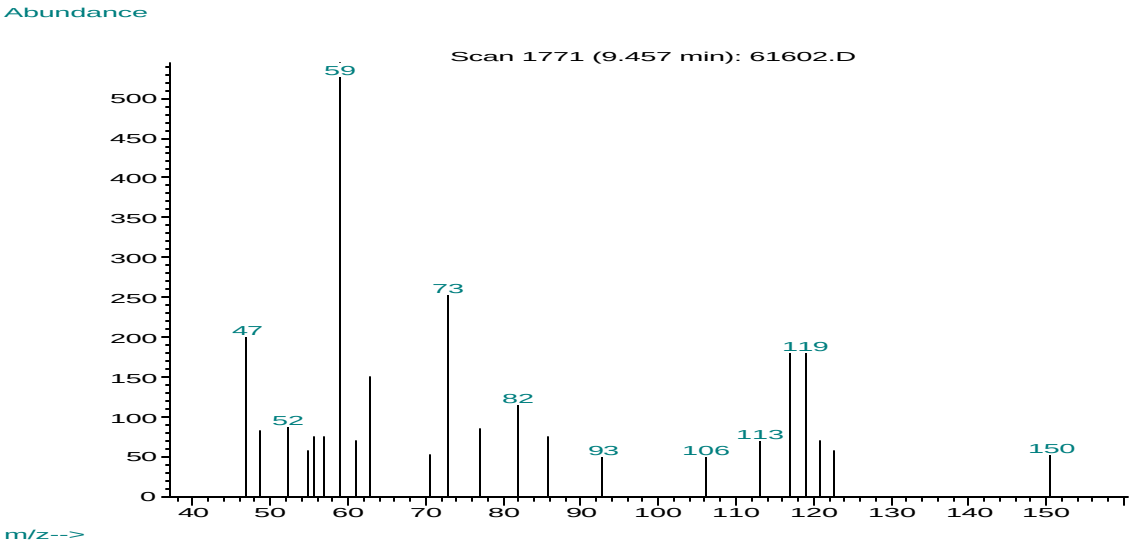

Figure 2.20 Spectra of methyl TCAA on GC-MSD, EI mode, retention time 9.5.

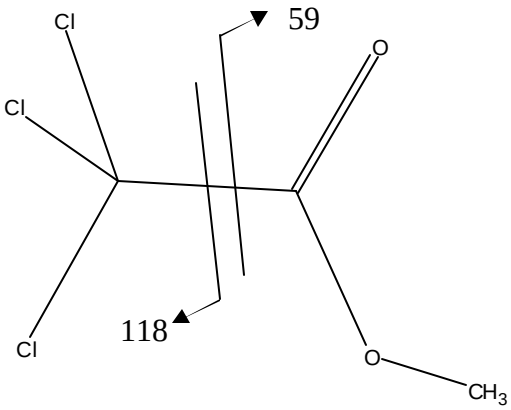

Figure 2.21 Structure of TCAA ester, showing possible MS fragments with arrow directions and accompanying masses.

2.3.5 Fluorescence Methods

At least 4mL of sample were filtered through a 0.2µm Whatman (Florham Park, NJ) nylon syringe filter. Samples were filtered as soon as possible after sample collection into 10mL glass vials and stored in the freezer for up to one week until analysis.

Instrument performance was evaluated from daily working standards. One hundred, 250, and 500µg/L solutions of quinine hemisulfate were prepared in 25mL of LGW with 69µL of concentrated H_2SO_4. These standards were run and analyzed for linearity of

intensity at the 350/440 excitation/emission wavelengths at the start of each run of sample processing. Quartz cuvettes were used for sample and standards analysis and were rinsed 3x with LGW between samples. The 250 standard was re-analyzed at the end of the run.

The FL solutions exported excitation/emission wavelengths and accompanying intensities to Excel. The Ramen/Raleigh scattering were removed and data were transferred to SigmaPlot. Contour graphs were created in SigmaPlot as a visual representation of the fluorescence image.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Caffeine and Triclosan: Method Development.

The caffeine and triclosan SPE extraction method published by Thomas and Foster (2004) was initially tested without any adjustments to determine recovery of analytes and clean-up of environmental sample extracts. Dirty extracts and low analyte recovery necessitated further experiments to obtain a method with sample extracts sufficiently clean to preserve the analytical instruments and to achieve acceptable analyte recoveries

3.1.1 Initial test of Thomas and Foster Method

Oasis HLB 200mg/6cc (Waters, Milford, MA) cartridges were evaluated for the recovery of caffeine and triclosan from environmental samples using an elution and conditioning method based on work by Thomas and Foster (2004). Duplicate 500mL aliquots of WWTP1 US were spiked with 1µg/L caffeine and 0.2µg/L triclosan (Samples A and B). Cartridges were conditioned with 3mL hexane, 3mL EtOAc, 3mL MeOH, and 5mL 10mM pH 7 phosphate buffer at rate of 15mL/minute. After conditioning, the aqueous samples were passed at a rate of approximately 5mL/minute, after which the cartridges were rinsed with 5mL of 10mM phosphate buffer, allowed to dry for a half hour each under vacuum and nitrogen, and then rinsed with 2mL hexane. The cartridges were eluted with 3mL EtOAc, blown down to dryness, reconstituted in 100µL ACN and derivatized with 50µL BSTFA and 50µL pyridine containing 12mg/L HCB, internal standard (IS). The derivatized extracts were analyzed using GC-ion trap-MS, EI mode, with the GC method by

Ollers et al. (2001) as shown in Table 3.1. Extracts were very dark and triclosan was not detected, suggesting the need for clean-up prior to extraction and further tests to increase recovery. Areas were integrated under the ion- extracted chromatogram peak using Varian MS WorkStation software. Relative area was normalized to HCB area.

Table 3.1 Variability caffeine and triclosan analysis in WWTP1 US by GC-ion trap-MS, EI mode. Targeted ions are: HCB-284, caffeine-194 and triclosan-347.

	HCB Area	Caffeine Area	Relative Area	Triclosan Area
Sample A	19575	33286	1.70	ND
Sample B	12037	20844	1.73	ND
Avg	15806	27065	1.7	
RPD	48	46	1.8	

ND= not detected

3.1.2 Testing Three SPE Methods with WWTP Effluent for Recovery

Five hundred mL aliquots of WWTP1 effluent were extracted with three different SPE methods to test effectiveness of method clean-up on both filtered and unfiltered samples. Method 1 was described in section 3.1.1. Method 2 was a combination SAX/HLB method. SAX cartridges (500mg/6cc) were conditioned with 2mL methanol followed by 2mL citric acid buffer. Citric acid buffer was prepared with 41.5g citric acid monohydrate and 200mL 1M NaOH in 1L LGW, then diluted to 40mM with a pH between 4.6 and 4.7.

The SAX cartridges were washed with 2mL 0.04M citric acid buffer and 2mL 0.1M sodium acetate and then connected in series with conditioned 60mg/3cc HLB cartridges. The 0.1M sodium acetate solution was prepared with 8.40g of anhydrous sodium acetate measured into a 1L volumetric flask containing LGW. The HLB cartridges were separately conditioned with 3mL 40% MeOH in LGW, 3mL LGW and 2mL 10% MeOH/ 2% ammonium hydroxide in LGW before connection to the SAX cartridges. The WWTP1 effluent samples were passed over cartridges in tandem (SAX then HLB) and the SAX was

removed. The HLB cartridge was dried under vacuum for one hour, dried under nitrogen for 15 minutes, and then eluted with 6mL 10% MeOH in MtBE. Method 3 evaluated HLB cartridges using the conditioning and elution of Method 2.

Extracts from all methods were very dark and, therefore, were not blown down or derivatized. For an accurate measure of turbidity, solvent blanks (either EtOAc or 10% MeOH in MtBE) and the extracts were analyzed using a Beckman single beam UV Spectrophotometer, model DU 650 (Fullerton, CA) at 254nm. The comparative absorbances are shown in Table 3.2. The lowest absorbance was from filtered 2 samples, extracted using SAX/HLB. Method 2 was then tested with another elution solvent in an attempt for cleaner extracts.

Table 3.2 Average absorbance of solvents and SPE extracts measured on a UV spectrophotometer.

	Absorbance			Average
Ethyl acetate blank	0			0
Filtered Method 1	0.51	0.52	0.52	0.52
Filtered Method 1	0.5036	0.5101	0.5118	0.5085
10% Methanol in MtBE blank	0			0
Filtered Method 2	0.2734	0.2732	0.2732	0.2733
Unfiltered Method 2	0.3315	0.3323	0.3325	0.3321
Unfiltered Method 3	0.3048	0.3054	0.3056	0.3053
Filtered Method 3	0.5526	0.553	0.553	0.5529
Rerun 10% methanol in MtBE blank	0			0

3.1.3 Testing Effectiveness of Acetonitrile as Elution Solvent

Caffeine and triclosan were spiked into 500mL LGW at 5 µg/L each and extracted with Method 1, described in section 3.1.1, and Method 2, described in section 3.1.2, using both the original elution solvent (either EtOAc or 10% MeOH in MtBE) and ACN. All extracts were blown down to dryness, reconstituted with 100µL ACN, derivatized with only 50µL BSTFA, and analyzed by GC-ion trap-MS. Visually, the extracts were clear. A solvent standard was prepared at the theoretical 100% concentration for comparison.

The results of this experiment are shown in Table 3.3. Extracts were derivatized without pyridine, so IS was not present to normalize results. Qualitatively, Method 1 was more effective at recovering both analytes than Method 2. Caffeine from Method 2 was reported as not detected (ND) since area counts were below 500.

Table 3.3 Caffeine and triclosan areas from LGW extracted with SPE using Methods 1 and 2 and evaluating two different elution solvents. Areas represent area under chromatogram peak, integrated using MS WorkStation software.

	HCB Area	Caffeine Area	Triclosan Area
Solvent Standard	ND	38044	268827
Meth 1 original	ND	128536	109150
Meth 1 ACN	ND	140958	71414
Meth 2 original	ND	ND	41954
Meth 2 ACN	ND	ND	57979

ND= not detected

3.1.4 Testing SPE Methods 1 and 3 with New Elution Solvent

Experiments in section 3.1.3 testing SPE extraction of spiked LGW showed that EtOAc, the original elution solvent from Method 1, was effective at recovering analytes but resulted in extracts that were too dark to analyze on the GC-ion trap-MS. Testing ACN as an alternative elution solvent resulted in slightly higher areas for caffeine but lower triclosan areas, as shown in Table 3.3. An elution solvent mixture, with a similar polarity to EtOAc, was prepared to maximize recovery of both analytes and to yield cleaner extracts. Solvent polarities are shown in Table 3.4. Solvent mixture polarities were calculated from solvent polarities and the concentration of the solvents in the mixtures.

Table 3.4 Polarity of different elution solvents.

Solvent	Polarity
Ethyl Acetate	4.4
Acetonitrile	5.8
MtBE	2.5
MeOH	5.1

One hundred mL LGW aliquots were spiked with 2µg/L caffeine and triclosan. Several methods were tested: Method 1, described in section 3.1.1; Method 3, described in section 3.1.2; and a fourth method using Strata X cartridges that differs in conditioning and elution solvents. Method 1 was tested using three elution solvents: (a) EtOAc (b) 1:1 MeOH: ACN (polarity = 5.45) and (c) 3:7 MtBE: ACN (polarity = 4.8). A solvent standard was prepared at the theoretical 100% concentration.

The Strata X cartridges were conditioned with 3mL MeOH and 3mL LGW. After the sample was passed, the cartridges were washed with 3mL 5% MeOH in LGW, dried under vacuum for 30 minutes, and eluted with 3mL 1:1 MeOH:ACN.

The HLB cartridges, conditioned according to Method 1 and eluted with EtOAc, were used to test further clean up. Silica gel cartridges (200mg/3cc) (Supelclean, Supelco, Bellefonte, PA) were conditioned with 10mL 65:35 hexane:acetone (Ternes et al. 1999). The HLB cartridge extract was loaded onto the conditioned silica gel cartridges, which were then eluted with 6mL of 65:35 hexane:acetone. The extracts were blown to dryness, reconstituted in 200µL ACN and derivatized with BSTFA and pyridine containing IS. Results are shown in Table 3.5. Both analytes were recovered from Method 1 with different solvents. The variability in the HCB areas was unexplainable. Comparing absolute areas, there was no significant difference between the elution solvents in Method 1. Comparing relative areas, MtBE:ACN as an elution solvent was most effective. Although silica gel visually cleans up the extracts, triclosan appears to get lost in the process.

Table 3.5 Caffeine and triclosan recoveries from method 1 with different elution solvents and silica gel clean-up compared to Strata X. Areas are integrated under the extracted ion peak from the chromatogram.

	HCB Area	Caffeine Area	Caff- Rel Area	% recov	Triclosan Area	Tric- Rel Area	% recov
Solvent Standard	22628	15103			16869		
Meth 1 MeOH: ACN	54829	18999	0.35	52	18702	0.34	46
Meth 1 MtBE: ACN	32130	18853	0.59	88	17290	0.54	72
Strata X	39435	12415	0.31	47	ND		
Meth 1 EtOAc with Silica	14720	6620	0.45	67	ND		

3.1.5 Evaluating SPE Extraction Method on Environmental Samples

Method 1 using 3:7 MtBE:ACN as elution solvent (renamed Method 1a) was tested on duplicate 100mL aliquots of WWTP1 US and LGW. Aliquots were spiked with 1.5µg/L caffeine and triclosan. A solvent standard was prepared for comparison at the 100% theoretical concentration. Results show that the method was effective for recovery of analytes in LGW and surface water, as shown in Table 3.6. Percent recovery was calculated as a comparison of the relative area in the analyte compared to the relative area of the solvent standard. Percent recovery provided a qualitative estimation of the method efficiency to optimize the method parameters. Recoveries over 100% for surface water were due to background concentration of analytes in the matrix, since no unspiked US was run for correction.

Table 3.6 Caffeine and triclosan recovery from spiked LGW and WWTP1 US water. Percent recovery is compared to solvent standard.

	HCB Area	Caff Area	RPD	Rel Area	% recov	Tric Area	RPD	Rel Area	% recov
Solv std	32623	16401		0.50		17581		0.54	
Avg LGW	28977	11899	12	0.41	83	13841	4.7	0.48	89
Avg WWTP1 US	27912	15349	1.3	0.55	110	14753	5.1	0.53	98
Avg	29280	14179		0.49		14953		0.51	
RPD	6.8	17		16.7		11		6.9	

3.1.6 Evaluating Silica Gel Clean-Up from Surface Water

Triplicate 100mL WWTP1 DS, spiked with 1.5µg/L caffeine and triclosan, and one un-spiked LGW aliquot were used to test several clean up methods in combination with Method 1a (described in section 3.1.5). Physical clean-up was performed by filtration with either 1.5µm glass microfiber filter (GMF) or ultrafine filter (UF) before SPE. Silica gel cartridges, using the conditioning and elution method described in section 3.1.4, were tested as clean up after HLB. The HLB elution extract was passed over conditioned silica gel cartridge. The eluant from the silica gel cartridge was captured in a test tube, derivatized, and analyzed. Since the results from Table 3.6 suggest triclosan is lost through the silica gel clean-up, the "wash" step, as HLB extract was passed over silica, was analyzed to determine analyte break through. A solvent standard, at theoretical 100% recovery (75µg/L), was prepared concurrently. After derivatization, extracts were analyzed with GC-ion trap-MS, EI mode.

Visually, there was no significant difference between extract color from different size filters. Results of filtration and silica gel clean-up are shown in Table 3.7. Although silica gel visually cleans extracts, detection of analytes from silica wash indicated analytes were eluting in the wash step. Filtration with 0.45µm (UF) yielded no significant difference than 1.5µm filtered (GMF) sample and therefore, was not worth the extra time this filtration step required. From this experiment, further clean-up methods were tested to ensure cleaner extracts to maintain instrument performance.

Table 3.7 Caffeine and triclosan recoveries from various clean-up methods, analyzed with GC-ion trap-MS, EI mode. Areas were integrated under the peak of the chromatogram with MS WorkStation software.

	HCB Area	Caffeine Area	Rel Area	% recov	Triclosan Area	Rel Area	% recov
Solv std	6197	558	0.09		1831	0.30	
LGW	6953	660	0.09	105	1268	0.18	61
Avg UF	7453	2767	0.37	412	1539	0.21	70
Avg GMF	7199	2569	0.36	396	1506	0.21	71
Avg Si extract	6432	350	0.06	63	ND		
Avg Si Wash	7232	879	0.11	119	1366	0.21	70

3.1.7 Evaluating SPE Methods on Septic Tank Effluent

Method 1a, described in section 3.1.5, was tested on grab samples from a household (HOUSE) and IWS office septic tank (IWS). Several batches of approximately 20mL of sample were poured into 40mL vials and centrifuged (2500rpm, 25° C for 15 minutes). The supernatant was filtered with 1.5µm filters and combined into triplicate 100mL aliquots. Filtered supernatant and triplicate 100mL LGW aliquots were spiked with 1.5µg/L caffeine and triclosan.

Post clean-up with silica gel was evaluated with another conditioning and washing solvent. Silica gel was conditioned with 4mL 3:7 MtBE: ACN. The HLB cartridges were eluted onto conditioned silica gel cartridges, which were captured in a test tube. Silica gel cartridges were rinsed with 2mL 3:7 MtBE:ACN into the same test tube. These extracts were blown down to dryness, reconstituted in 200µL ACN and derivatized with BSFTA and pyridine containing HCB. This method (renamed Method 1b) visually removed all the color from the extracts. A solvent standard containing caffeine and triclosan was concurrently prepared at the concentration representing 100% recovery of 0.5mg/L taken through the entire extraction process.

The caffeine peaks from all extracts were short and broad, and therefore, difficult for Varian WorkStation software to integrate. Peaks were manually integrated after smoothing

the chromatograms and the results are shown in Table 3.8. Percent recovery was compared to solvent standard and was not corrected for background concentrations of analytes in environmental samples, since unspiked samples were not analyzed. It is unclear why triclosan was not detected in any HOUSE extracts. The solvent standard and LGW were re-analyzed with duplicate injections to provide a more accurate indication of method recovery, as shown in Table 3.9.

Table 3.8 Caffeine and triclosan recoveries from Method 1b, analyzed using GC-ion trap-MS, EI mode.

	HCB Area	Caffeine Area	Rel Area	% recovery	Triclosan Area	Rel Area	% recovery
Solv std	5941	1523	0.26		3323	0.56	
LGW	5943	860	0.14	60	2452	0.41	75
IWS	5724	14906	2.60	1015	3321	0.58	104
HOUSE	5887	118625	20.15	7860	ND		

Table 3.9 Caffeine and triclosan recoveries from LGW method test of Method 1b, GC-ion trap-MS, EI mode.

	HCB Area	Caffeine Area	RPD	% recovery	Triclosan Area	RPD	% recovery
Solv std	11440	3606			7479		
Avg LGW	11755	3322	55	90	6048	7.5	79

3.1.8 Caffeine Derivatization Test

Since caffeine peaks were broad and difficult to integrate, it was hypothesized that derivatization caused a reaction in caffeine that resulted in the tailing peaks. Caffeine was detectable without derivatization, and therefore, caffeine standard both derivatized and underivatized were compared for peak shape and area. Duplicate 200µL aliquots of ACN were spiked with 700µg/L caffeine. One sample was derivatized and both were analyzed by GC-ion trap-MS. Derivatization did not significantly affect area, signal strength, or peak shape, as shown in Table 3.10.

Table 3.10 Results of derivatization experiment. Area and signal to noise ratio were calculated by MS WorkStation software.1

	Caffeine area	Caffeine S:N
Caffeine	1365	43
Caffeine derivatized	1386	44

3.1.9 Comparison of HLB and Strata X Cartridges Using Method 1B

Triplicate 250mL aliquots of LGW were spiked with 2µg/L of caffeine, triclosan and caffeine-d3. Aliquots were extracted using SPE Method 1b, as described in section 3.1.7. SPE HLB and Strata X SPE cartridges were compared. The deuterated caffeine, caffeine-d3, was acquired mid-way through the project and was used as a caffeine surrogate to test the recovery of caffeine through the extraction method. Table 3.11 shows caffeine and triclosan recovery from HLB and Strata X SPE cartridges. Strata X more effectively recovered caffeine and deuterated caffeine than HLB, but HLB was more effective for triclosan. Overall, recoveries from Strata X were less variable in analyte recovery.

Table 3.11 Results of HLB- Strata X cartridge comparison, analyzed with GC-ion trap-MS, EI mode.

	HCB Area	% CV	Caff Area	% CV	Rel Area	Tric Area	% CV	Rel Area	Caffeine-d3 Area
HLB	10505	7.8	1509	26	0.14	17817	15	1.72	930
Strata X	10498	9.7	2608	14	0.25	13316	10	1.28	1384
Strata vs HLB			61		56	-41		-48	28
RPD			42		55	3.9		10	63

3.1.10 Evaluating Methods on Septic Tank Samples

Triplicate pump tank samples collected from the septic system at the IWS office were spiked with 2µg/L caffeine, triclosan, and deuterated caffeine (caffeine-d3). For each cartridge type, there were duplicate unspiked aliquots. In Table 3.12, spiked samples are labeled HLB-sp; Strata-sp and unspiked samples are labeled HLB and Strata. Instead of centrifuging septic tank samples, samples were successively filtered with 2.7µm and 1.5µm

filters. Filtered samples were extracted using Method 1b, as described in section 3.1.7. Relative areas were compared to HCB area, as shown in Table 3.12.

Table 3.12 Caffeine and triclosan areas from septic tank samples, GC-ion trap-MS, EI mode. Areas are integrated under the chromatogram peak. Relative areas are normalized to the HCB area.

Sample	HCB Area	Caff Area	% CV/ RPD	Rel Area	Tric Area	% CV/ RPD	Rel Area	Caff-d3 Area
Avg Hlb	10939	6065	18	0.55	3889	15	0.36	3110
Avg Hlb-sp	11887	30637	22	2.56	9416	34	0.79	5922
Avg Strata	11443	25327	18	2.2	4255	23	0.37	4905
Avg Strata-sp	11831	24194	14		7250	38		4477
Average	11592							4723
Stdev	708							1528
CV	6.1							32

Comparing relative areas in Table 3.12, Strata X recovered more caffeine than HLB. Strata X unspiked absolute areas were higher than HLB, but HLB recovery from spiked samples was higher. Overall, the two cartridge types were comparable for analyte recovery. Strata X was chosen for practicality and ease of using one cartridge instead of two cartridges in series, as had been the case for HLB.

3.1.11 Method Development Summary

The caffeine and triclosan SPE method was altered from work by Thomas and Foster (2004). Experiments were conducted to increase caffeine recovery and extract clean-up to yield extracts that would not compromise the performance and longevity of the instruments.

The main changes from the original method are outlined below. Strata X SPE cartridges were utilized instead of HLB. A 3:7 MtBE:ACN solvent mixture was used to elute the Strata X cartridges, instead of EtOAc. SPE extract was subjected to post-treatment using silica gel cartridges, conditioned and washed with the same solvent mixture. As they became available, deuterated caffeine and triclosan were spiked into the samples to

calculate losses of non-deuterated compounds through the methods and concentrations across matrices. Overall, recovery of spiked LGW through the method was 90% for caffeine and 79% for triclosan.

3.2 Application of Caffeine and Triclsoan Method on Environmental Samples

Caffeine and triclosan were proposed as indicators for failed on-site wastewater treatment systems. These compounds have high prevalence in the domestic waste stream, and prior literature has demonstrated high removal from WWTPs (Seiler et al. 1999, Singer et al. 2002). The main routes of triclosan and caffeine removal are aerobic biodegradation, therefore, it was hypothesized that under the anaerobic conditions in septic tanks, little degradation of these compounds will occur. In the case of failed on-site systems, triclosan and caffeine concentrations were expected to be high enough in the tank that even with aerobic environmental degradation, these compounds would be detectable downstream. Caffeine was proposed as a surrogate of hydrophilic compounds and triclosan as a surrogate for hydrophobic compounds. Their behavior can represent a wider range of chemicals with similar properties.

A method was developed to extract caffeine and triclosan simultaneously from varied matrices, which was used to process all the samples. A summary of the analytical method follows: deuterated caffeine and triclosan were spiked into all samples at environmental concentrations before filtration with 1.5µm filter. The Strata X SPE cartridges were conditioned with hexane, MtBE, MeOH and phosphate buffer. Strata X cartridges were eluted onto silica gel cartridges with 3:7 MtBE:ACN. Silica gel cartridges were washed with the same solvent mixture and the extract was then blown to dryness,

reconstituted in ACN, derivatized with BSTFA and pyridine spiked with HCB. Extracts were analyzed by GC-MSD, targeting ion m/z 284 for HCB, 194 for caffeine, 197 for caffeine-d3, 347 for triclosan and 352 for triclosan-d3. Concentrations were calculated using the deuterated standard method, which applies the ratio of deuterated to non-deuterated areas obtained from a solvent calibration curve to environmental samples. An example calibration curve is shown in Appendix 2.

3.2.1 Caffeine and Triclosan Occurrence from Surface Water Surrounding WWTPs

For comparison, three WWTPs in North Carolina were selected for sampling. These WWTPs represented differing treatment capacities, population served sizes, and both UV and chlorine disinfection. These plants provided an indication of the concentration ranges from WWTPs throughout North Carolina and were sampled throughout the year for an indication of seasonal variability. WWTP samples were collected upstream, from within plant effluent, at the point of discharge into the receiving water (if this point was accessible) and downstream of the WWTP discharge. Specifics on sampling locations and procedures are shown in Methods section 2.2.2 and 2.3.1.

For an indication of possible analyte concentrations from a range of WWTPs, the results of the three treatment plants were compiled. Samples were collected from WWTP1 on 6 dates, WWTP2 on 3 dates and WWTP3 twice. The results are presented to portray the range of concentrations over the sampling period by using box and whisker plots. The whiskers represent the 10 and 90 percentile values. The box displays the 25 to 75 percentile values, with the median value represented by the line in the box. This type of graph represents the data range, since the box encapsulates 50% of the values and the whiskers indicate outliers. The actual data points are overlaid on the box and whisker plots, since

sample size was not always large enough to be statistically represented by a box and whisker plot. All data points are shown, however, sometimes the points are not distinguishable due to high concentration of dots in a small range. Discrepancies in sample sizes between caffeine and triclosan was due to non- detection of analytes. Maximum analyte concentrations detected in the effluents were 1.2µg/L caffeine and 1µg/L triclosan, as shown in Figures 3.1 and 3.2.

Overall, the majority of sample concentrations were in a narrow range with high outliers. Outliers could be due to temporal variation in analyte influent concentration or rainfall events that introduce other pollution sources into the surface water and may flush wastewater through the plant without proper treatment. One set of samples for WWTPs 2 and 3 were collected during a rain event, where plants experienced higher than average WWTP flows and could lead to outliers. The likelihood of triclosan to sorb to particles and variable sample storage time could contribute to concentration variation, since only the dissolved component of triclosan was measured with this method.

Other sources of concentration variation could be due to other non-point sources entering into the receiving water. For example, WWTP1 has higher upstream concentrations of both caffeine and triclosan than the other two plants, possibly due to failed on-site wastewater system pollution or community storm-water runoff upstream of the WWTP discharge. This plant represented the situation where caffeine was ubiquitous in the watershed, due to anthropogenic influences. Around WWTP1, upstream concentrations ranged from BD-400ng/L for caffeine and BD-300ng/L triclosan.

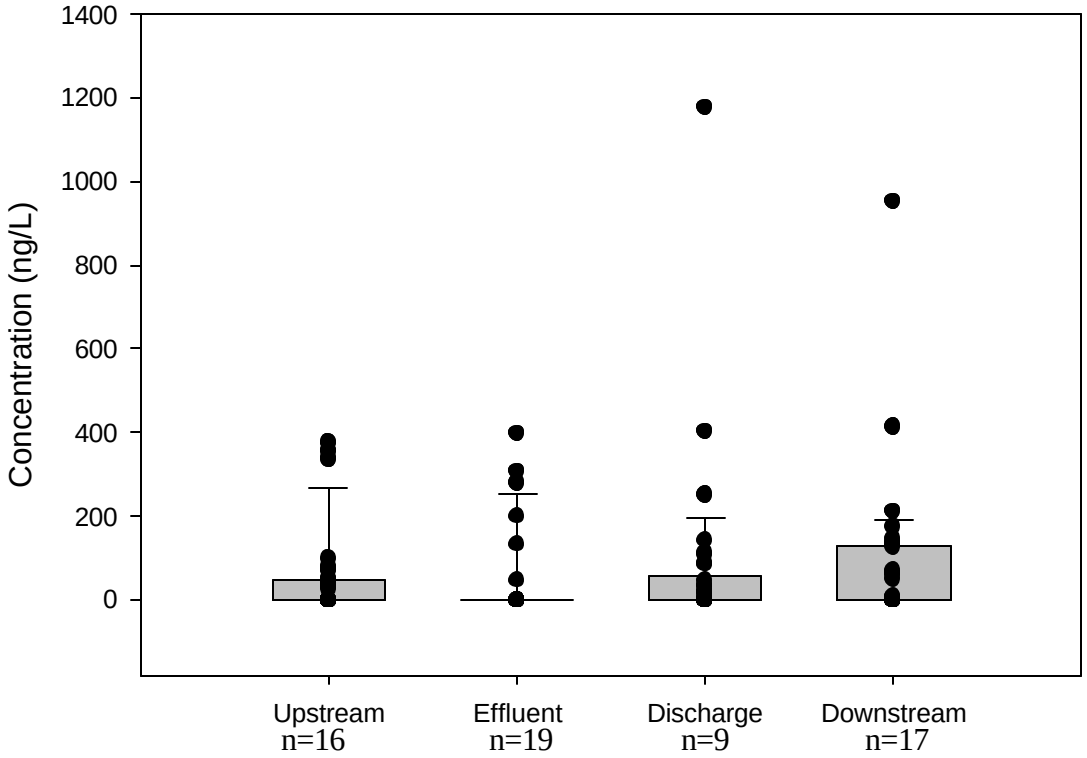

Figure 3.1 Caffeine concentration ranges compiled from three WWTPs and surrounding surface water.

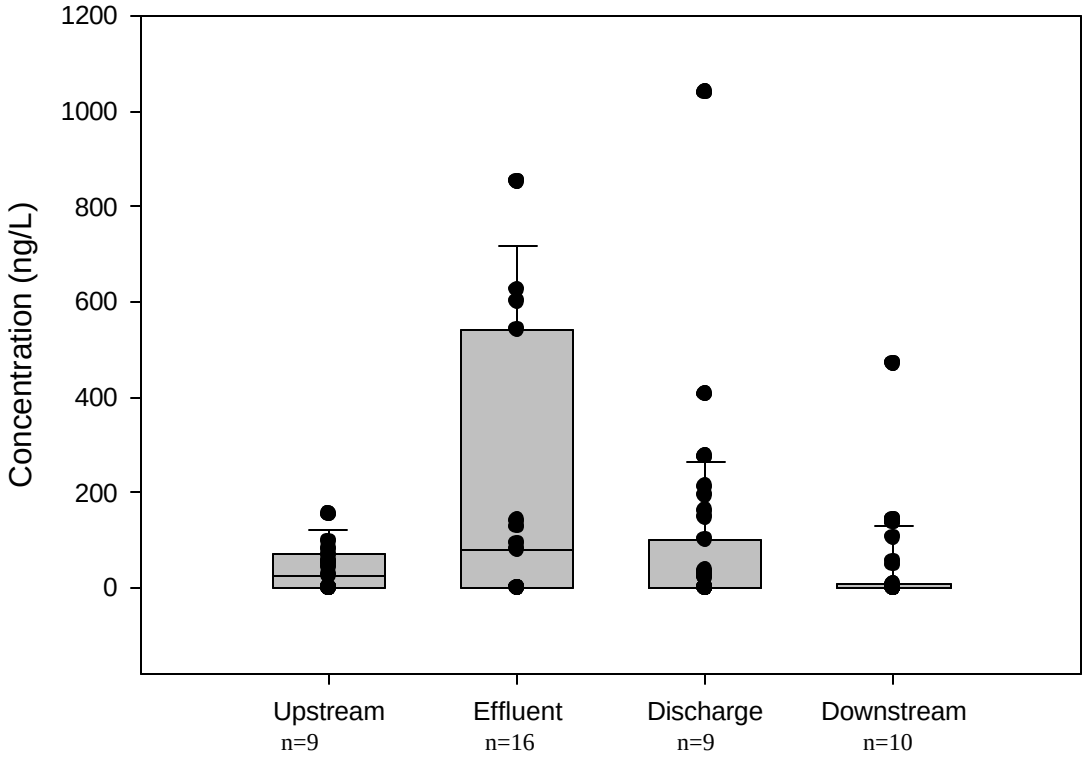

Figure 3.2 Triclosan concentration ranges compiled from three WWTPs and surrounding surface water.

3.2.2 Caffeine and Triclosan Occurrence in On-Site Wastewater Systems

Samples were collected from the septic tanks of a number of on-site wastewater treatment systems to obtain a representative sample of analyte concentrations found in septic tanks across North Carolina, with different influent sources. This occurrence data were used to test the hypothesis that caffeine and triclosan were present in septic tank waste at higher concentrations than from WWTP effluents.

Septic tank results were compiled from the advanced treatment systems and households, as shown in Figure 3.3. The details of site selection are described in Methods section 2.2.1. Samples were collected over the course of the year for temporal and seasonal variability. IWS and the high school system were sampled four times and households were sampled on three dates. Even though samples were analyzed in triplicate, some samples were lost during processing or were not sufficiently clean for instrument analysis.

The wide concentration range from the septic tanks was most likely due to sample collection before and after lunch time at the high school system, which yielded a large range of influent concentrations. The other tanks have good precision, although this may be due to insufficient sample size to capture the variability of analytes in the waste stream. Caffeine and triclosan septic tank concentrations were in the µg/L range, as compared to the ng/L range detected from WWTP effluents, as seen in Figures 3.1 and 3.2.

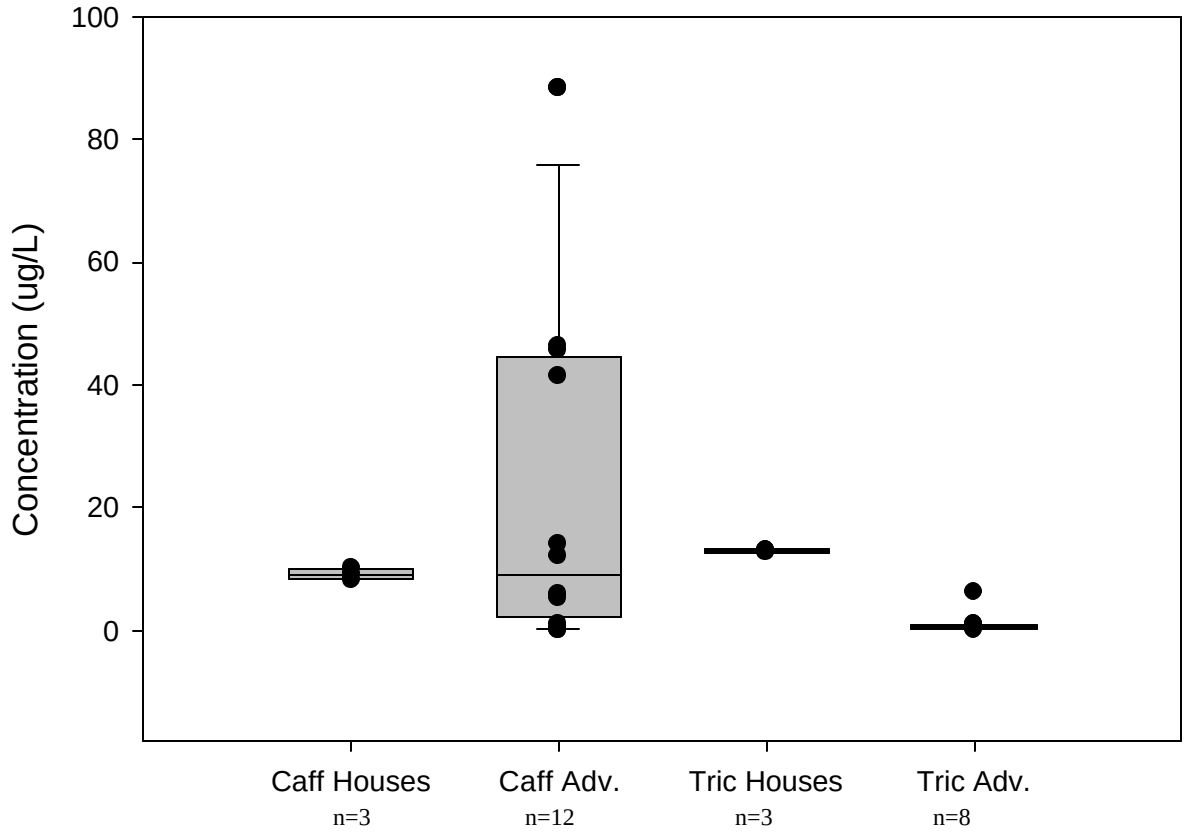

Figure 3.3 Comparison of caffeine and triclosan occurrence from septic tanks, both households and advanced treatment.

Advanced treatment systems were hypothesized to be comparable to functioning on-site wastewater treatment system, since they both employ similar treatment processes: settling, aerobic, and anaerobic degradation. Samples were collected throughout the high school advanced treatment system for an indication of caffeine and triclosan degradation through a functioning system (Sampling locations are shown in Figure 2.2). Figure 3.4 shows average caffeine and triclosan concentrations through the high school system, with error bars representing the standard deviation. Outliers were removed from consideration.

After aerobic and anaerobic treatment in the vegetated sand filter (1x), caffeine and triclosan concentrations were greatly reduced from septic tank effluent. After further aerobic degradation, concentrations (Pre-UV) were comparable to background levels as

seen in upstream samples. Detection of caffeine at the same concentration up and downstream suggested that the system did not contribute additional analytes into the river.

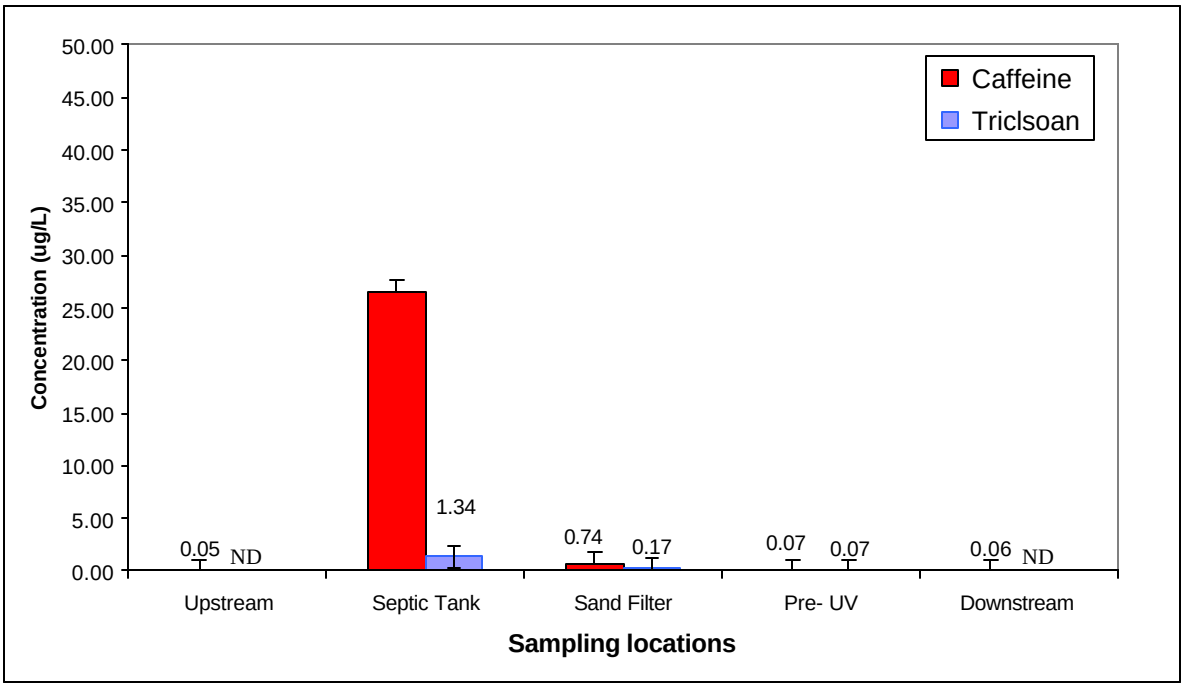

Figure 3.4 Average caffeine and triclosan occurrence throughout the high school advanced treatment system. (US n= 5 caffeine, 0 triclosan; Effluent n= 10, 1x n= 5, Pre-UV n= 5, and downstream n= 5 caffeine, 0 triclosan)

Extrapolating the results shown in Figure 3.4 to a functioning on-site wastewater treatment system imply that effluent from a functioning system (estimated by Pre-UV concentrations) would contain analyte levels in the concentration range of WWTP effluent and not septic tank effluent. It appears that effluent from a functioning system would not be a significant source of caffeine or triclosan to surface waters. Extrapolation of caffeine and triclosan to the broader spectrum of chemicals they represent indicates effective degradation of both hydrophilic and hydrophobic compounds within functioning septic systems.

Comparison of the analyte concentrations in WWTP and septic tank effluents show an order of magnitude difference, as seen in Figures 3.5. The average of all three WWTP effluent concentrations was 230ng/L caffeine (n=30) and 300ng/L triclosan (n=31). In

contrast, average septic tank concentrations, including both households and advanced treatment, were 22µg/L caffeine (n=16) and 6.8µg/L triclosan (n=23). Extrapolation of advanced treatment to a functioning septic system suggested that effluent from a functioning system would be an average of 70ng/L for both analytes.

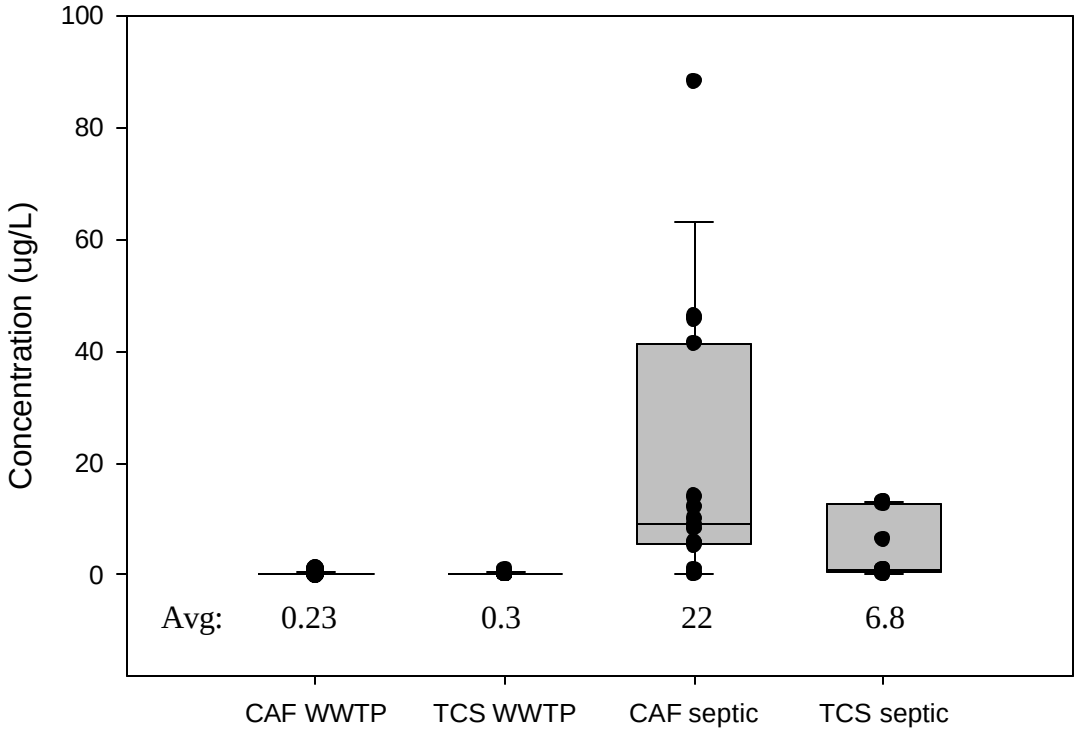

Figure 3.5 Comparison of average caffeine and triclosan concentrations between wastewater treatment plants and septic tank effluent.

3.2.3 Estimating Caffeine and Triclosan Persistence Downstream

Septic tank caffeine and triclosan concentrations were high enough that it was hypothesized that in the event of a septic system failure, where effluent flowed out from the tank into the environment, caffeine and triclosan would travel overland to surface water, be detectable downstream and persist at levels above WWTP effluent. This assumption was tested with dilution calculations and laboratory experiments.

The persistence of compounds downstream of a WWTP and a failed system were estimated using degradation equations presented by Morrall et al. (2004) and average concentrations from both wastewaters. The following equation was used to estimate the time and stream distance of analyte degradation:

$$C_T(t) = C_{T(0)}e^{-k_T t} \quad (3.1)$$

where C_T = total chemical concentration (ng/L), $C_T(t)$ = chemical concentration at time t (ng/L), $C_{T(0)}$ = initial chemical concentration (ng/L), k_T = total chemical first-order loss rate (h^{-1}), and t = time (h). Initial concentrations were the average concentrations from septic tanks and WWTP effluent, k_T was 0.06/h (Morrall et al. 2004), and average stream flow (m^3/s) was estimated from Morgan Creek USGS monitoring station from 2004-2005 at a rate of 0.078 m^3/s. (http://nwis.waterdata.usgs.gov/nc/nwis/qwdata/?search_site_no=02097464).

Table 3.13 shows calculations of triclosan removal via sorption and settling from both average septic (6772 ng/L) and WWTP effluent (293 ng/L) concentrations. Using these calculations in combination with the SPE PQL of 60ng/L, triclosan was below the detection limit from WWTP effluent discharge into surface waters after 25 hours, or 2 miles. Triclosan from septic tanks was estimated to be detectable for over 75 hours and 6 miles downstream. Additionally, triclosan from septic tanks was estimated to be detectable for 50 hours and 4 miles downstream of failed septic system at a concentration above the average WWTP effluent. These estimations may overestimate detectable time and distances since they do not include photolysis or microbial degradation. However, these calculations provide an indication of the persistence of triclosan in water downstream of potential septic system failure.

Table 3.13 Triclosan removal calculations from average WWTP and septic tanks.

Time (hours)	C_T WWTP (ng/L)	Distance (miles)	C_T septic (ng/L)
0	293	0	6772
1	276	0.1	6378
5	217	0.4	5017
10	161	0.8	3717
20	88	1.6	2040
25	65	2.0	1511
30	48	2.4	1119
40	27	3.1	614
50	15	3.9	337
60	8.0	4.7	185
70	4.4	5.5	102
75	3.3	5.9	75
80	2.4	6.3	56
90	1.3	7.1	31
100	0.7	7.9	17

Caffeine persistence was estimated from Equation 3.1 with estimated caffeine biodegradation rate constants of $1.25x10^{-4}$-$2.92x10^{-4}$ h^{-1} (Buerge et al. 2006). Equation parameters were: $k_T = 1.67x10^{-4}$ h^{-1}, average stream flow = 2.77 or 0 078 . m^3/s and C_T was the average caffeine concentrations from WWTPs (226 ng/L) and septic tanks (22232 ng/L). Results are shown in Table 3.14.

Table 3.14 Biodegradation calculations of caffeine from WWTP and septic effluent.

Time (hours)	C_T WWTP (ng/L)	Distance (miles)	C_T septic (ng/L)
0	226	0	22232
1	226	0.1	22228
10	226	0.8	22195
50	224	3.9	22048
100	222	7.9	21865
500	208	39.4	20454
1000	191	78.7	18819
5000	98	393.6	9662
10000	43	787.1	4199

A laboratory experiment was conducted to estimate dilution of septic tank discharged into surface waters. High school advanced treatment septic tank samples were diluted with upstream surface water collected by the on-site treatment system. Triplicate dilutions were

prepared at 1:20, 1:50, and 1:100 in 250mL aliquots. Average septic tank flow rates of 69.3 gallons/person/day, estimated by Mayer et al. (1999), were used assuming a four person household for an average of 277.2 gpd. Assuming that 100% of effluent from a failed tank reached surface water, dilution calculations at this range represent small streams, shown in Table 3.15, which may not accurately represent the size of streams near failed septic systems. Analytes were considered detectable if the concentration was higher than the SPE method PQL of 60ng/L for caffeine and triclosan. Although there was only a five-fold increase in the dilution factor between 1:20 and 1:100 dilutions, there was a ten-fold decrease in caffeine concentration, which implied that dilution and degradation of analytes may not be linear. A non-linear relationship creates uncertainty regarding estimates from Tables 3.13 and 3.14.

Table 3.15 Dilution experiment with effluent from the septic tank at the high school system.

	Caffeine Conc (µg/L)	RPD	Triclosan conc (µg/L)	RPD	Stream flow (ft^3/s)	Stream flow (m^3/s)
Effluent (n=2)	8.1	1.8	0.68	7.1		
1:20 Dilution (n=2)	0.32	25.2	0.05	10	0.009	2.55×10^{-4}
1:50 Dilution (n=1)	0.09		-		0.021	5.95×10^{-4}
1:100 Dilution (n=1)	0.03		-		0.043	1.22×10^{-3}
Upstream (n=1)	0.18		0.06			

3.2.4 Detection of Caffeine and Triclosan in Surfacing Septage

Occurrence data was collected to test the assumptions presented for caffeine and triclosan as potential indicators of on-site wastewater failure pollution: that caffeine and triclosan would be present in septic tanks at higher concentrations than WWTPs, caffeine and triclosan in the septic tank effluent would persist in the event of a system failure, analytes have the potential to travel overland and would be detectable downstream.

Triplicate samples were collected from both the septic tank and pooled surface sewage next to the septic tank of a failed septic system in a mobile home park. This system

was identified by the Orange County Health Department. Effluent had been flowing straight out of the tank from the broken drainage pipe for several days before sample collection. Average concentrations of caffeine and triclosan in the septic tank and pooled sewage are shown in Figure 3.6 (n=3), with error bars representing standard deviation. Both analytes were detected in the sewage on the ground even after several days of potential microbial degradation and photolysis. Analytes in ponded sewage have the potential to travel to surface water via overland flow or rainfall. Even though analyte concentrations in the surface sewage were considerably lower than the septic tank, they remained higher than maximum WWTP effluent concentrations. These results verify that a failed septic system would release these analytes into the environment.

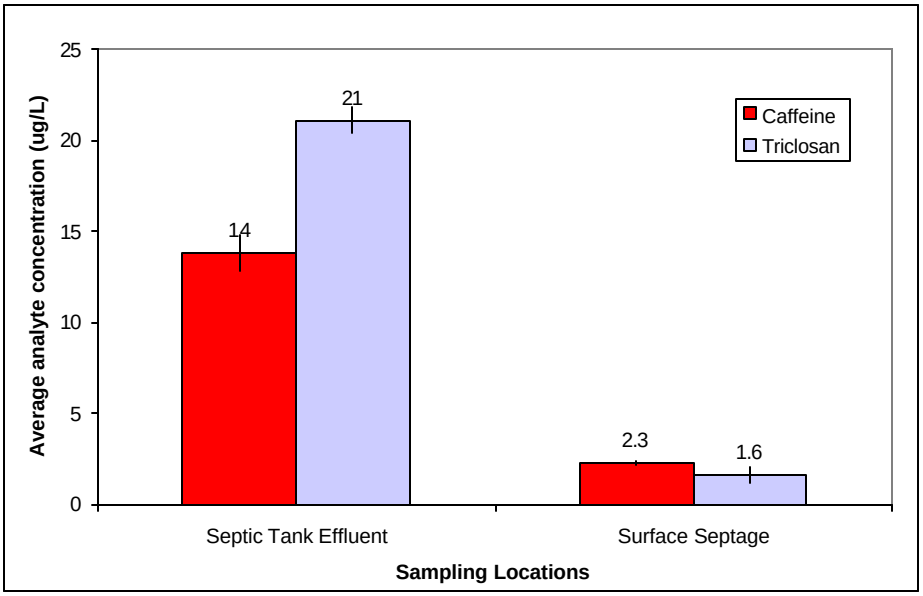

Figure 3.6 Average caffeine and triclosan occurrence in septic tank effluent and ponded surface sewage.

3.2.5 Detection of Caffeine and Triclosan in Surface Water Downstream of Failed Septic System

A second failed septic system, near a stream, was identified with the help of the Orange County Health Department. This system failed due to soil clogging in the drainfield.

Therefore, the effluent had undergone some aerobic and anaerobic degradation before surfacing and running off into surface waters. At the time of inspection, a small pool of surfacing sewage was visible on the surface at the far end of the drainfield. Grab samples were collected from within the septic tank and upstream of a nearby stream. These samples were analyzed in triplicate. During a rain event several days later, up and downstream samples were collected to capture potential pollution. Triplicate samples were collected at three sampling times in an attempt to capture the rising limb of the stream hydrograph. The rising limb was the expected time of highest runoff and, therefore, highest pollution potential. Without a stream gauge to accurately assess stream stage, stream height was measured during sampling. Stream height continued to rise throughout the sampling period, so it was assumed that the stream would continue to rise as it continued raining. Figure 3.7 shows the locations of the sampling sites. The failed system is approximately 180 meters (0.11 miles) from the stream over land and approximately 290 meters (0.19 miles) upstream of the downstream sampling location.

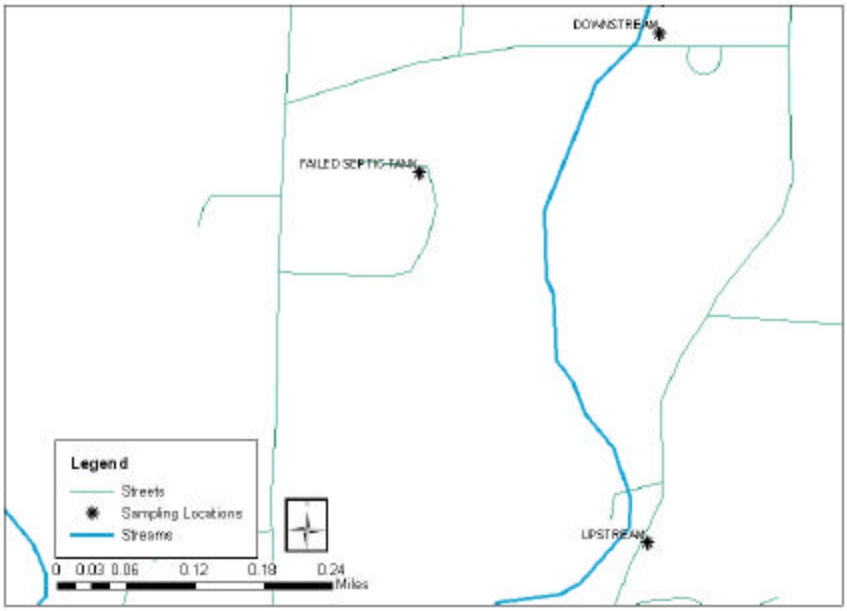

Figure 3.7 Sampling locations around failed septic system.

Even with large distances of overland flow and in-stream movement downstream, caffeine was detected in all triplicate samples from the last sample collection in the rain event. Septic tank and stream caffeine and triclosan concentrations are shown in Figure 3.7, with error bars representing standard deviation (n=3). Average caffeine concentration downstream of the failed system was 270ng/L, which was slightly higher than the average caffeine concentration from WWTP effluents and higher than concentrations found downstream of WWTPs.

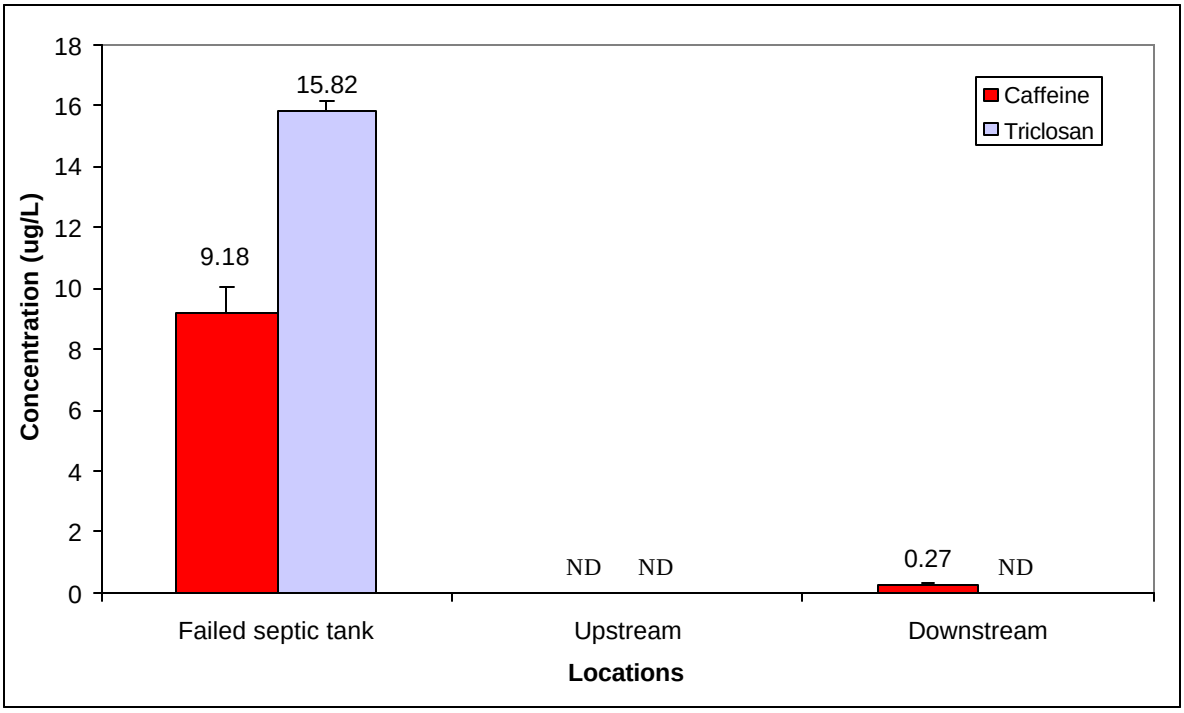

Figure 3.8 Average caffeine and triclosan occurrence in a failed septic tank and downstream surface water (n=3).

The detection of analytes in both sewage pooled on the surface and downstream of failed systems implies the ability of analytes to migrate overland and persist downstream, despite possible environmental degradation through sorption, settling, photolysis and microbial degradation.

3.3 DCAA and TCAA: Method Development

Literature findings of HAA formation in drinking water treatment can be extrapolated for an indication of HAA formation during chlorination of wastewater effluent. HAA formation is limited by the amount of nitrification in the effluent. If the wastes are highly nitrified, chlorine will first interact with ammonia to form chloramines and then NOM to form DBPs, such as HAAs. Research by Sirivedhin and Gray (2005) indicated that waters with high DOC, such as wastewaters, have a higher disinfection by-product formation potential (DBPFP) than natural waters with exposure to chlorine. Krasner et al. (2005) showed that HAAs can form during chlorine disinfection of municipal WWTP effluent. However, only low levels of HAAs were detected due to high concentrations of ammonia in the waste. These findings suggest that HAAs will form at detectable concentrations as wastewater effluent is chlorinated and will persist downstream of municipal WWTP discharges.

This hypothesis was tested on waters surrounding WWTP1, which chlorinated and de-chlorinated the effluent. Upstream, effluent, and downstream samples were collected and extracted using EPA method 552. Early applications of this method showed DCAA and TCAA concentrations below the PQL of 2µg/L. An additional calibration point was added to lower the PQL to 1µg/L, but, many DCAA and TCAA concentrations were still below this value. In order to quantify low DCAA and TCAA concentrations, method development was undertaken to increase the concentration factor during extraction by several methods: concentration of solvent extraction volume, larger volume liquid-liquid extraction (LLE) and solid phase extraction (SPE).

3.3.1 Evaluating Effect of Extracting Solvent Concentration by Blow-down

HAAs exist in the environment in acid form, and they need to be derivatized to methyl esters for detection using gas chromatography. In order to test the effectiveness of solvent blow down as a concentration step, experiments were first performed to determine if greater recovery resulted from blowing down the acid or ester form. Esters were diluted from the haloester 6 standards stock mixture containing methyl DCAA at 600µg/L and methyl TCAA at 200µg/L. Since methyl DCAA and methyl TCAA were present in the mixture at different concentrations, separate dilutions were prepared to obtain similar concentrations for both analytes. Mix A contained 110µg/L methyl TCAA and 330µg/L methyl DCAA and mix B contained 11µg/L methyl TCAA and 33µg/L methyl DCAA. Mix C had 40µg/L methyl TCAA and 120µg/L methyl DCAA and mix D was 4µg/L methyl TCAA and 12µg/L methyl DCAA. All solutions were prepared in 2mL volumetric flasks containing MtBE. The low concentration solutions were transferred to 10mL centrifuge tubes, blown down to approximately 200µL under nitrogen (visually assessed) and analyzed on the GC-ECD alongside aliquots of the original solutions to calculate recoveries. During a separate evaluation, it was determined that visual approximation of 200µL was off by an average of 24µL. Table 3.16 shows the results of this experiment, which indicate ester form blow down was more effective at lower concentrations. Initial concentrations are the concentrations before blow down. If 100% of the TCAA and DCAA esters remained after blow down, then the concentrations in the blown down solution would be 110 and 120µg/L, respectively. Expected areas for blown down concentration was the area of the 110 or 120µg/L solution. Percent recovery (% recov) was calculated by:

$$\text{Percent recovery} = \frac{\text{(detector response of blow down solution)}}{\text{(detector response of the concentrated source solution)}} \times 100 \qquad (3.1)$$

Table 3.16 DCAA and TCAA ester recovery from blow down, analyzed by GC-ECD. Expected areas of blown down solvents were the areas of the non- blown down solvent mixtures.

	Blown Down	Initial DCAA conc (µg/L)	Methyl DCAA Area	Expected Area	% recov	Initial TCAA conc (µg/L)	Methyl TCAA Area	Expected area	% recov
Mix A		330	13317			110	18763		
Mix B	Y	33	12038	13317	90	11	8920	18763	48
Mix C		120	5035			40	2548		
Mix D	Y	12	5975	5035	119	4	2889	2548	113
Avg					**108**				**81**
% CV					28				81

Evaluation of the effect of blow down on the acids was undertaken on duplicate 1µg/L solutions prepared in 2mL volumetric flasks of MtBE (Acid replicate A and B). Solutions were transferred to 10mL centrifuge vials, and blown down to approximately 200µL under nitrogen, which was visually assessed. Blown down extracts were derivatized with 25µL diazomethane/MtBE for 15 minutes at 4°C, quenched with a few grains of silicic acid, and analyzed on the GC-ECD alongside derivatized solutions of the original 1µg/L. Table 3.17 shows the recovery of DCAA and TCAA using this technique. If no loss of acid had occurred during blow-down, the area response of the concentrated solution would be given by Equation 3.2:

$$\text{Area of concentrated solution} = \text{area of source solution} \times \text{concentration factor} \qquad (3.2)$$

For this experiment, the estimated concentration factor was nine, since 1.8mL was reduced to approximately 200µL. This recovery calculation was made from a single point measurement rather than from a calibration curve, which could generate error. Although acids were derivatized into esters for analysis, results are presented as acids to distinguish when using ester stock.

Table 3.17 DCAA and TCAA recovery from blow down of acid form, analyzed by GC- ECD. Expected areas were extrapolated from Equation 3.2

	Blown down	DCAA Area	Expected Area	% recovery	TCAA Area	Expected Area	% recovery
Acid replicate A		4083			4889		
Acid replicate A	Y	23734	36747	65	70148	44001	160
Acid replicate B		3544			4320		
Acid replicate B	Y	19062	31896	62	60339	38880	155
Avg				**62**			**157**
RPD				8			3

There was a discrepancy in acids and esters concentrations, which may be due to degradation of the ester stock. To calculate accurate concentrations, 500µg/L acids were prepared in 2mL MtBE, derivatized and analyzed on the GC-ion trap-MS, CI mode. Analyte area of derivatized acids were compared to a solution of 555µg/L esters, which accounted for the mass difference between the acids and esters and represents the concentration if all the acids were recovered. Actual concentrations of ester stock were estimated as TCAA: 240µg/L and DCAA: 370µg/L.

The ester portion of the above experiment was repeated in duplicate, using dilution calculations based on estimated ester stock concentration. DCAA and TCAA recovery is presented in Table 3.18. Dilutions of the stock solution were prepared at 12µg/L and 120µg/L using corrected concentrations for both analytes. Duplicates were prepared of lower concentration mixtures for analysis of precision in blow-down. Mix A contained 120µg/L methyl TCAA and 185µg/L methyl DCAA, while mixes B and C had 12µg/L methyl TCAA and 18.5µg/L methyl DCAA. Mix D had 120µg/L methyl DCAA and 78µg/L methyl TCAA and mixes E and F contained 12µg/L DCAA and 7.8µg/L methyl TCAA. Internal standard, 1-2, dibromopropane, was added after blow down at a concentration of 50µg/L to ensure that variability in detector response was not due to injection error. If 100% of the 12µg/L ester

was recovered after blow-down, the chromatographic response would be equivalent to that of ten times the concentration (i.e. 120µg/L). Comparing the results of Tables 3.17 and 3.18, DCAA area was in the order of magnitude expected from 1µg/L acid areas but TCAA was enhanced. Comparing the average recoveries in Table 3.15 and 3.18, the repeat experiment yielded lower average recoveries.

Table 3.18 Repeat of esters blown down and recovery experiment, analyzed by GC-ECD. Expected area was calculated using Equation 3.2.

	Blow down	Initial DCAA conc (µg/L)	IS Area	Methyl DCAA Area	Exp Area	% recov	Initial TCAA conc (µg/L)	Methyl TCAA Area	Exp Area	% recov
Mix A		185		601603			120	1584094		
Mix B	Y	18.5	22347	627945	601603	104	12	775520	1584094	49
Mix C	Y	18.5	29825	671833	601603	112	12	823547	1584094	52
Mix D		120		256187			78	616720		
Mix E	Y	12	49980	166159	256187	65	7.8	195076	616720	32
Mix F	Y	12	33612	206164	256187	80	7.8	246326	616720	40
Avg			**33941**			**90**				**43**
% CV			34			24				21

3.3.1.1 Solvent Blow Down After LLE

Twenty mL duplicates of LGW and WWTP1 upstream (WWTP1 US) water samples were spiked with 2µg/L DCAA and TCAA and processed using LLE extraction (Brophy et al. 2000). MtBE extract was transferred to 2mL volumetric flasks, extract was derivatized and 200µL was transferred using a micropipetter to an insert inside a 2mL auto-sampler vial for GC-ECD analysis. The remaining volume was transferred to a 10mL centrifuge vial, and blown down to approximately 200µL under nitrogen, which was visually assessed. Fifty µg/L IS was added and transferred to an auto-sampler insert for GC-ECD analysis. Results are shown in Table 3.19. Expected area was calculated using Equation 3.2. For this experiment, the concentration factor was nine, since 1.8mL was blown down to approximately 200µL.

Table 3.19 Average DCAA and TCAA recovery from spiked LGW and WWTP1 US following LLE and blow-down, analyzed by GC-ECD.

	Blow down	IS Area	DCAA Area	Exp Area	% Recov	TCAA Area	Exp Area	% recov
Avg LGW		254602	40643			185724		
Avg LGW	Y	562878	141753	365787	39	542924	1671516	32
Avg WWTP1 US		224248	45663			208208		
Avg WWTP1 US	Y	682825	215565	410967	52	787217	1873872	42

The results show lower recovery, particularly for DCAA in acid form, than when the ester standard is blown down. However, this could be partially explained by less than 100% recovery of the acid during extraction. Experiments of ester form blow down from extracts of LGW and surface water yield lower recoveries than experiments in pure solvent (Section 3.3.1) and a similar effect could be happening with the acid forms. Blow down of internal standard yielded areas 2-3 times as large as non-blown down extracts, rather than the nine-fold expected concentration factor, but this was most likely due to volatility losses of the compound. Recovery was higher for DCAA when blowing down in ester form than as an acid, but for TCAA it was higher when blown down in acid form. However, blowing down as acids was less variable, practically easier, and avoided the presence of magnesium sulfate and silicic acid granules, which were used for derivatization, during blow-down. For these reasons, HAAs were blown down in acid form.

3.3.2 Larger Volume Liquid-Liquid Extraction

Liquid-liquid extraction (LLE) was evaluated using a larger aqueous volume. Duplicate 100mL LGW aliquots were spiked with 2µg/L of DCAA and TCAA and sample pH was adjusted to a value below 1 with 7.5mL of concentrated H_2SO_4. The sample was transferred to a 250mL separatory flask, 20mL MtBE as extraction solvent was added,

followed by 6mg of sodium sulfate to salt out the acids. Extraction was conducted by capping the separatory flask, inverting several times, and allowing the water and organic phases to separate for approximately five minutes. After separation, the aqueous layer was drained and the remaining MtBE organic solvent layer was collected into 40mL glass vials. The extract was then blown down under nitrogen to slightly less than 2mL, transferred to a 2mL volumetric flask and adjusted to volume with MtBE. One hundred µg/L IS was added to the extracts, which were then derivatized according to the method by Brophy et al. (2000) and the resulting esters analyzed on the GC-ECD. The total concentration factor was 50, which, assuming 1005 recovery, would generate an ester concentration in the final extract of just over 100µg/L.

Table 3.20 shows the experiment results: average of 10% for DCAA and 35% for TCAA. Expected response was calculated using the trendline equation from absolute areas of the calibration curve, which had been prepared by smaller volume LLE. The fact that the estimation of 125µg/L was obtained from the smaller volume LLE adds uncertainty to the results. However, general recovery from LLE extraction should be comparable, despite volume differences. Method losses could be due to concentration of extraction solvent in acid form after LLE. Results from Table 3.18, of spiked LGW extracted using LLE for estimated extract concentration of 10µg/L, show DCAA area around 40,000 counts, which was larger than this area for estimated concentration of 100µg/L. This discrepancy implies that larger volume LLE was not as effective at recovering DCAA.

Table 3.20 DCAA and TCAA recovery from larger volume LLE with extraction solvent blow down, analyzed by GC-ECD.

	IS Area	DCAA Area	Expected results	% recovery	TCAA Area	Expected results	% recovery
LLE A	546499	26309	254883	10	355110	750140	47
LLE B	605535	27897	254883	11	166366	750140	22
Avg	**576017**	**27103**		**11**	**260738**		**35**
RPD	10	5.9		5.9	72		72

Recovery of DCAA and TCAA extracted through larger volume LLE was retested. Triplicate 100mL aliquots of LGW were spiked with 2µg/L DCAA, TCAA and DCAA-d2 were extracted as described above, derivatized and analyzed on the GC-MSD along with a solvent standard prepared at the theoretical 100% recovery concentration. Table 3.21 shows the results of the retest of analyte recovery through larger volume LLE extraction. Percent recovery was the average area of LGW extracts compared to the average standard area for that ion. Recovery was higher than the first experiment, but DCAA-d2 ion m/z 86 has very low recovery. There are practical limitations to LLE; it was more time consuming than SPE and there were a limited number of separatory flasks available to perform multiple sample extractions.

Table 3.21 DCAA (ions m/z 83 and 85), TCAA (ions m/z 117 and 119) and DCAA-d2 (ions m/z 84 and 86) recovery from larger volume LLE analyzed on GC-MSD.

	83- % recovery	85- % recovery	117- % recovery	119- % recovery	84- % recovery	86- % recovery
Avg LGW	75	58	58	42	62	7.5

3.3.3 Solid Phase Extraction (SPE)

Several SPE cartridges and conditioning/elution methods were tested to concentrate DCAA and TCAA from aqueous samples and remove interfering humics and other organics.

The use of C18 cartridges (Alltech, Deerfield, IL) was based on the method of Yoo et al. (1992). Duplicate 250mL unfiltered aliquots of WWTP1 US were spiked with 1µg/L of

both DCAA and TCAA, 2mL MeOH was added, and the solution was acidified to pH < 1 with concentrated H_2SO_4. The C18 cartridges were conditioned with 3mL MtBE, 3mL MeOH, and 2 x 3mL LGW. The sample was passed at an approximate flow rate of 1mL/min. Then the cartridges were dried under vacuum and nitrogen for 20 minutes each before eluting with 2 mL MtBE containing 50µg/L IS. The extract was transferred to 2mL volumetric flasks, adjusted to volume with MtBE + IS, derivitized with diazomethane as in the method by Brophy et al. (2000), and analyzed with GC-ECD. The overall concentration factor was 125, yielding an expected HAA concentration in the final extract of 125µg/L, assuming 100% recovery.

Table 3.22 shows the results of this initial SPE experiment and demonstrates the variability. Expected results were extrapolated from the absolute area trendline of the calibration curve of LGW extracted with smaller volume LLE to calculate estimated area for 125µg/L in the extract. Estimating concentration from a different extraction method adds uncertainty to the recovery calculations. Additionally, the areas of all analytes are larger than the areas in Table 3.20, although assuming 100% recovery, they should all be 125µg/L. These increased areas, especially for internal standard, were hypothesized to be coming from other compounds co-eluting from the cartridge bed with similar retention times to DCAA and the internal standard.

Table 3.22 DCAA and TCAA analysis and recovery from surface water extracted by C18 SPE, analyzed by GC-ECD.

	IS area	DCAA area	Expected areas*	% recovery	TCAA area	Expected areas*	% recovery
SPE A	1440925	19962	316698	6.3	85078	918000	9.3
SPE B	7752675	906178	316698	286	275934	918000	30
Avg	**4596800**	**463070**		**146**	**180506**		**20**
RPD	137	191		191	106		106

* obtained from calibration curve obtained by LLE using 20mL LGW and 4mL MtBE

Of all the concentration methods tested, SPE appears to be the most efficient and was further investigated. However, there were no comparable standards to accurately calculate percent recovery and the problems of increased internal standard area and high variability among results needed to be corrected.

3.3.3.1 Increasing C18 recovery with two cartridges in-series

In order to enhance SPE recovery, two C18 cartridges were tested in series. Two identical C18 cartridges were individually conditioned as described in section 3.3.3, connected in series leaving LGW in the lower cartridge before adding aqueous sample into the upper cartridge. In-series cartridges were each separately eluted, as described in sect. 3.3.3, into one test tube and compared to the elution of a single cartridge using the same extraction method. Samples were analyzed using GC-ion trap-MS, CI mode. Each method was tested on triplicate 100mL LGW aliquots (A-C) spiked with 3µg/L DCAA and TCAA, with expected concentration in final extract was 375µg/L. However, no standard was prepared for comparison. One single cartridge extract was spilled in processing. While DCAA was detectable in all five samples, TCAA was only visible in the chromatogram of the sample labeled 'In-series-C'. Analyte areas are shown in Table 3.23. There were many extra peaks in all chromatograms, which may have prevented the detection of TCAA. If the result of this sample was considered an outlier and removed from consideration, the average area increased and the variance decreased. (Results with outliers removed are bolded). Results confirmed tandem C18 cartridges were more effective at analyte recovery than single cartridges.

Table 3.23 DCAA and TCAA recovery from C18 SPE. Analyzed by GC-ion trap-MS, CI mode.

	DCAA Area	Average	RPD/ % CV	TCAA Area
Single - A	46699	43344	16	ND
Single - C	39989			ND
In-series - A	82711	67638	31	ND
In-series - B	76441			ND
In-series - C	43762	**79576**	**7.9**	120862

ND= not detected

3.3.3.2 Comparison of SAX, C18 and HLB Cartridges

SAX, C18 and HLB SPE cartridges were tested for analyte recovery. Triplicate 100mL LGW aliquots spiked with 3µg/L DCAA and TCAA were tested for each method. Sample size was reduced from 250mL, used in previous experiments, based on research by Martinez et al. (1998), which suggested that higher recovery could be achieved with smaller sample sizes. The C18 cartridges were conditioned and eluted as described in section 3.3.3. One µL MeOH was added to each LGW aliquot, which was then acidified to below pH 1 with concentrated H_2SO_4.

Bond Elute SAX cartridges (Varian, Palo Alto, CA), used as a pre-treatment, were conditioned using the method of Martinez et al. (1998) and used in combination with either C18 cartridges conditioned as described in section 3.3.3 or Oasis 60mg/3cc HLB (Waters, Milford, MA) cartridges conditioned as described below. The SAX cartridges were conditioned with 5mL MeOH and 5mL LGW at pH 0.5 (adjusted with concentrated H_2SO_4). The LGW aliquots were then passed over the SAX cartridges at approximately 15mL/min. The SAX cartridges were not allowed to dry after conditioning and were connected in series with either HLB or C18 cartridges. Each SAX cartridge was washed with 2.5mL LGW at pH 0.5 onto the HLB or C-18 cartridge. The SAX cartridge was removed and the C18 or HLB cartridge was dried before elution.

A final method was based on Loos and Barcelo (2001). LGW aliquots were acidified to pH 1.8 with concentrated H_2SO_4. The HLB cartridges (60mg/3cc) were conditioned with 5mL MeOH and 3mL LGW at pH 2.5, adjusted with H_2SO_4, at a flow rate of approximately 1mL/min. After separate conditioning, two HLB cartridges were stacked in series and the LGW aliquot was loaded at a rate of 5mL/min. The cartridges were then separated and each cartridge was washed with 1mL LGW at pH 2.5. The cartridges were dried under both vacuum and nitrogen. Three plastic syringe volumes of air were forced through to ensure dryness. Finally, the cartridges were eluted with 4mL of MeOH:acetone (50:50 v:v), which was blown to dryness under nitrogen, reconstituted in 2mL MtBE + IS and derivatized. In later experiments, cartridges were eluted with MtBE + IS to avoid blow down and reconstitution losses.

Samples were analyzed using GC-ion trap-MS, CI mode, but a large contamination peak interfered with analyte detection. Figure 3.9 shows the chromatograms extracted for ions m/z 143 and 177 for DCAA and TCAA esters, respectively. The chromatogram for analysis of a 1mg/L ester standard is shown for comparison of analyte retention times. Qualitative analysis suggested the smallest contamination resulted from SAX-C18 or Loos and Barcelo HLB methods.

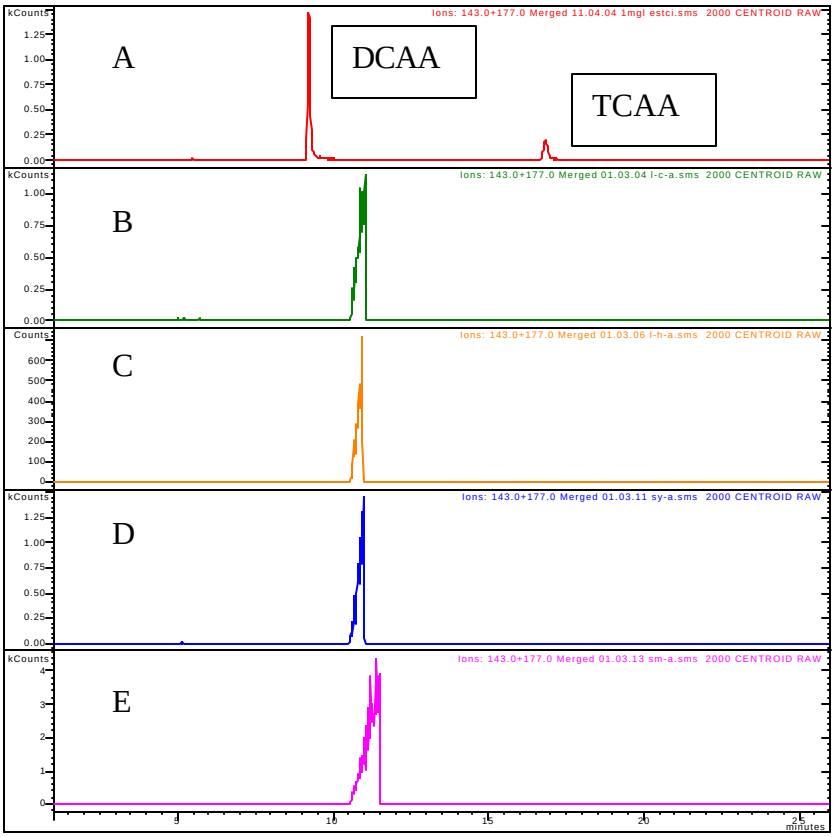

Figure 3.9 Ion chromatographs, extracted for ions m/z 143 (DCAA ester) and 177 (TCAA ester), of the following methods: (A) 1mg/L esters, (B)Loos C18, (C)Loos HLB, (D)SAX-C18, (E)SAX -HLB, using GC-MS CI mode.

3.3.3.3 Comparison of SAX-C18 SPE and Sample Volume

Martinez et al. (1998) suggested that analyte recovery from tap water samples may be affected by pH and sample size. The effect of sample size was further tested using duplicate 50 and 100mL aliquots of LGW, which were spiked with 8µg/L and 4µg/L, respectively, of DCAA and TCAA. SPE extraction was performed using the SAX-C18 method described in 3.3.3.2, derivatized and analyzed by GC-ion trap-MS, CI mode. A dilution containing 200µg/L DCAA and TCAA, the theoretical 100% recovery concentration, was prepared, derivatized and analyzed by GC-ion trap-MS for comparison. Internal standard was not detectable with GC-ion trap-MS, CI mode. Table 3.24 shows DCAA and TCAA recovery comparing sample extraction size. Comparing average areas, 100mL sample recovered more

analytes than 50mL. This experiment would need to be repeated with surface water to assess if NOM from surface or wastewater was retained by the cartridge bed sites, resulting in lower recovery as analytes passed un-retained. The extremely high areas and RPDs from LGW samples suggested contaminants co-eluted from the C18 cartridges.

Table 3.24 DCAA and TCAA recovery from SAX-C18 SPE comparing aqueous sample size.

	DCAA Area	Avg	RPD	TCAA Area	Avg	RPD
HAAs 200µg/L	487176			2317869		
SAX-C18-100mL-a	71014	127444	89	32961	18698	153
SAX-C18-100mL-b	183874			4435		
SAX-C18-50mL-a	8712	12684	63	21213	24157	24
SAX-C18-50mL-b	16655			27100		

3.3.3.4 Recoveries from WWTP1 US with SAX-C18 and HLB

SPE methods from 3.3.3.2 for SAX-C18 and HLB, along with the use of the HLB conditioning and elution method with C18 cartridges, were used for the extraction of surface water using MtBE + IS as the elution solvent. Duplicate 100mL samples of WWTP1 DS were spiked with 4µg/L each DCAA and TCAA, extracted, derivatized, and analyzed by GC-ion trap-MS, CI mode. A dilution containing 200µg/L DCAA and TCAA, the theoretical 100% recovery concentration, was prepared, derivatized and analyzed by GC-ion trap-MS for comparison. Table 3.25 shows the analyte areas for qualitative comparison between SAX-C18 and the Loos and Barcelo method using HLB or C18 cartridges, since internal standard was not detected by GC-ion trap-MS. Qualitatively, Loos and Barcelo HLB method had the highest recovery and reasonable precision.

Table 3.25 DCAA and TCAA recoveries from WWTP1 US using SPE cartridges and methods. Analyzed by GC-ion trap-MS, CI mode.

	DCAA area	Avg	RPD	TCAA area	Avg	RPD
HAAs 200µg/L	487176			2317869		
SAX-C18-a	58760	51899	26	650835	391334.5	133
SAX-C18-b	45038			131834		
Loos-C18-a	20080	20386.5	3		25752	100
Loos-C18-b	20693			25752		
Loos-HLB-a	264499	245534.5	15	1790553	1726225	7
Loos-HLB-b	226570			1661896		

3.3.3.5 Contaminant Peak Identification

Each step of the method was separately assessed as a potential source of contamination. Two mL MtBE and 2mL derivatized MtBE were analyzed on the GC-ECD and no contamination was visible. The HLB cartridges were conditioned as described in section 3.3.3.2 and C18 cartridges conditioned as described in section 3.3.3. Two mL MtBE was passed over cartridges and captured. A 200µL aliquot was transferred, using a micro-pipetter, to an auto-sampler insert, and the remainder was derivatized. Both were analyzed on the GC-ECD and GC-ion trap-MS and no contamination peaks were visible from either instrument.

3.3.3.6 Determining Recovery of DCAA-d2 through SPE Method

Triplicate 100mL aliquots of WWTP1 effluent (WWTP1 eff), along with 100mL of LGW, were spiked with 800ng/L DCAA-d2, which was obtained mid-way through the project. The surrogate was assumed to behave similarly to non-deuterated compounds in terms of partitioning and sorption and, therefore, could be used to test recovery of non-deuterated compound.

Samples were filtered with 1.5µm Whatman filters and acidified with concentrated H_2SO_4 to pH<0.5 before SPE. The HLB cartridges were individually conditioned using the

Loos and Barcelo (2001) method, described in section 3.3.3.2, connected in series and the aqueous samples were loaded. After loading was complete and the cartridges were dried, cartridges were separated, washed, dried under vacuum and nitrogen, and eluted using the method described in section 3.3.3.2 with MtBE + IS as the elution solvent. The eluted extract of approximately 4mL was split into two aliquots. Two mL were removed using a syringe, derivatized and analyzed by GC-MSD. These extracts were compared to a standard prepared at 20µg/L, the concentration assuming 100% recovery. Standards, LGW and WWTP1 effluent extracts at this concentration level were labeled as "a" extracts in Table 3.26. Without measuring the volume, the remainder of the extract was left in the centrifuge tube and blown down to dryness, reconstituted in 250µL MtBE for an exact volume, derivatized as described in section 3.3.1, and analyzed by GC-MSD along with a standard at 160µg/L, the theoretical 100% concentration. Standard, LGW and WWTP1 effluent extracts at this concentration were labeled "b" extracts in Table 3.26.

The results of this experiment are shown in Table 3.26. However, there are several problems with these results. It was expected that the area responses for Standard B ions m/z 84 and 86 would be eight times larger than Standard A, but this was not seen. The concentration factor was based on the assumption that 2mL of remaining extract were blown down, but actual volume was not measured and could have been less than 2mL. This possible overestimation of the concentration factor yielded uncertainty in the recovery calculations. Contamination peaks were visible in extract chromatograms, which may have interfered with detection of targeted ions. An unspiked WWTP effluent sample was not extracted, so results could not be corrected for background concentrations of analytes. Table 3.26 presents average results with the outliers removed and percent recovery compared to solvent standard.

For ion m/z 86, average recovery from WWTP1 effluent was 95% for "a" extracts and 99% for "b" extracts. However, these recoveries could be over-estimated due to the presence of background analytes. Results from LGW were low and inconsistent. Higher areas from WWTP effluent samples suggested that other compounds may be co-eluting off the cartridge bed. After this experiment, further experiments were conducted on pre-treatment and SPE cartridges to increase recovery.

Table 3.26 DCAA-d2 recovery test from WWTP1 effluent. Analyzed by GC-MSD.

	84 area	% CV/ RPD	% recovery	86 area	% CV/ RPD	% recovery
Std a	491			13019		
LGW a	634		129	639		5
Avg WWTP1 eff a	24979	29	**3879**	15222	27	**95**
Std b	19882			23932		
LGW b	2165		11	1831		8
Avg WWTP1 eff b	1749	60	**9**	23713	90	**99**

3.3.3.7 Comparing SPE Cartridges for Analyte Recovery

For each cartridge type tested, triplicate 100mL LGW aliquots were spiked with 800ng/L DCAA-d2, DCAA, and TCAA. A standard at 40µg/L, the theoretical 100% recovery concentration, was simultaneously prepared for comparison. The Loos and Barcelo (2001) method, described in section 3.3.3.2, was tested using MtBE + IS as elution solvent on both HLB and Phenomenex SDBL cartridges. Triplicate SAX cartridges were evaluated comparing sample pH adjustment and elution solvents of pH adjusted LGW or 1:9 MeOH:MtBE. Sample pH was adjusted in an effort to remove humic material from the surface and wastewaters and to test the effectiveness of recovery of HAAs in protonated or deprotonated form.

Two SAX methods were evaluated. For SAX method 1, the cartridges were conditioned with 5mL MeOH and 5mL LGW. These samples are labeled SAX1 in Table

3.28. Samples labeled SAX 1a-c were eluted with 2mL 1:9 MeOH:MtBE, extracts were blown to dryness, reconstituted with 250µL MtBE, added internal standard, and derivatized with 25µL of diazomethane. Samples labled SAX 1d-f were conditioned as above but were eluted with 2mL LGW at pH 0.5. For all extracts from SAX method 1, LLE was used to extract HAAs. Three mL of MtBE was added to extract analytes, which were vortexed for one minute. The MtBE layer was transferred to 10mL centrifuge vial, blown to dryness under nitrogen, reconstituted with 250µL MtBE and derivatized with 25µL diazomethane.

For the second SAX method, sample pH was adjusted to 2.5 with concentrated H_2SO_4 and the cartridges were conditioned with 5mL MeOH and 5mL LGW at pH 2.5. Samples labeld SAX 2a-c were eluted as SAX 1a-c, which was described above. Samples labeled SAX 2d-f were eluted and extracted as SAX 1d-f, as described above. Derivatized extracts were analyzed in GC-ion trap-MS, CI mode. After analysis, samples were recapped, stored and reanalyzed using GC-MSD SIM.

HLB and SAX 1a-c did not yield detectable results, as shown in Table 3.27. Sample SAX 1f was considered an outlier and removed from analysis. Percent recovery was compared to the area of the solvent standard. Internal standard was not detectable with GC-MS, CI mode. In most samples, there were many background noise peaks around the time of DCAA and DCAA-d2, with 127 as the main ion, that interfered with analyte area integration. This background noise was also visible in SAX 1d/e, however, analyte peaks are separate from background contamination, as shown in Figure 3.10.

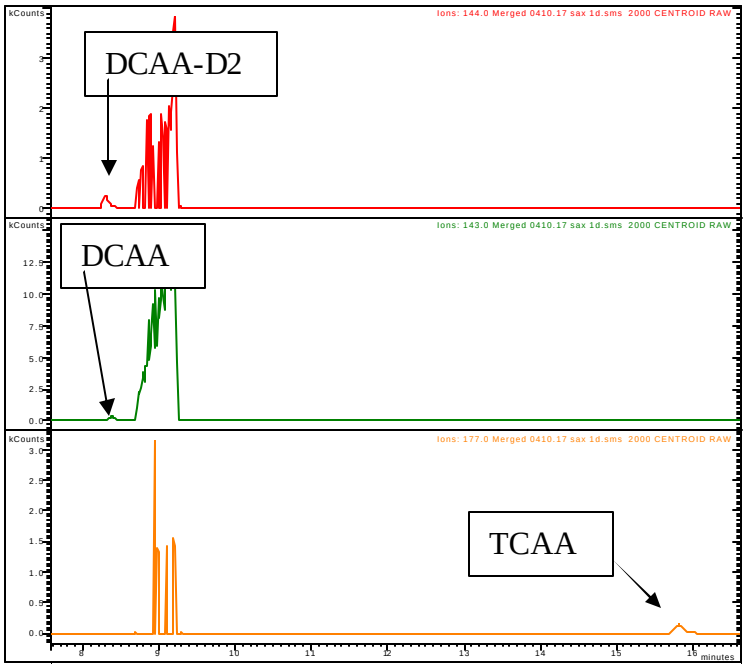

Figure 3.10 SAX 1D chromatograph, targeted ions: DCAA-d2: 144, DCAA: 143 and TCAA: 177, GC-MS CI mode.

Table 3.27 DCAA (ion m/z 143), TCAA (ion m/z 177) and DCAA-d2 (ion m/z 144) recovery from HLB, SDBL and SAX with pH adjustment SPE extraction, GC-MS CI mode.

	Avg DCAA- % recovery	RPD/ % CV	Avg TCAA- % recovery	RPD/ % CV	Avg DCAA-d2- % recovery	RPD/ % CV
HLB						
SDBL	5	84	6	166	7	61
SAX 1a-c						
SAX 1d/e	20	34	26	95	43	32
SAX 2a-c	3	69	6	92	7	85
SAX 2d-f	2	0	2	90	3	57

Duplicates of each method type were re-analyzed with GC-MSD, as shown in Table 3.28. TCAA was not detected in the solvent standard due to high background noise, and therefore, percent recovery cannot be calculated. For DCAA, highest recovery results from SAX 1d/e, as shown in GC-ion trap-MS results in Table 3.27.

Table 3.28 Results from re-analysis with GC-MSD. 83 and 85 are DCAA and 84 and 86 are DCAA-d2.

	83- recov	85- recov	84- recov	86- recov
HLB	9	12	8	1
SDBL	17	8	8	26
SAX 1a/b				1
SAX 1d/e	51	59	60	
SAX 2a/b	3	3	7	1
SAX 2e/f	54			20

3.3.3.8 SAX and HLB SPE Comparison Without Blow-down

Analytes were spiked at a higher concentration and were extracted by SPE without subsequent blow down of the extract, so that SPE extraction efficiencies could be compared between SAX and HLB cartridges. Triplicate 100mL aliquots of LGW and WWTP2 US surface water were spiked with 4µg/L DCAA, TCAA and DCAA-D2. These aliquots and one unspiked WWTP2 US aliquot were tested without sample pH adjustment, since the results described in section 3.3.3.7 suggest that no pH adjustment yields the highest recovery. SAX cartridges were conditioned and eluted as for samples SAX 1d-f, described in section 3.3.3.4 and results are shown in Table 3.29, labeled SAX LGW and SAX US.

Two HLB methods, labeled HLB A and B, were each tested with triplicate 100mL LGW aliquots spiked with 4 µg/L DCAA, TCAA and DCAA-D2, and pH adjusted to 0.5 with concentrated H_2SO_4. For method HLB A, 60mg/3cc HLB cartridges were separately conditioned with 5mL MeOH and 5mL LGW at pH 0.5. The two cartridges were connected in series, aqueous samples were loaded, the cartridges were separated and washed with 1mL LGW at pH 0.5. For method HLB B, 60mg/3cc HLB cartridges were conditioned with 5mL MeOH, 3mL LGW at pH 2.5, two cartridges were connected in series, aqueous samples loaded, cartridges were then separated and washed with 1mL LGW at pH 2.5. All cartridges were dried under vacuum and nitrogen for half an hour. Cartridges that had been connected in series from both HLB A and B were each eluted with 2mL 50:50 MeOH: LGW into one test tube. LLE was used to extract HAAs using 2mL MtBE, and extracts were vortexed for one minute. After layers separated, the MtBE extract was transferred to a 2mL volumetric flask, derivatized and analyzed by GC-MSD.

Simultaneously, an experiment was conducted to assess DCAA, TCAA and DCAA-d2 recovery by LLE after SPE. Two hundred µg/L DCAA, TCAA and DCAA-d2 were spiked into 2mL LGW adjusted to pH 0.5 with concentrated H_2SO_4. LLE was conducted with 2mL MtBE, as described above. Final extracts were analyzed on GC-MSD and compared to standards of 100 and 200µg/L of DCAA, TCAA and DCAA-d2 prepared in MtBE + IS and derivatized.

Table 3.29 presents average recovery compared to appropriate standard average for each method. GC-MSD SIM targeted ions m/z 83 and 85 for DCAA, 117 and 119 for TCAA and 84 and 86 for DCAA-d2. Recoveries from LLE were over 100%, which may be affected by the lack of linearity of the half and full standards. Outliers were removed from average recoveries. Recoveries of SAX upstream were corrected for analytes detected in the upstream blank. SAX upstream show higher recoveries than SAX LGW, which could be due to matrix effects, such as high levels of salts and other anionic compounds that might co-elute with analytes.

Table 3.29 DCAA (ions m/z 83 and 85), TCAA (ions m/z 117 and 119), and DCAA-d2 (ions m/z 84 and 86) recovery from HLB, SAX and LLE, GC-MSD.

	83- avg recovery	85- avg recovery	117- avg recovery	119- avg recovery	84- avg recovery	86- avg recovery
HLB A	87	86	374	24	36	9
HLB B	115	69	112	411	83	72
SAX LGW	85	76	57	74	83	72
SAX US	99	72	98	80	102	95
LLE LGW	131	109	120	107	154	92

Extracts were reanalyzed for comparison on GC-ion trap-MS, CI mode, as shown in Table 3.30. Average recovery was calculated by comparing analyte areas to a solvent standard at concentration of 100% recovery. Even though results from each instrument were

normalized to the solvent standard analyzed by the same instrument, there was discrepancy between recoveries from GC-ion trap-MS and GC-MSD.

Table 3.30 DCAA, TCAA and DCAA-d2 recovery from HLB, SAX and LLE, GC-MS, CI mode.

Sample	DCAA recovery	TCAA recovery	DCAA-D2 recovery
HLB A	43	24	38
HLB B	91	84	98
SAX LGW	75	67	79
SAX US	77	74	88
LLE LGW	96	85	104

From both GC-MSD and GC-MS results, HLB B has the highest recovery overall, compared to HLB A and SAX LGW. However, prior experiments show contamination peaks in the chromatograms and difficulty detecting DCAA and TCAA, even in standards. Although HLB B method appears to be the most effective for analyte recovery from LGW, more experiments need to be conducted to improve recovery efficiency of analytes from more complex matrices.

3.3.3.9 DCAA and TCAA Method Concentration Summary

Detection of HAAs from complex matrices, such as surface and wastewaters, is complicated because the concentrations of HAAs were low and many interfering compounds must be removed. Three general concentration methods were tested: blowing- down acid and ester forms of HAAs, using larger volume LLE and several SPE extraction cartridges and methods. Due to time constraints, method development work on HAA concentration method was discontinued. HAA results presented in Section 3.4 were extracted with LLE using the Brophy et al. (2000) method and larger volume LLE.

3.4 DCAA and TCAA Occurrence in Environmental Samples

HAAs were hypothesized to form during the chlorination of wastewater before discharge, were proposed as indicators of WWTP pollution into receiving waters. To validate this hypothesis, samples around WWTPs were tested for HAA occurrence within WWTP effluent and their persistence downstream. It was assumed that concentrations of chlorine within septic tanks would not be high enough to cause HAA formation and analyzed septic tank samples were used to test this assumption. Samples were processed using the Brophy et al. (2000) LLE and larger volume LLE methods.

3.4.1 DCAA and TCAA Occurrence from Surface Waters Surrounding WWTPs

Two WWTPs that chlorinate and dechlorinate wastewater before discharge were sampled for HAA formation in WWTP effluent and persistence in downstream surface water. WWTP2 was sampled five times over the summer and fall and WWTP2 was sampled twice in the fall, with duplicate samples processed. HAAs were extracted using the Brophy et al. (2000) LLE extraction method. The PQL for this method was 1µg/L, however, many of the environmental sample concentrations were below this PQL. The trendline equation from the calibration curve was used to extrapolate areas below this PQL, if the RPD between duplicate calibration points was below 10%. Negative concentration estimations were considered to be zero. An example calibration curve is shown in Appendix 2.

Figures 3.11 and 3.12 present the occurrence of DCAA and TCAA from WWTP1 and surrounding surface water. Because nitrification occurs in WWTP1 effluent, low HAA concentrations were expected from WWTP1. Chlorine is likely to react first with ammonia, to form chloramines, before interacting with NOM to form HAAs. The widest concentration range for both analytes occurs from the effluent, which may reflect variance in wastewater

ammonia present at the time of chlorination and, therefore, varied DCAA and TCAA formation. DCAA and TCAA appear to persist downstream, at concentration ranges similar to WWTP effluent. Low DCAA concentrations upstream implied that downstream concentrations resulted from WWTP effluent.

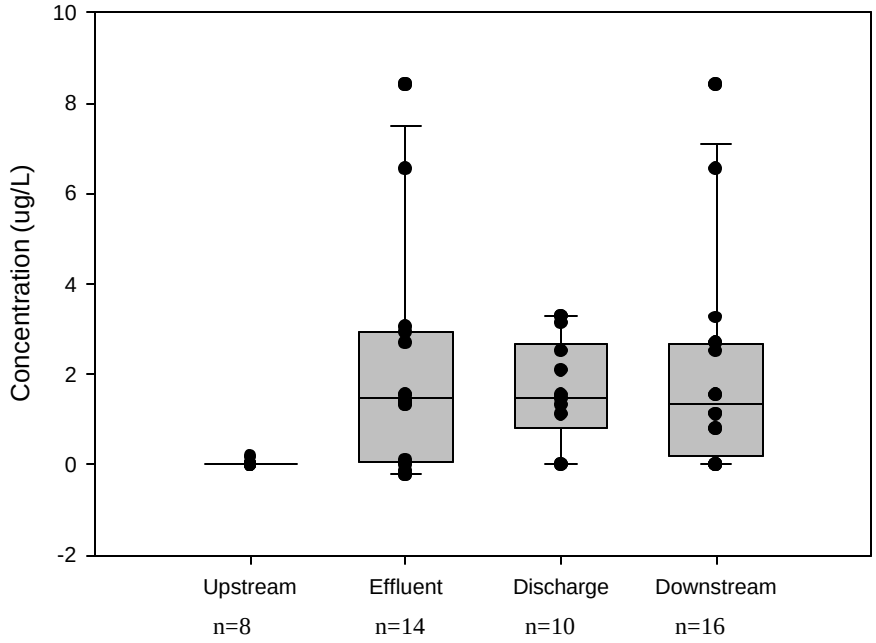

Figure 3.11 DCAA occurrence in samples from WWTP 1 and surrounding surface water.

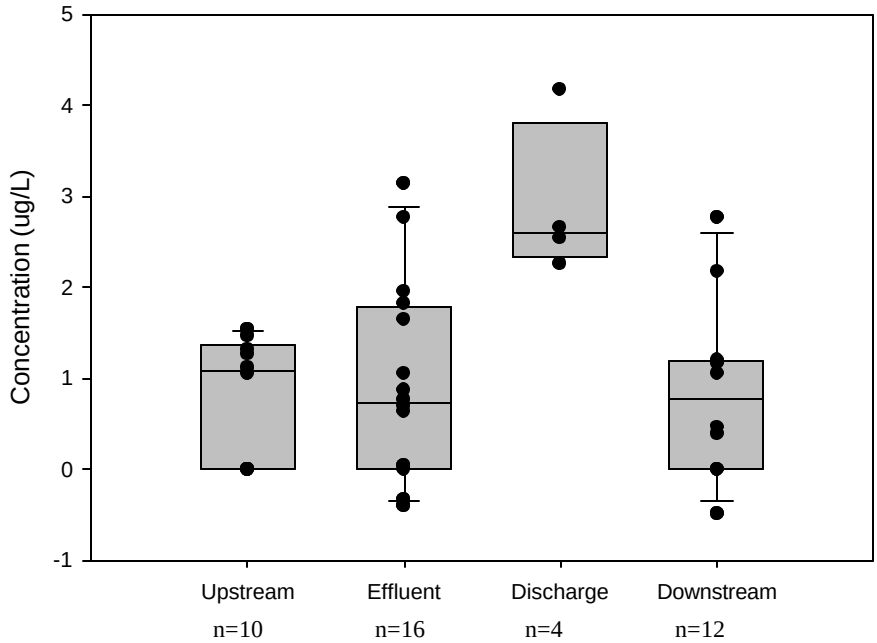

Figure 3.12 TCAA occurrence in samples from WWTPs 1 and 2 and surrounding surface water.

WWTP2 nitrified the wastewater effluent, so higher HAA concentrations were expected from WWTP2 effluent than from WWTP1, as seen in Figures 3.13 and 3.14. Because TCAA was not detected upstream, HAA occurrence downstream from WWTP2 can be correlated to WWTP effluent discharge. DCAA appears to have degradaed downstream more than TCAA, although both analytes were detectable downstream. TCAA had a higher range from effluent, which could be due to sampling variability. However, persistence of TCAA downstream added strength to usage of HAAs as proposed indicator.

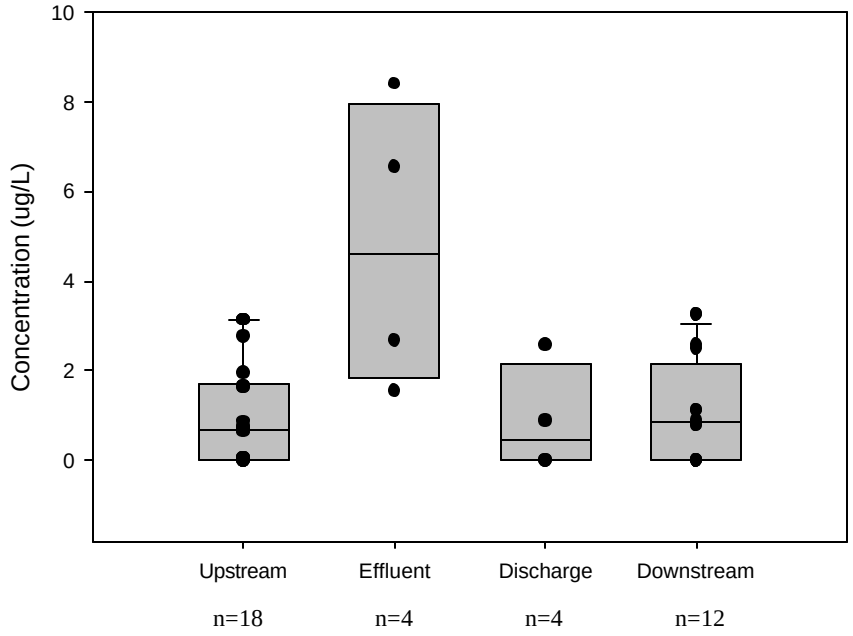

Figure 3.13 DCAA in samples from WWTP 2 and surrounding surface waters.

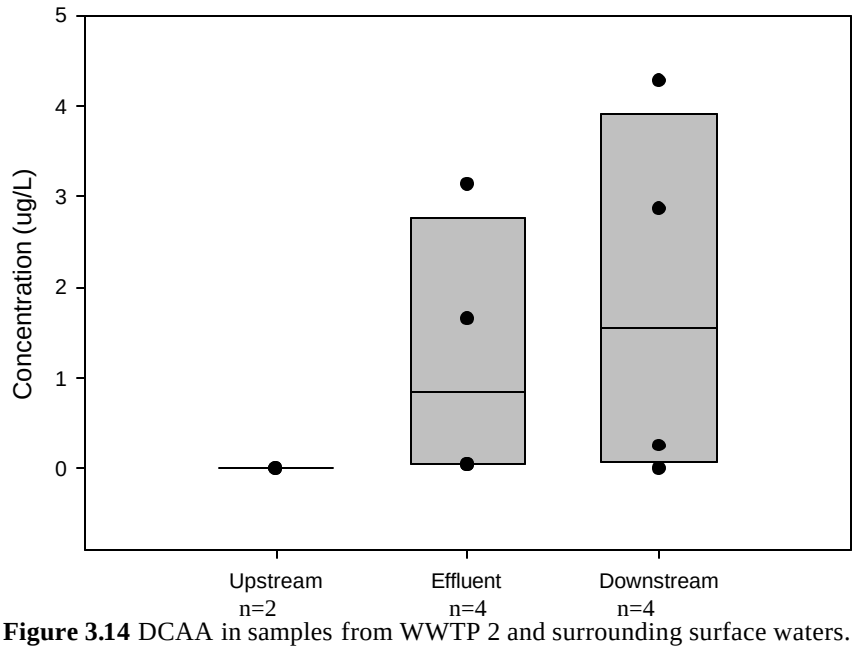

Figure 3.14 DCAA in samples from WWTP 2 and surrounding surface waters.

Occurrence data from both treatment plants were compiled to represent possible DCAA and TCAA ranges from chlorinating WWTPs as shown in Figures 3.15 and 3.16. Variability in effluent HAA concentrations could be due to daily fluctuations in factors that influence HAA formation, such as DOC in wastewater, chlorine dose, amount of nitrification and chlorine contact time. DCAA showed a wide range of sample concentrations, although the majority of the concentrations were between 1-3µg/L. DCAA concentrations downstream are significantly higher than upstream levels. Although not conclusive, these results show that HAAs formed as effluent was chlorinated, persisted after dechlorination and were detectable downstream. Although HAAs were only detected at low concentrations in effluents, these compounds persisted downstream of WWTPs. However, the low downstream concentrations show the need to develop a method with a lower PQL in complex matrices.

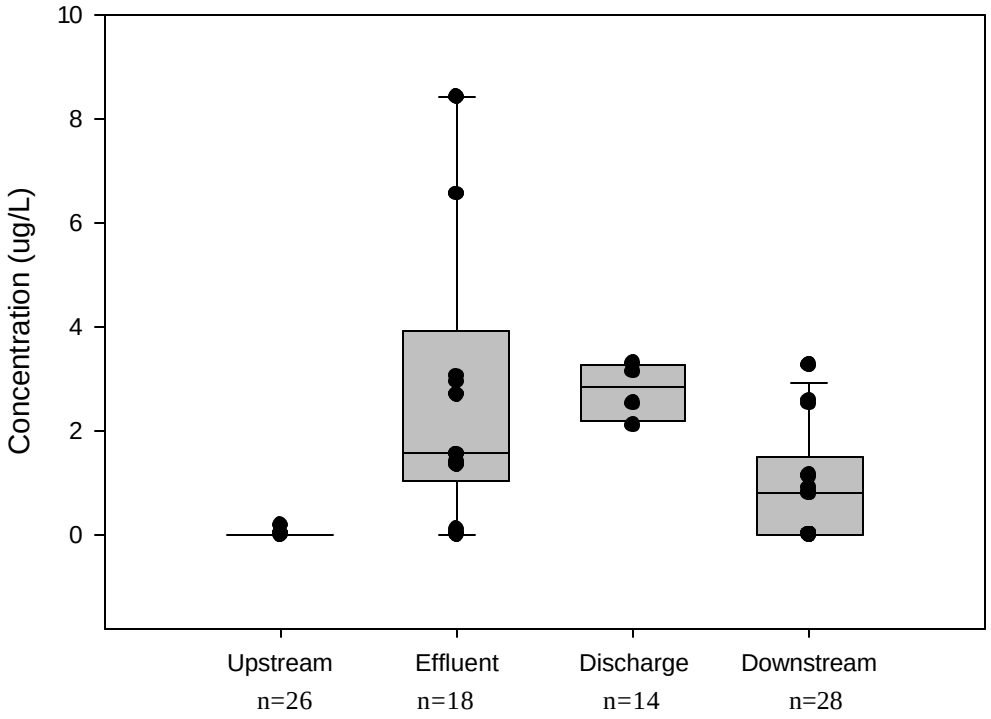

Figure 3.15 DCAA occurrence compiled from WWTP 1 and 2 samples and surrounding surface waters.

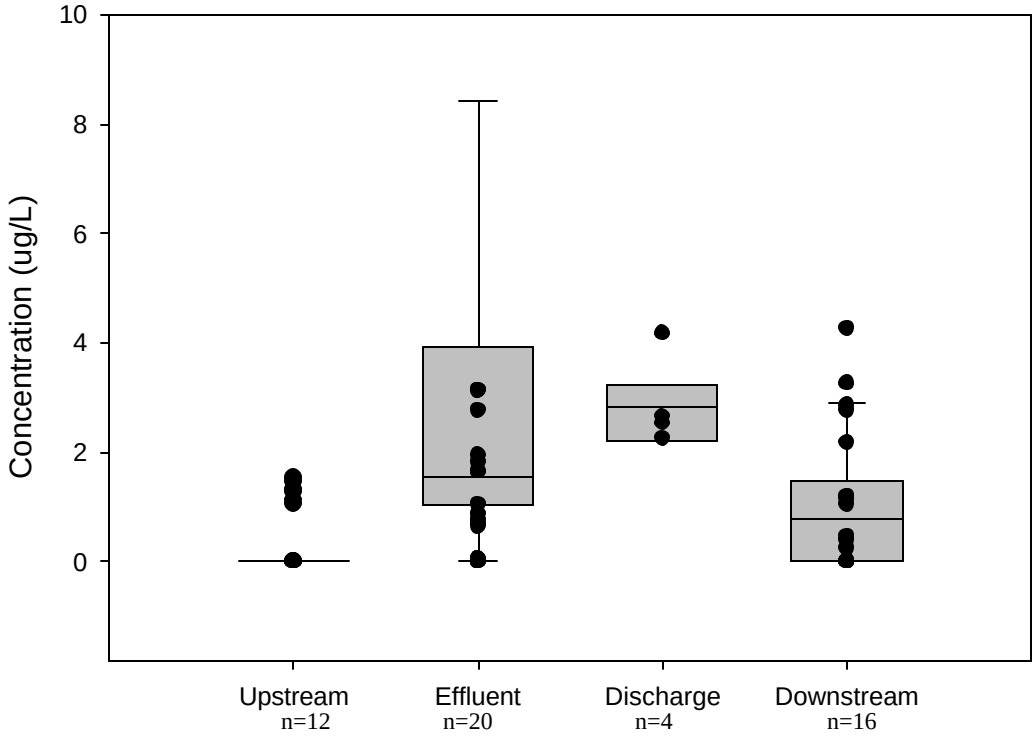

Figure 3.16 TCAA occurrence compiled from WWTP 1 and 2 samples and surrounding surface waters.

3.4.1.2 DCAA and TCAA Extracted with Larger Volume LLE

In an attempt to detect HAAs at lower levels, 100mL samples from WWTPs 2 and 3, collected on two sampling dates in May, were extracted using larger volume LLE with 20mL MtBE. The MtBE organic layer was blown to dryness and reconstituted in 2mL MtBE + IS, for a concentration factor of 50. Concentrations were calculated from the trendline of a solvent calibration curve. The combined occurrence data from WWTPs 2 and 3 are presented in Figures 3.17 and 3.18. The results are not directly comparable to Figures 3.15 and 3.16 because different samples were processed from different dates, different WWTPs were sampled and the difference in concentration calculations. Despite these differences, concentration ranges are similar, which indicates the precision of LLE method. Larger volume LLE extraction in combination with solvent concentration appears to enable detection of HAAs at lower levels.

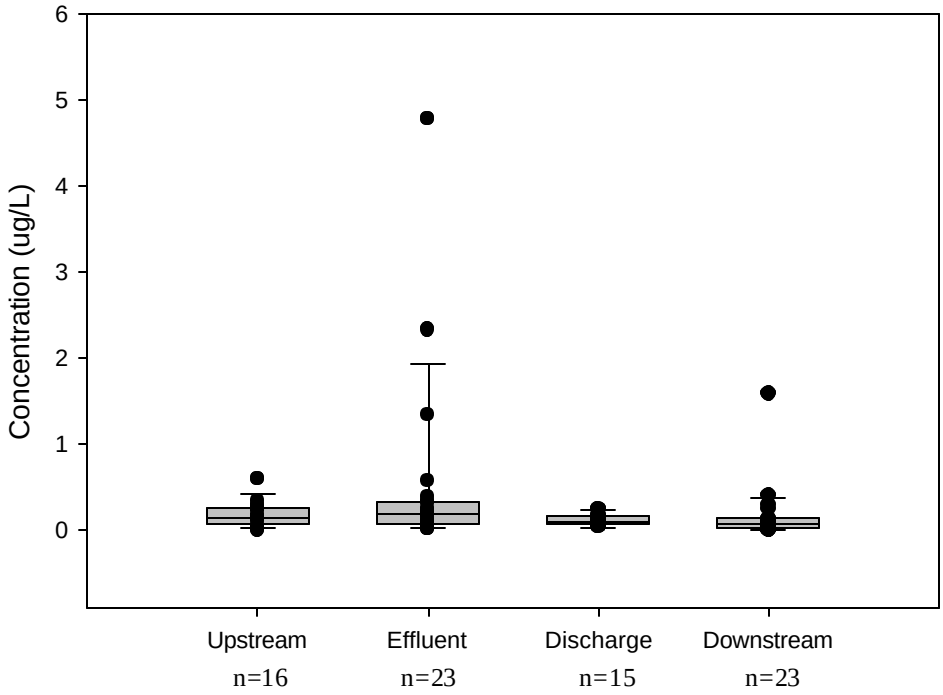

Figure 3.17 DCAA and TCAA occurrence from WWTP samples extracted using larger volume LLE.

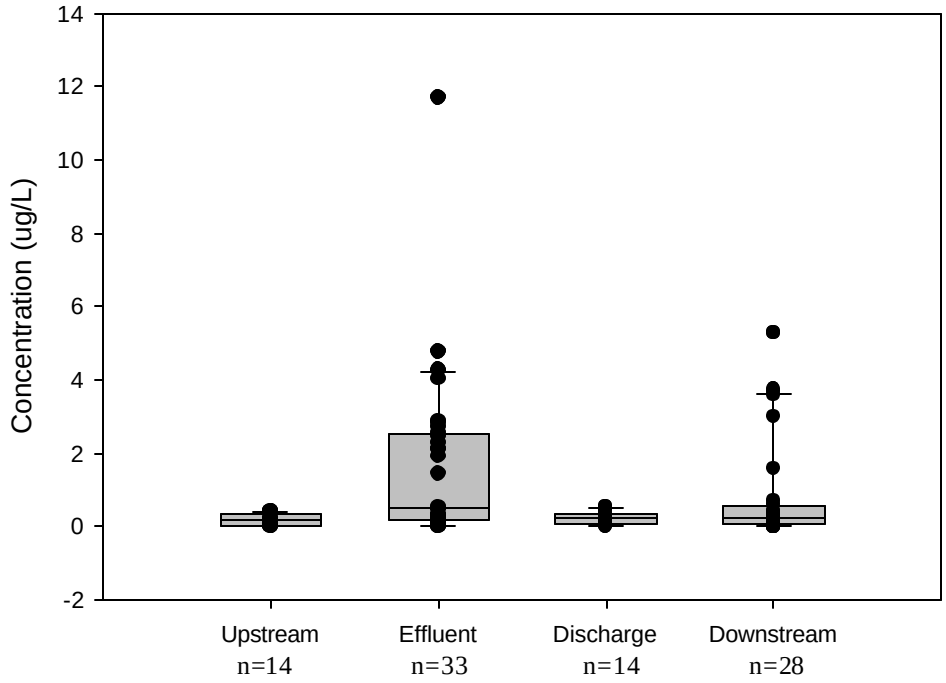

Figure 3.18 DCAA and TCAA occurrence from WWTP samples extracted using larger volume LLE.

3.4.2 DCAA and TCAA Occurrence in On-Site Wastewater Treatment Systems

It was hypothesized that DCAA and TCAA would not be detected in household septic tanks, either because most households do not disinfect the septic system or use enough chlorine cleaning products for HAA formation. To test this assumption, advanced treatment and household septic tank samples were analyzed for an indication of HAA concentrations in septic tanks. Septic tank samples were processed using the Brophy et al. (2000) method of LLE with 20mL sample and 4mL MtBE.

The majority of DCAA and TCAA concentrations from septic tanks were lower than from WWTP effluent, as shown in Figure 3.19. The high school advanced treatment system and approximately half the houses sampled were on city water and HAAs detected in septic tanks were likely due to HAA occurrence in tap water. The concentration ranges from WWTPs and septic tanks overlapped and were not distinguishable with the current method.

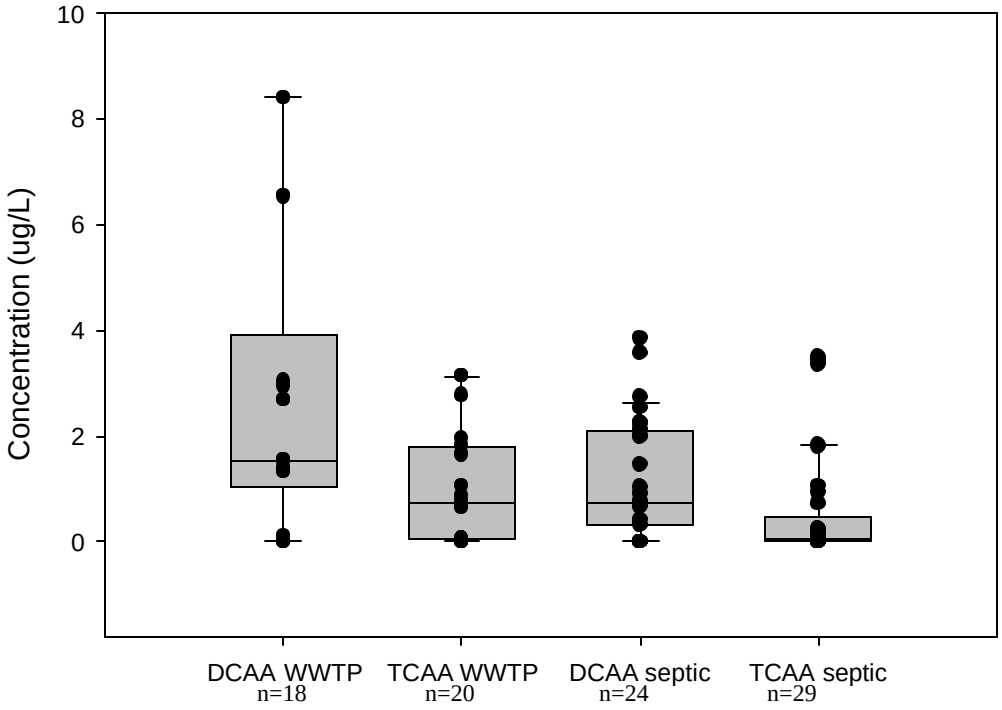

Figure 3.19 DCAA and TCAA occurrence in septic tanks and WWTP effluent.

A precise and accurate method for HAA detection at low levels needs to be perfected for clearer distinctions between septic and WWTP effluent concentrations. Although DCAA and TCAA concentrations in WWTP and septic tank effluent have similar ranges, the occurrence data confirm detectable levels of DCAA and TCAA in WWTP effluent which persist downstream.

3.5 Fluorescence Results

By comparing the regions and intensity of fluorescence from water samples, fluorescence spectrophotometry has the ability to provide on-line and real-time analysis of water samples. There are five main fluorescence regions: regions I and II represent aromatic proteins, region III encapsulates fulvic acids, region IV represents soluble microbial by-products and region V humic acids. Region IV, which indicates the presence of tryptophan,

has been linked to the presence of wastes (Baker 2001). In this study, the usefulness of fluorescence as a tool to distinguish WWTP and failed septic system pollution was tested with effluent samples.

Environmental samples were filtered with a 0.2µm filter and stored at 4°C for up to a week before measuring fluorescence. Fluorescence data were acquired by FL Solutions software and intensity data were transferred to Microsoft Excel, which sorted the data. The Ramen/ Rayleigh scattering, which naturally occurs in the fluorescence image of all water samples, was manually removed using Excel. For sample comparison, intensities from the highest points within each fluorescence region were selected. After cleaning the data in Excel, intensity data were transferred to SigmaPlot, which was used to produce EEM contours and box and whisker plots.

3.5.1 Use of EEM (Fluorescence) as an Indicator

Samples from all three WWTPs and surrounding surface waters were analyzed using fluorescence spectrophotometry. WWTP2 was sampled on two dates, but WWTPs 1 and 3 were sampled once. An example of the series of samples analyzed is shown in Figure 3.20. Although these EEMs show one sample, they represent a trend that was repeated from all the samples at each location. The scale was not normalized, and up- and downstream contours represent 250 intervals to allow greater comparison in regions I and II, while effluent and discharge have 500 intensity intervals. Section 1.6 describes the fluorescence regions in more detail, but the general regions are highlighted on the effluent EEM. A drawback to fluorescence is the need to compare downstream samples to upstream samples in order to assess pollution, since each natural water has a unique fluorescence.

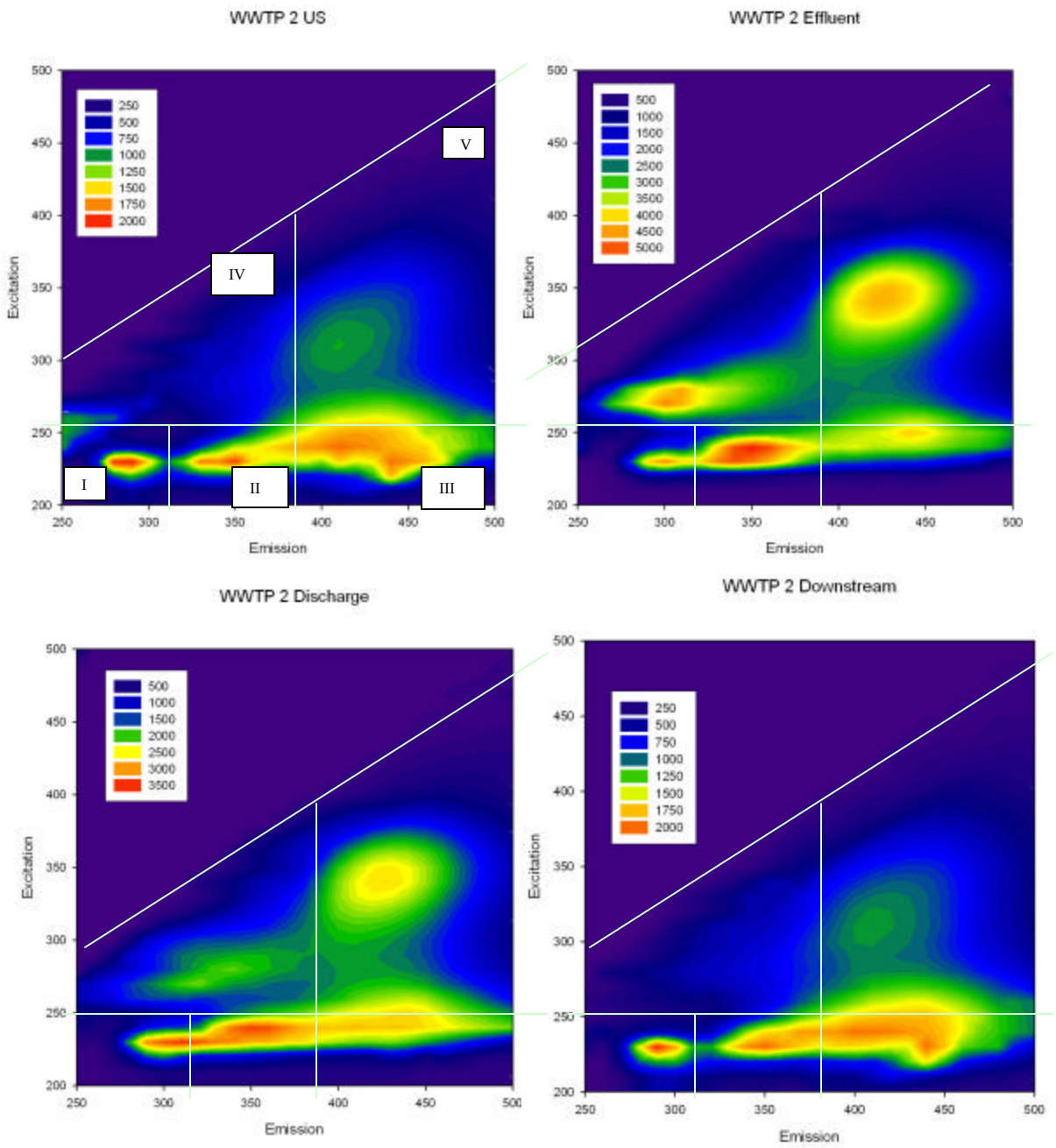

Figure 3.20 EEM images of fluorescence intensities in sampling locations around WWTP2. Note differences in legend scale. Region I: Aromatic Protein. Region II: Aromatic Protein II. Region III: Fulvic acid-like. Region IV: Soluble microbial by-product-like. Region V: Humic acid-like.

The effluent sample had higher intensities than the natural water samples in all regions, especially in the microbial by-product region (IV), which characterizes waste-impaired waters. The intensity of every spectral region degraded with dilution in surface water until the intensities downstream were indistinguishable from upstream in all regions,

even though the region II in downstream EEM has a wider range of intensities than the upstream EEM. The similarity in intensity ranges through all fluorescence regions between up and downstream samples and the lack of tryptophan detection downstream indicate that wastewater discharge was not identifiable downstream 0.4 miles below the effluent discharge.

The intensity ranges for all three WWTPs and two septic tank samples were compiled by fluorescence region and compared in a box and whisker plot, shown in Figure 3.21. Only minimum and maximum values are presented in regions with less than three values. The higher intensities and wider intensity ranges found in wastewaters, both septic and WWTP effluents, distinguish these samples from natural surface water. The wide range of intensities from WWTPs may reflect daily differences in the amount of wastewater treatment on each sampling date, based on treatment plant factors such as flow, retention time and chlorine dose. However, more samples are necessary, especially from septic tank samples, to accurately portray the range of intensities.

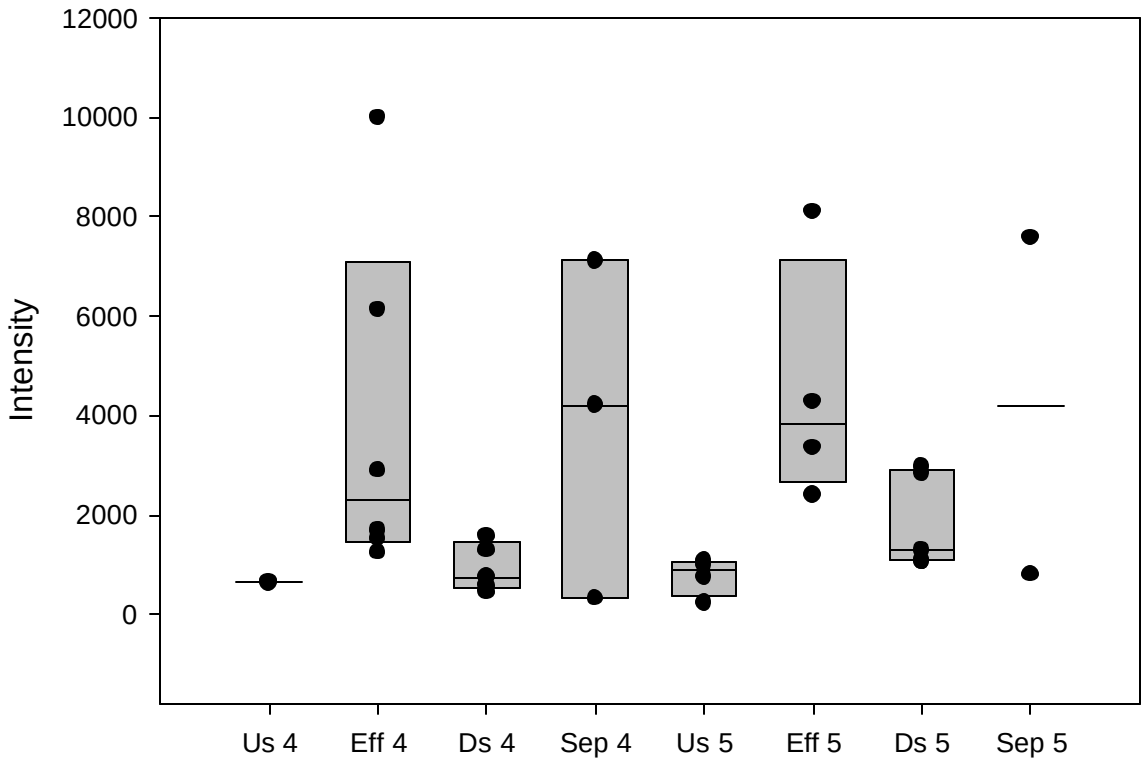

Figure 3.21 Fluorescence intensity ranges compiled for WWTPs, surrounding surface waters and septic tanks. Region 4 is soluble microbial by-products and region 5 is humic acid. US n= 4, EFF n= 4, DS n=7, septic n=2.

Side-by-side comparison of EEM images from septic (high school tank) and WWTP effluents visually highlight the differences in fluorescence regions and intensities, as shown in Figure 3.22. Septic effluent shows higher intensities in the tryptophan/tyrosine (Region II-soluble microbial by-product-like) and in Region V (humic acid-like). The difference in the amount of treatment and treatment processes within the septic tank and WWTP are shown in the EEM images. However, the overlapping intensity ranges indicated that their effluents may not be distinguishable in the environment.

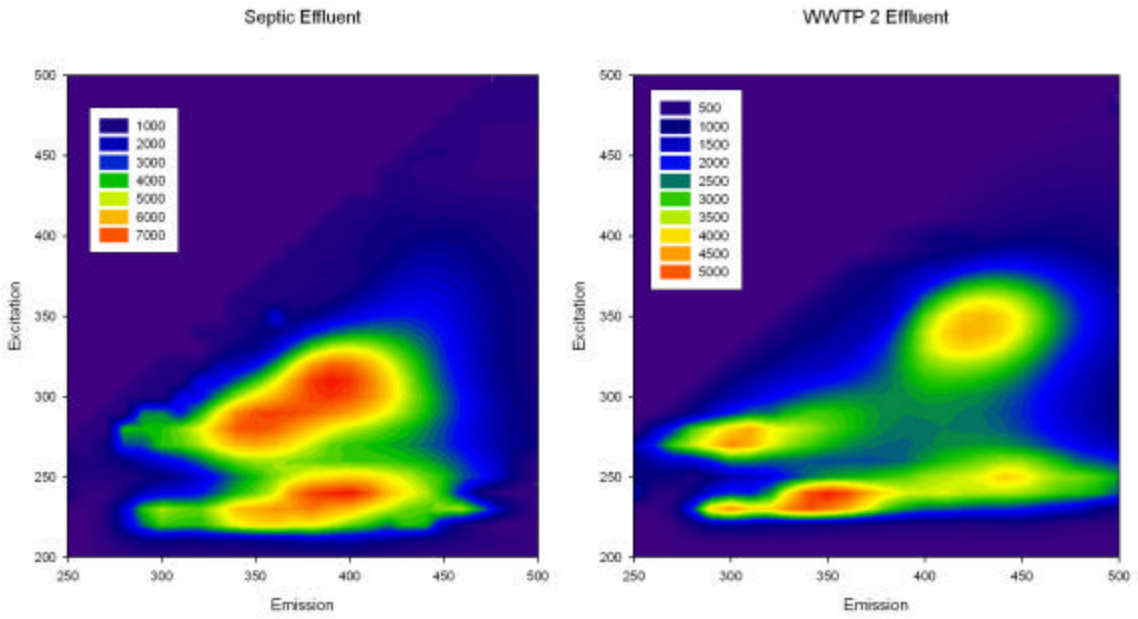

Figure 3.22 EEM images for WWTP and septic effluent. Note difference in scale between the two images.

For an indication of the fluorescence spectrum of effluent from a functioning septic system, one sample from each location through the high school advanced treatment system was analyzed to provide a snapshot of fluorescence intensities. Tryptophan, which often indicates waste, is seen in the septic effluent and after one pass through the sand filter, as shown in Figure 3.23. There are little differences in humic-like and fulvic-like intensities between different surface and wastewater samples.

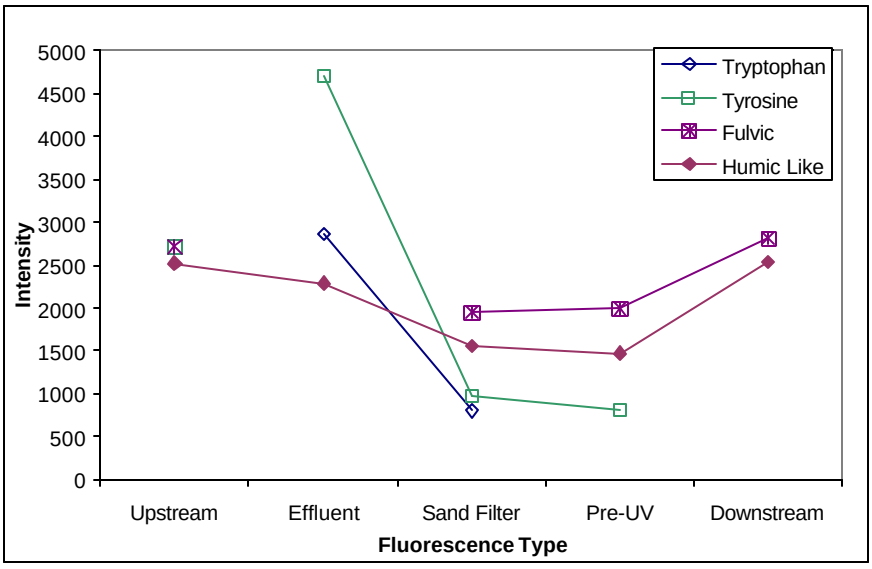

Figure 3.23 Fluorescence intensities across the advanced treatment high school system.

3.5.2 Fluorescence Intensity in Septic Tank Effluent Dilution Experiments

As an indication for the distance downstream that the fluorescence spectra would be impacted by a failed on-site system, high school advanced treatment septic tank samples were diluted with surface water collected upstream of the system. Dilutions were prepared at 1:2, 1:5, 1:10 and 1:20. Average septic tank flow rates of 69.3 gallons/person/day, estimated by Mayer et al. (1999), were used assuming a four person household for an average of 277.2 gallons/day. Assuming that 100% of effluent from a failed tank reached surface water, dilution calculations at this range represent small streams, shown in Table 3.32. Results of the dilution experiment are shown in Figures 3.24 and 3.25. The high intensity in septic effluent became diluted by upstream waters. The spectral region representing the presence of tryptophan was absent from upstream, but remained detectable in all dilutions, implying the presence of waste in the water.

Table 3.32 Equivalence of dilution factor to stream flow.

Dilution Factor	Effluent(g/d)	Calculated streamflow (ft^3/s)
2	277.2	0.000858
5	277.2	0.002144
10	277.2	0.004289
20	277.2	0.008578

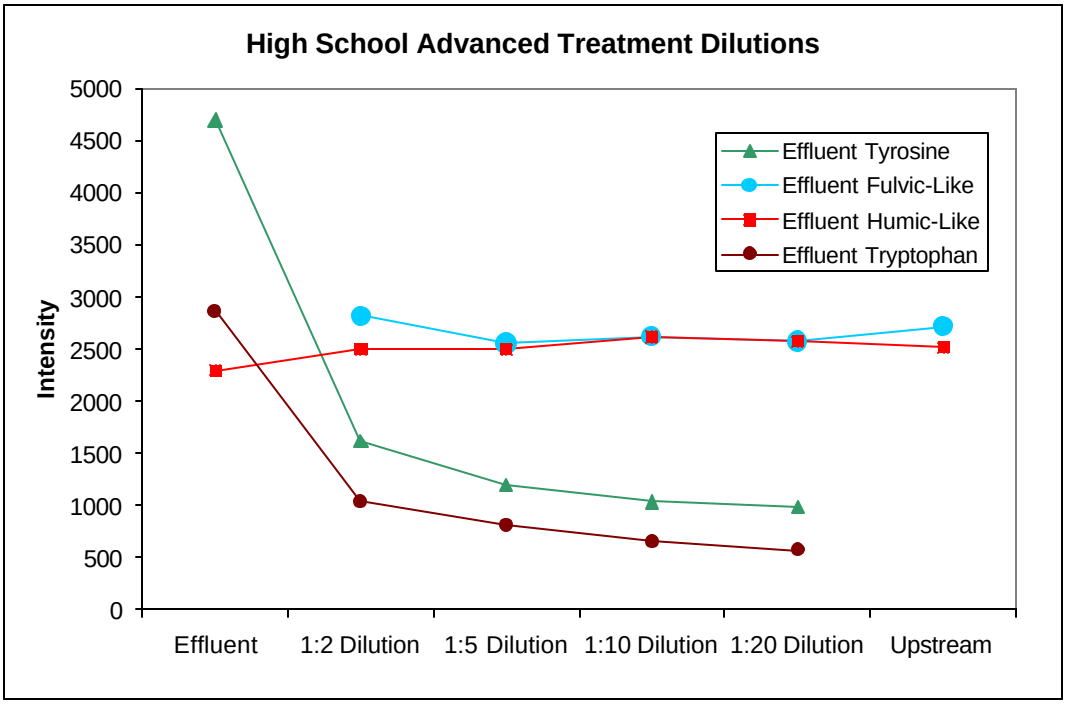

Figure 3.24 Fluorescence intensities of septic tank effluent diluted with upstream surface water.

The EEM contours from the dilution experiment are shown in Figure 3.25. The legend shows the intensities, the scale of which is different for each plot. Visually, by the 1:10 dilution, the EEM more closely resembles the upstream than the septic effluent sample.

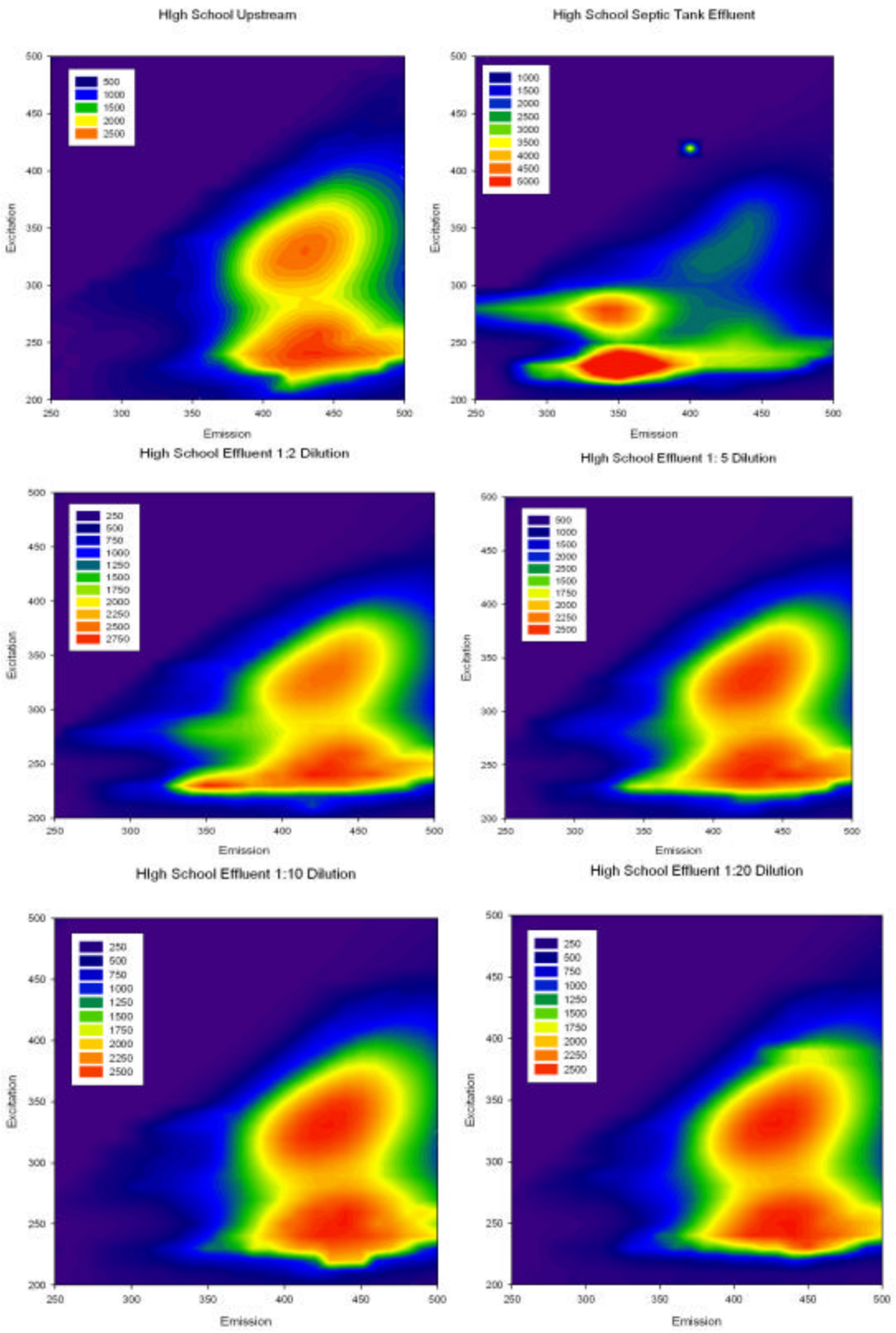

Figure 3.25 EEM contours showing fluorescence intensity for high school septic tank diluted samples. Note septic tank effluent scale only reaches 5000 for comparability, but intensities are much higher.

CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS

4.1 Summary and Conclusions

The objective of this research was to identify chemical indicators that could be sampled from surface waters to distinguish between point and non-point sources of pollution, specifically municipal WWTPs and failed on-site septic systems. Potential indicators were chosen based on prevalence, chemical properties and previous occurrence data indicating likelihood of detection. Caffeine and triclosan were proposed as indicators of failed septic systems and HAAs, specifically DCAA and TCAA, as indicators of the presence of effluent from conventional WWTPs. Fluorescence spectrophotometry was investigated as an additional tool to differentiate pollution sources in the aquatic environment.

An SPE method was perfected for simultaneous analysis of caffeine and triclosan in different wastewater samples. Deuterated standards were spiked into sample aliquots at the start of processing for determination of analyte loss through the method and concentration determination. Strata X cartridges were washed with hexane, MtBE, MeOH and a phosphate buffer. These cartridges were eluted with 3:7 MtBE:ACN solvent mixture onto conditioned silica gel cartridges, which were eluted with the same solvent mixture. Extracts were blown down to dryness, reconstituted in ACN, and derivatized with BSTFA and pyridine containing HCB, as internal standard. Extracts were analyzed by GC-MSD.

The effluents from three WWTPs in North Carolina, of varied size disinfecting with either chlorine or UV, were sampled in cold and warm water seasons and analyzed for a sense of

target analyte concentration ranges. Caffeine and triclosan were detected in both WWTP and septic tank effluent samples. Caffeine in WWTP effluents (n=29) ranged from below detection (BD) to 1.2µg/L (average = 0.23µg/L) and triclosan (n=30) from BD to 1.63µg/L (average = 0.3µg/L). These analytes were detected downstream (0.27-3.6 miles) of effluent discharges from WWTP, but at lower concentrations (from BD to 0.8µg/L caffeine and from BD to 0.7µg/L triclosan). Septic tank samples were collected from households and two advanced treatment systems, an office and a high school. Caffeine (n=15) from septic tank effluents ranged from 5.4 to 88.3µg/L and triclosan (n=20) from 110ng/L to 16.2µg/L.

The high school advanced treatment septic system was sampled throughout the entire treatment process for an indication of the effect of a functioning system on caffeine and triclosan degradation. Through the combination of aerobic and anaerobic degradation processes, both analytes were degraded from high concentrations in the septic tank (average caffeine (n=7): 26µg/L and triclosan (n=7): 1.5µg/L) to an average of 70ng/L for both compounds after treatment, which can be extrapolated to concentrations after drainfield treatment. These high rates of degradation suggest that a functioning septic system, which employs settling, aerobic and anaerobic treatment processes through the combination septic tank and drain field, would effectively remove compounds and not be a source of surface water pollution.

On the other hand, high concentrations of caffeine and triclosan are expected to persist in the septic effluent as it flows from a failed system into nearby surface waters and then be detectable downstream. For this project, septic system failure was defined as a problem between the tank and the drainfield, so that effluent still undergoes settling and anaerobic degradation in the septic tank. Failed septic systems were located with the help of the Orange County Health Department. Samples were collected from within septic tanks, pooled surface sewage and from a

nearby stream during a rain event. Both indicator analytes were detected in the pooled sewage (n=3) at average concentrations of 2.3µg/L caffeine and 1.6µg/L triclosan. From a different failed system, caffeine was detected in samples collected downstream of a failed septic system during a rain event at an average of 270ng/L, which is a higher concentration than the average value of WWTP effluents sampled. These occurrence data show that triclosan and caffeine remain in high concentrations in surfacing effluent from failed septic systems and that if the septage were to travel overland to surface waters, their presence in surface waters far removed from WWTP discharges would be an indication of non-point pollution.

DCAA and TCAA were extracted from environmental samples using the Brophy et al. (2000) method of LLE using 20mL sample aliquots and 4mL MtBE. Initial extractions of wastewaters revealed low concentrations of DCAA and TCAA around or below the PQL of 1 µg/L, so several methods were tested to lower the detection limit of the analytical methods. Solvent extraction concentration was tested using blow down for HAA acids and methyl esters. Larger volume LLE was utilized with 100mL of sample and 20mL of MtBE. The MtBE extract was blown to dryness and reconstituted in 2mL MtBE for derivatization and analysis. HLB cartridges were tested in series conditioned with MeOH and LGW at pH 0.5. Sample pH was adjusted to pH 0.5, passed over two cartridges in series, which were eluted with MtBE or 50:50 MeOH:LGW. Initial results of concentration methods were inconclusive but larger volume LLE was used to extract environmental samples and yielded lower detection.

The HAAs were harder to distinguish between WWTP and septic systems. It was hypothesized that HAAs would be formed as WWTP effluent is chlorinated, dependent on the amount of in plant nitrification. It was also assumed, by contrast, that insufficient chlorine would be present in septic tanks to form HAAs. Samples were collected from three chlorinating

WWTPs over the course of a year. DCAA (n=18) ranged from BD to 8.4µg/L and TCAA (n=20) from BD to 3.1µg/L in these effluents. The range in septic tank effluent samples for DCAA (n=22) was BD-2.6µg/L and TCAA (n=29) was BD-1.8µg/L. Despite difficulty detecting HAAs at low environmental concentrations, DCAA and TCAA were detected in WWTP effluents and in surface waters downstream of the point of discharge. Their observance upstream of the point of discharge indicates the WWTP effluent as the source of these compounds. These results suggest that HAAs are formed during chlorination of WWTP effluent at a slightly higher level than from septic systems. However, detection of HAAs in surface water does not conclusively indicate WWTP pollution.

Fluorescence spectrometry is a useful tool due to ease of sample preparation, quick analysis and ability for on-line sampling. Detection of tryptophan fluorescence indicates influence of waste. Hand-held fluorescence detectors can be taken into the field for real-time detection of pollution.

EEM fluorescent spectra were collected for WWTP and septic tank effluents, as well as surface waters surrounding WWTP discharges and throughout the advanced septic treatment system. Fluorescence regions and their intensity levels can be used to characterize pollution in surface waters, especially in Region IV (soluble microbial by-product-like) and Region V (humic like). When septic tank effluent samples are diluted with surface water, the EEM spectra becomes indistinguishable from upstream but tryptophan intensities are still detectable. Presence of tryptophan in the fluorescence spectra of downstream water samples would be a good indication of wastes, even if the EEM spectra are indistinguishable. The usefulness of fluorescence may be limited to close distances from discharge sources.

The proposed indicators can be used in combination with other monitoring tools to help identify a surface water pollution source. For example, fluorescence spectrometry is a useful initial tool. High intensities in fluorescence regions and presence of tryptophan provide an indication of influence of waste. The next step could be to test these fluorescence samples for caffeine and HAAs and use the relative results to suggest the pollution source as WWTP or failed septic systems. When used in combination with maps identifying other potential pollution sources, these tools have the ability to verify a surface water pollution source.

An indicator tool with the ability to distinguish between point and non-point sources of surface water pollution is an invaluable benefit to both public health and environmental management. Source identification enables more directed, efficient remediation and reduces further water quality degradation. Protection of surface water quality is especially important in North Carolina, where these waterways are often the primary sources of drinking water.

4.2 Recommendations

Developing one indicator to specifically identify pollution sources is a very complex issue. This research is part of a larger project identifying indicators of other types of non-point source pollution. Research into analyte concentrations and runoff from other non-point sources will enable further distinction between sources.

Additional sampling sites and increased number of samples are necessary for a more precise quantification of analyte concentrations from both WWTPs and septic systems. Sampling from more failed septic systems, especially pooled sewage and downstream of such systems would substantiate the concentration ranges in these sources and the effectiveness of these compounds as indicators. If a failed system was identified near a stream, sampling during

both rain events and dry periods would provide information to better quantify effluent overland flow as compared to transport during runoff, which could be tested by installing auto-samplers. Additional sampling sites at further distances downstream of WWTPs would provide field estimations to test the die-off calculations. Further research into compound sorption and microbial degradation would enable a more precise determination of degradation and persistence downstream. Analysis of compounds from suspended solids would provide a more accurate understanding of compound transport and degradation downstream of pollution sources.

Additionally, studies of personal care product use and their degradation/ removal during wastewater treatment would provide insight into other potential indicator compounds that are in high prevalence. This information would be useful for determining influent concentrations and estimating elimination rates from septic systems.

Since DCAA and TCAA were detected at low concentrations in WWTP effluents, further method development for their analysis in complex matrices is necessary to determine whether they persist downstream of conventional WWTP discharge and could be proposed as indicators of surface water pollution from such sources.

APPENDIX 1.

Table A1. Selected properties of chemicals.

	Caffeine	**Triclosan**	**Dichloroacetic Acid**	**Trichloroacetic acid**
Molecular Formula	$C_8H_{10}N_4O_2$	$C_{12}H_7Cl_3O_2$	$C_2H_2Cl_2O_2$	$C_2HCl_3O_2$
Kow units	0.01 - 0.07[1]	4.2-4.8[2]	1.33	0.92
Water solubility	10-15mg/mL at 23℃	10mg/L at 20℃[3]	10g/100mL at 15° C[4]	10g/100mL at 22° C[5]
pK_a	10.4	8.1	0.51	1.26
Molecular weight	194	289.5	129	163
Melting point ℃	238	54-57.3	9.7	57
Boiling point ℃			194	196
CAS #	58-08-2	003380-34-5	79-43-6	76-03-9
Est Koc units	10	1.8×10^4	1.895	2.738
Notes		Chlorinated phenoxyphenol, highly stable, lipophilic compound[2]		

CAS = Chemical Abstract Service
Estimated Koc = soil organic partition coefficient

[1] Thomas and Foster (2005)
[2] Lindstrom et al. (2002)
[3] Morrall et al. (2004)
[4] NIST 2005a.Acetic Acid, Dichloro -,National Institute of Standards and Technology.http://webbook.nist.gov/cgi/cbook.cgi?Units=SI&cTG=on&cIR=on&cTC=on&cMS=on&cTP=on&cES=on&cTR=on&cPI=on&cDI=on&ID=C79436.2006.
[5] NIST 2005b.Acetic Acid, Trichloro -,National Institute of Standards and Technology.http://webbook.nist.gov/cgi/cbook.cgi?Units=SI&cTG=on&cIR=on&cTC=on&cMS=on&cTP=on&cES=on&cTR=on&cPI=on&cDI=on&ID=C76039.2006.(2005b)

APPENDIX 2. Sample Calibration Curves

Table A2.1 Example Caffeine and Triclosan Calibration Curve, with Deuterated standard RR Calculations. 284: HCB, 194: Caffeine, 197: Deuterated Caffeine.

Sample	Conc (µg/L)	284 time	284 area	194 time	194 area	RR	197 time	197 area	345 time	345 area	RR	352 time	352 area
ACN blank													
cal 2	0.06	25.525	157324				29.094	3851				33.873	5785
cal 3	0.12	25.525	167182	29.257	616	2825	29.129	2725	33.918	405	1263	33.888	4007
cal 4	0.30	25.524	152412	29.324	1091	3797	29.362	1436	33.91	2638	2640	33.872	4995
cal 5	0.60	25.526	143885	29.272	2410	3082	29.123	1954	33.894	7557	2967	33.866	6365
cal 6	1.20	25.531	142238	29.277	1901	1205	29.105	1972	33.897	15679	3310	33.868	5919
cal 6	1.20	25.529	142221	29.191	3435	3613	29.177	1188	33.897	16625	3260	33.867	6372
ACN blank													
Avg			150877			3329					3044		5574
Stdev			10082			453					309		918
% CV			6.7			14					10		17

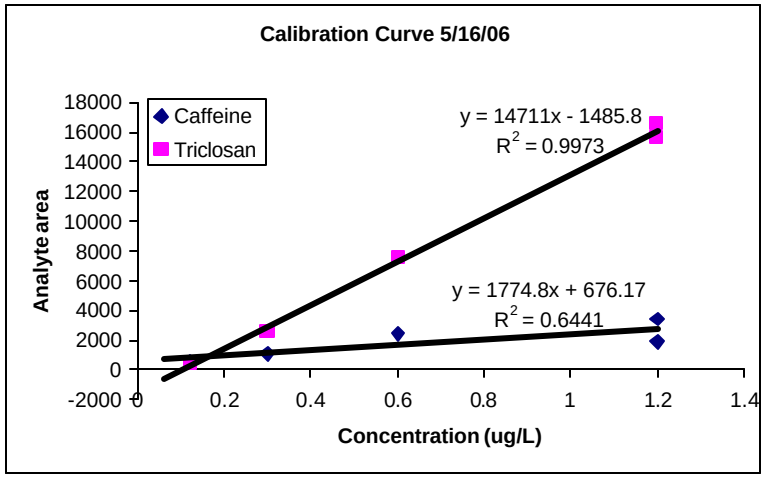

Figure A2.1 Calibration curve from 5/16/06

Table A2.2 Example calibration curve for HAAs, analyzed July 8 by GC-ECD.

Sample	Conc (µg/L)	IS Area	DCAA Area	Rel Area	Avg	RPD	TCAA Area	Rel Area	Avg	RPD
Cal 1A	0	146583								
Cal 1B	0	149164								
Cal 2A	2	162745	50252	0.309	49334	4	86476	0.531	85109	3
Cal 2B	2	152388	48415	0.318			83741	0.550		
Cal 3A	5	155224	91747	0.591	83135	21	217764	1.403	207721	10
Cal 3B	5	155099	74523	0.480			197678	1.275		
Cal 4A	10	161923	151380	0.935	135637	23	445413	2.751	471335	-11
Cal 4B	10	162138	119893	0.739			497257	3.067		
Cal 5A	25	166597	270773	1.625	256831	11	1070083	6.423	972415	20
Cal 5B	25	160314	242888	1.515			874747	5.456		
Cal 6A	50	165456	466659	2.820	457713	4	1747008	10.6	1759800	-1
Cal 6B	50	167666	448766	2.677			1772591	10.6		

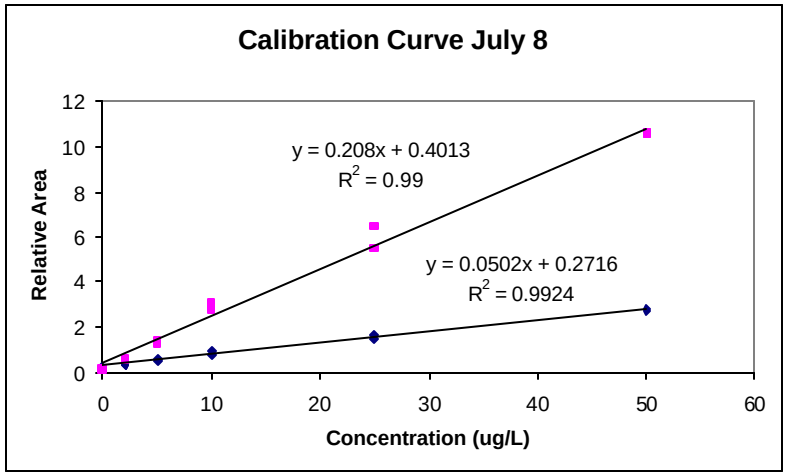

Figure A2.2 DCAA and TCAA calibration curve analyzed 7/8/06 by GC-ECD.

APPENDIX 3. Sample chromatograms

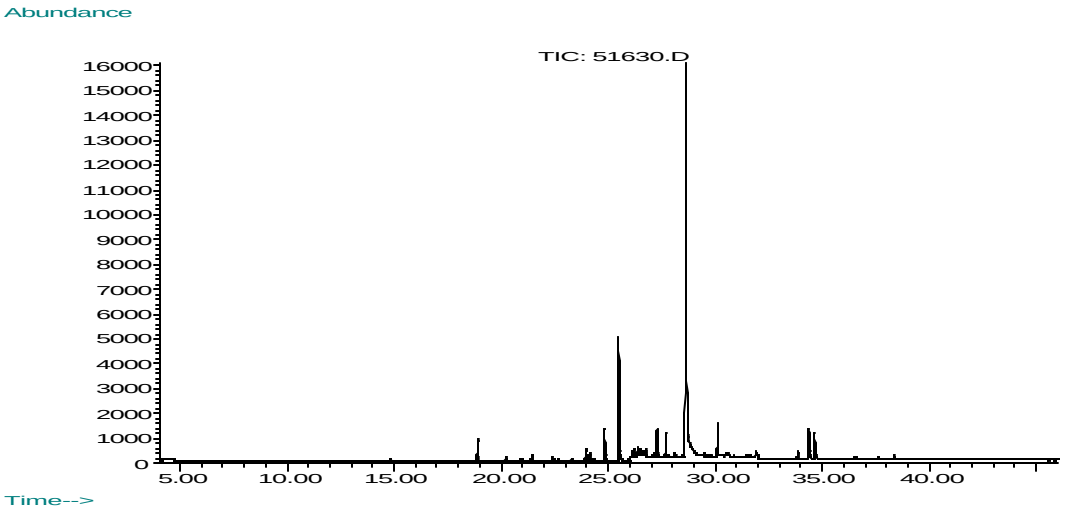

Figure A3.1 Sample total ion chromatogram from a high school septic tank effluent, analyzed by GC-MSD, 5/16/06.

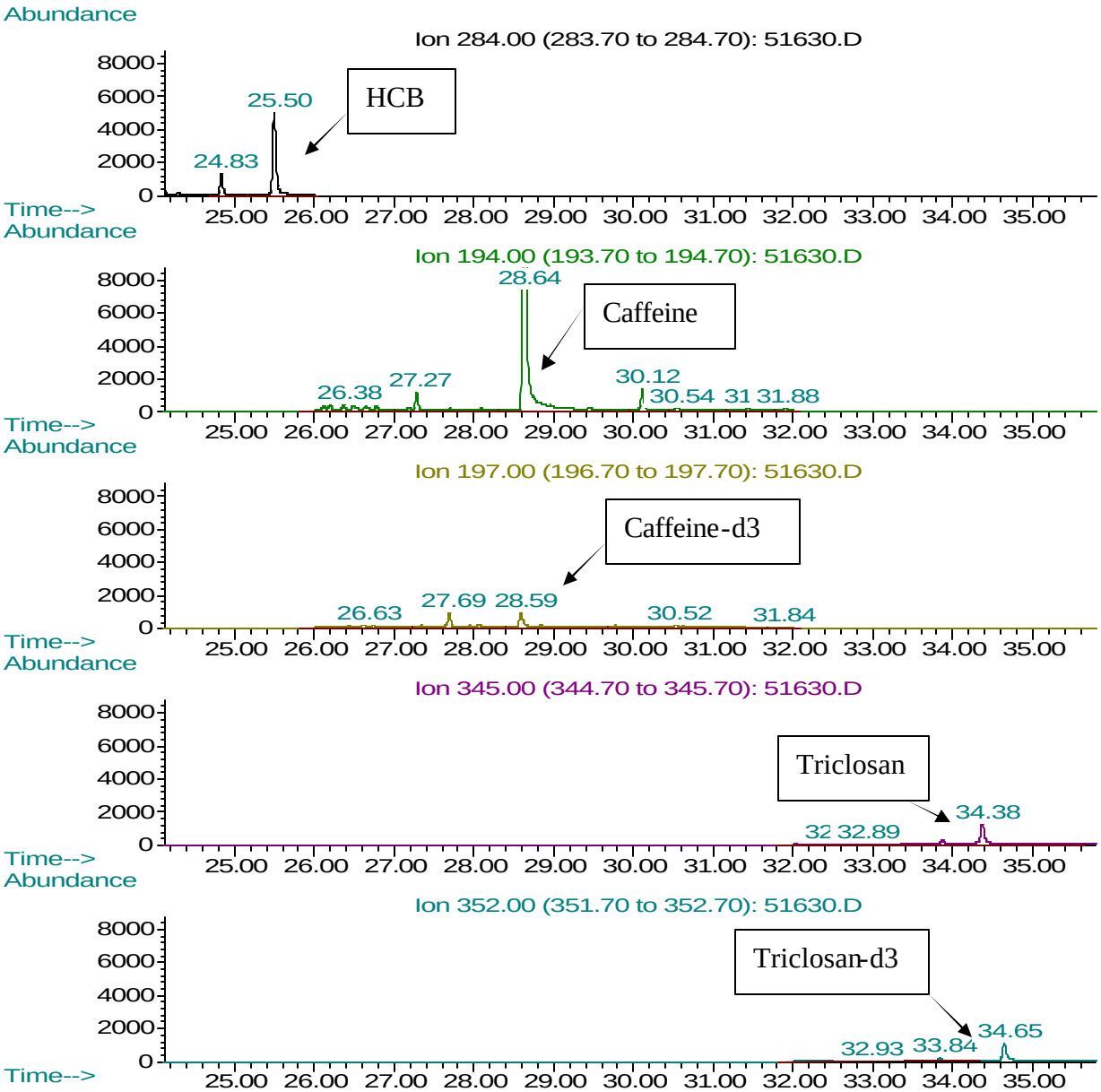

Figure A3.2 Example of extracted ion chromatograms from the high school septic tank effluent Extracted ions are m/z 284 for HCB, 194 for caffeine, 197 for deuterated caffeine, 345 for triclosan and 352 for deuterated triclosan.

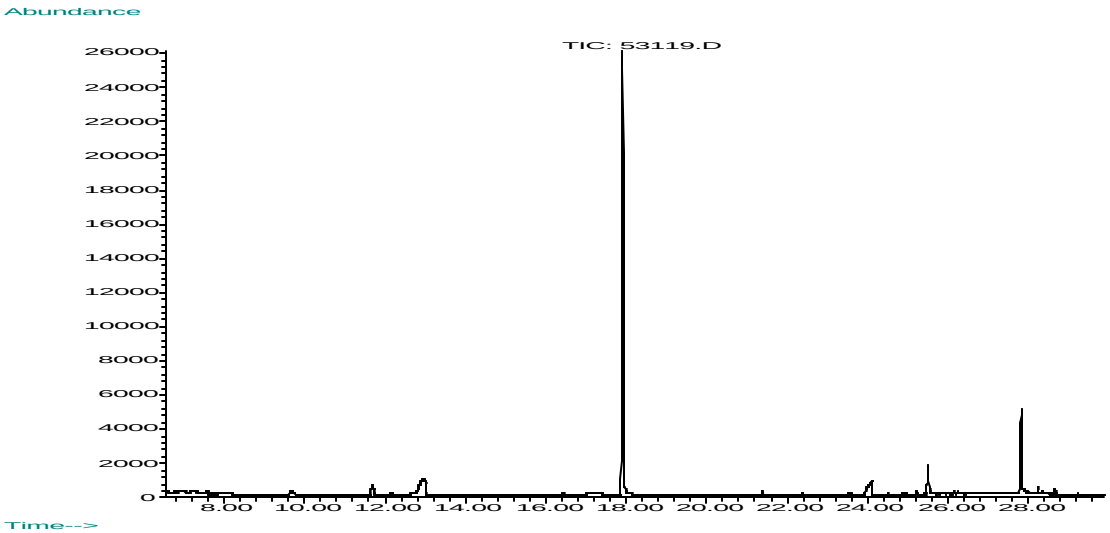

Figure A3.3 Total ion chromatogram from WWTP2 effluent processed 5/31/06 using larger LLE extraction, analyzed by GC-MSD.

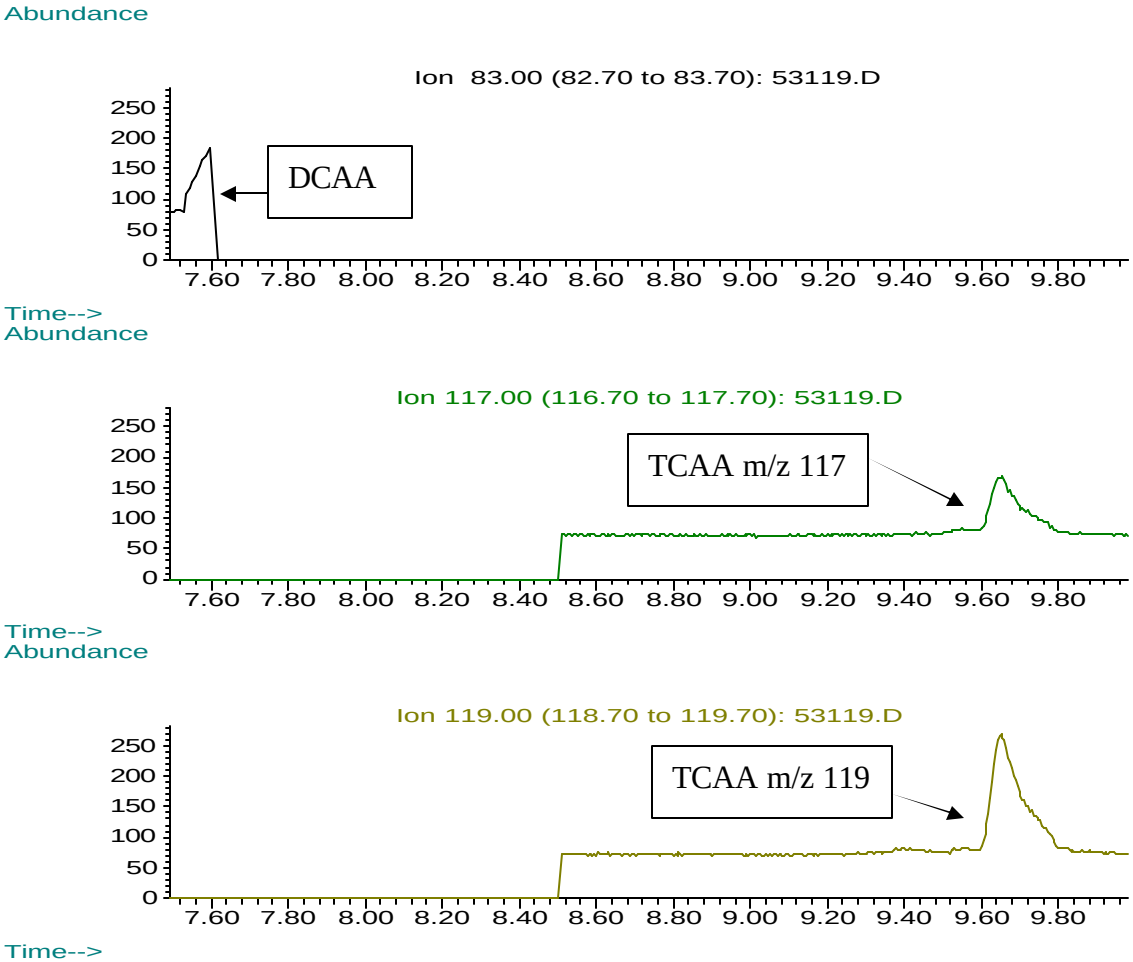

Figure A3.4 Extracted ion chromatogram from WWTP2 effluent processed 5/31/06 using larger LLE extraction, analyzed by GC-MSD. Extracted ions are m/z 83: DCAA, 117: TCAA and 119: TCAA.

Chromatogram Plots

File: ...cuments\research stuff\research data\2006 march\310.13 we blk a.sms
Sample: 310.13 we blk A
Operator: Talia
Scan Range: 1 - 3263 Time Range: 0.00 - 52.47 min.
Date: 3/10/2006 11:30 PM

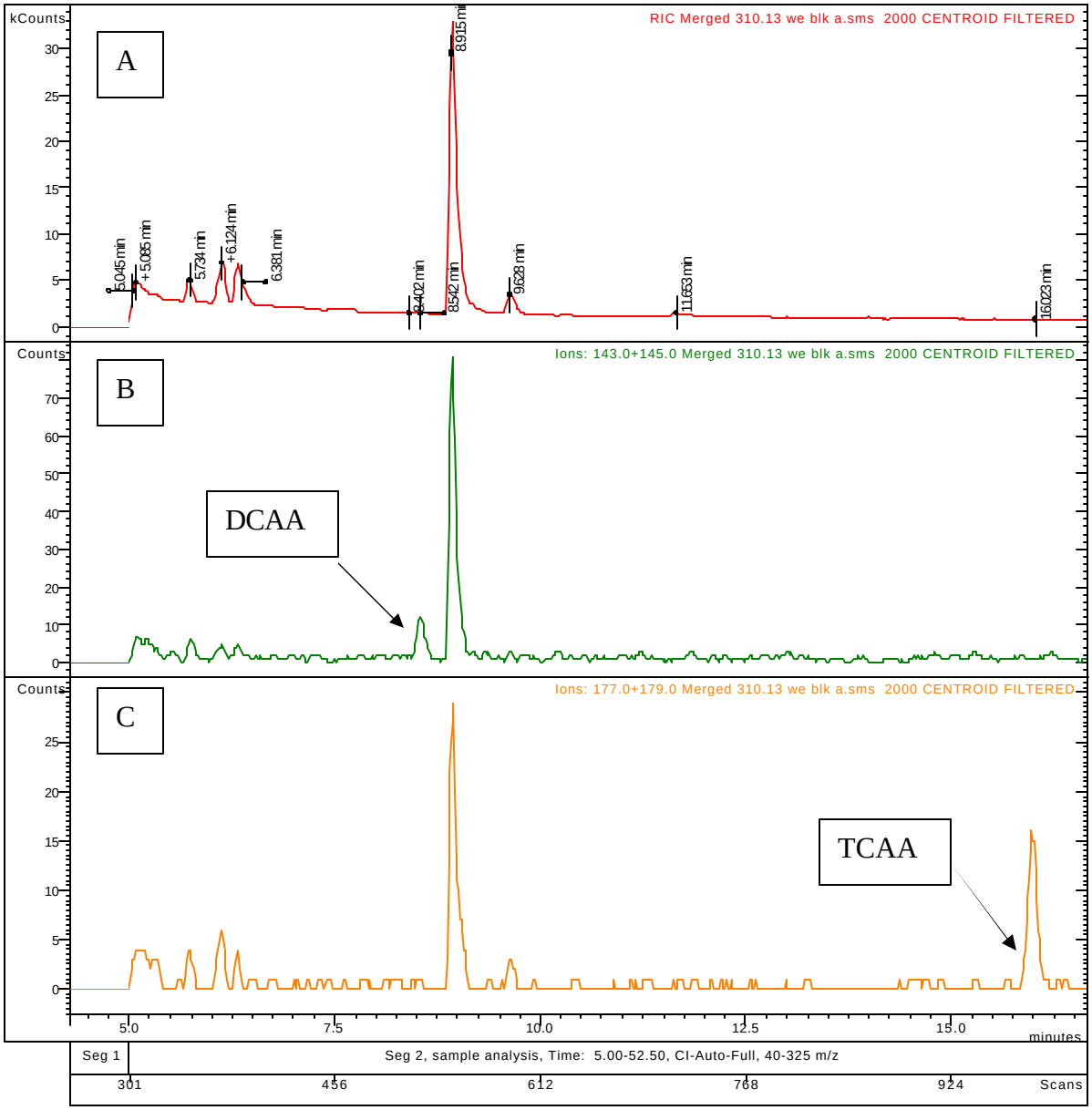

Figure A3.5 GC-ion trap-MS, CI mode, chromatogram from WWTP2 effluent processed 3/10/06. (A) Total ion chromatogram, (B) DCAA extracted with ions m/z 143 and 145, (C) TCAA extracted with ions m/z 177 and 179.

APPENDIX 4.

Table A4.1 Fluorescence Intensities.

		Tryptophan (275, 305)			Tyrosine (280, 360)			Fulvic-like (230-260, 380-500)			Humic-like (260-400, 380-500)			Soluble microbial products (260-400, 300-380)		
Date	Sample	Ex	Em	Inten.	Ex	Em	Inten.	Ex	Em	Inten.	Ex	Em	Inten.	Ex	Em	Inten.
11/23/2005	WWTP1 DS	280	310	1584				250	420	3455	320	400	2979			
11/23/2005	WWTP1 DS										290	417	2837			
11/23/2005	Septic	277	305	4210	290	361	7117	240	400	7542	310	390	7579			
11/23/2005	WWTP1 us							240	420	2534						
11/23/2005	WWTP1 we	279	310	1535				250	430	3812	320	400	3362			
8/18/2005	WWTP1 ds	280	310	125							286	415	244			
8/18/2005	WWTP1 we	280	310	100							320	400	153			
8/18/2005	septic 6-09	275	305	328				261	420	683	320	400	813			
8/18/2005	WWTP1							250	430	261	320	400	235			
5/3/2006	WWTP2 ds				280	350	1301	252	430	1653	320	410	1302			
5/3/2006	HS 1x				280	360	838	250	430	1635	330	413	1262	317	349	593
5/3/2006	HS D1				280	360	697	250	423	1560	320	420	1007			
5/3/2006	HS d2				280	360	676	240	408	1553	320	411	916	300	350	500
5/3/2006	HS d3				280	360	609	250	413	1509	320	413	876			
5/3/2006	HS d4				280	360	614	250	420	1551	320	410	857			
5/3/2006	WWTP1 us							240	410	1596	310	410	764			
5/3/2006	WWTP2 eff	280	300	6133	280	360	10000	250	450	5551	350	430	8101			
5/3/2006	WWTP2 D1				290	340	8770	250	440	3649	340	430	5166			
5/3/2006	WWTP2 D2				285	340	6846	260	440	3219	340	430	4269			
5/3/2006	WWTP2 D3				282	340	5378	250	440	2686	340	430	3421			
5/3/2006	WWTP2 D4				280	340	4779	260	440	2589	340	430	2911			
5/22/2006	HS ds							240	430	2807	330	430	2531			
5/22/2006	HS pu				280	360	816	250	430	1988	330	420	1472			
5/22/2006	HS 1x	275	305	803	280	360	969	250	430	1944	330	420	1550			
5/22/2006	HS pt	275	305	2857	280	343	4693				330	430	2286			
5/22/2006	HS pt d1	275	305	1035	280	360	1613	240	420	2814	330	430	2499			
		Tryptophan (275, 305)			Tyrosine (280, 360)			Fulvic-like (230-260, 380-500)			Humic-like (260-400, 380-500)			Soluble microbial products (260-400,		

														300-380)		
Date	Sample	Ex	Em	Inten.	Ex	Em	Inten.	Ex	Em	Inten.	Ex	Em	Inten.	Ex	Em	Inten.
5/22/2006	HS pt d2	275	305	817	280	360	1200	240	430	2553	330	430	2495			
5/22/2006	HS pt d3	275	305	648	280	360	1035	250	440	2620	330	430	2615			
5/22/2006	HS pt d4	275	305	569	280	360	979	250	440	2570	330	430	2568			
5/22/2006	HS us							240	430	2715	330	430	2516			
5/22/2006	WWTP3 us				280	360	624	240	415	1878	310	410	1079			
5/22/2006	WWTP3 eff	275	305	1257	280	360	1705	240	410	2888	330	410	2411			
5/22/2006	WWTP3 ds 1				280	360	1093	240	410	2550	320	410	1646			
5/22/2006	WWTP3 ds 2	275	305	457	280	360	581	240	428	1754	310	408	1088			
5/22/2006	WWTP2 us				280	360	667	240	410	1806	310	410	1007			
5/22/2006	WWTP2 eff				280	360	2900	250	440	4068	340	422	4286			
5/22/2006	WWTP2 dis				280	360	1816	240	430	2723	340	430	2607			
5/22/2006	WWTP2 ds				280	360	755				310	410	1083			

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