Effects of Wastewater Disinfection on Triclosan and Detection of a Hydroxylated Polybrominated Diphenyl Ether in Wastewater Effluent

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

Robert Noah Hensley

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Adviser:
William Arnold

June 2012

Acknowledgements

I would first like to thank my adviser, Bill Arnold. Your understanding, expertise, and dedication to your graduate students are exceptional. I doubt I would have found a better adviser anywhere in the world. I am thankful for all that you have done from supporting my education and finding funds to troubleshooting issues with my experiments. You have helped me with my goals and dreams, and I will be forever grateful.

My fellow graduate students have also been exceptional. I have appreciated the willingness of each one to help discuss results and revise experiments. Of particular note, I would like to thank Cale Anger for all of the insightful and encouraging conversations. Both Cale and Hao Pang were great to have as part of the triclosan team and were always willing to lend a helping hand. The following people helped with obtaining wastewater samples and this project could not have been completed without them: Karin North and Robert Hara at the Palo Alto Regional Water Quality Control Plant; Larry Rogacki, Robert Golden, and Paul VanLith at the Metropolitan Wastewater Treatment Plant; Michael Ross of the College of St. Benedicts's; and Paul Stock at the St. John's University Wastewater Treatment Plant. Peter Villalta, coordinator of the Mass Spectrometry Services at the Masonic Cancer Center, and Brock Matter were exceptional in their expertise and help with analyzing samples. Thanks are also due to the Department of Civil Engineering Graduate Fellowship and the National Science Foundation for funding my education and this research.

Further thanks goes out to my friends and family. My dad and mom have always supported and believed in me. The Clark's have also been integral in my success, both in the past and during my graduate studies. Finally, I give thanks to my heavenly Father who called me out of darkness and into the glorious light, giving my life meaning and helping me find my purpose.

Dedication

This thesis is dedicated to my wonderful wife Amy. You are the love of my life. You are the most compassionate, loving person I know. Thank you for working so hard to help me attain my dreams. I have appreciated your patience, love, and support and cannot imagine life without you. Thank you for everything that you have done for me and with me from spending long nights in my office, while I work in the lab until 3 am to running me back and forth from campus. I hope to work just as hard to help your dreams and ambitions turn into reality. This thesis marks the end of one journey and the start of another. Though we may not know what the future holds, we will always have each other. Together we will love others: providing increased access to safe, clean water and healthcare services to people around the world who are in need. May love be our legacy.

"Unable are the Loved to die
For love is immortality,
Nay, it is Deity –

Unable they that love – to die
For Love reforms Vitality
Into Divinity."

– Emily Dickinson

Abstract

Triclosan, an antimicrobial agent used in many personal care products, is chlorinated in wastewater treatment plants (WWTPs) giving rise to chlorinated triclosan derivatives (CTDs). Through photolysis in natural waters, these compounds yield specific polyhalogenated dibenzo-*p*-dioxins. Hydroxylated polybrominated diphenyl ethers (OH-PBDEs), which have natural and anthropogenic sources and are of similar structure to triclosan, may react similarly. It is important to understand the loads of these chemicals emanating from WWTPs and how disinfection practices influence these concentrations.

Using pre-concentration of water samples by solid phase extraction followed quantification using liquid chromatography tandem mass spectrometry, the yield of CTDs has been quantified at WWTPs using different disinfection treatments. Triclosan was detected in all wastewater samples from < 14 – 1609 pM (< 36 – 465 ng/L). The sum of CTDs in chlorinated effluents were higher than other effluents, ranging from 63 – 80 pM (21.5 – 27.2 ng/L). Wastewater from a WWTP that used reduced chlorination had 17.4 – 23.4 pM (3.9 – 4.7 ng/L) of total CTDs. In wastewater that was not disinfected or UV irradiated, concentrations of CTDs were 1.9 – 29.4 pM (0.7 – 10.1 ng/L). Laboratory ozonation of wastewater reduced total CTDs significantly from 10 pM (3.4 ng/L) to 1.5 pM (0.5 ng/L). The estimates of triclosan-derived dioxin loadings to US surface water ranged from 0.3 – 207 g TEQ/year (0.01 – 8.3% of US air emissions of dioxins). These estimates are significant due to their unique discharge directly into the aquatic environment.

The OH-PBDE, 6-OH-BDE-47, was detected in some extracts at the highest levels detected in wastewater yet – up to 33.6 pM (1.8 ng/L). This report is the first to confirm wastewater as a source of 6-OH-BDE-47 to a fresh water environment.

Table of Contents

List of Tables

List of Figures

List of Abbreviations and Notations

$^{13}C_{12}$-triclosan – Isotopically labeled triclosan
4-Cl-TCS – 4,5-chloro-2-(2,4-dichlorophenoxy)phenol
4,6-Cl-TCS – 4,5,6-chloro-2-(2,4-dichlorophenoxy)phenol
6-Cl-TCS – 5,6-chloro-2-(2,4-dichlorophenoxy)phenol
6-OH-BDE-47 – 6-hydroxy-2,2',4,4'-tetrabromodiphenyl ether
(A) – Anthropogenic in origin
(C) – Chlorination
CAS – Chemical Abstract Service
Co – Composite (24-hour) sample
CTD – Chlorinated triclosan derivative
DBP – Disinfection byproduct
DCDD – Dichloro-dibenzo-*p*-dioxin
EPA – Environmental Protection Agency
ESI – Electrospray ionization
FW – Freshwater
f.w. – Fresh weight
G – Grab sample
HAA – Halo acetic acid
LC-MS/MS – Liquid chromatography and tandem mass spectrometry
LOD – Limit of detection
LOQ – Limit of quantitation
l.w. – Lipid weight
MCDD – Monochlorinated dibenzo-*p*-dioxin
MeO-PBDEs – Methoxylated polybrominated diphenyl ethers
MGD – Million gallons/day
MS-Q^3 – Triple quadrupole mass spectrometer
MTBE – Methyl *t*-butyl ether
MWP – Metropolitan Wastewater Treatment Plant
(N) – No disinfection
Na – Naturally occurring compound
ND – Not detected
(O) – Ozonation
OH-PBDE – Hydroxylated polybrominated diphenyl ether
OH-PCB – Hydroxylated polychlorinated byphenyl
OH-PCDE – Hydroxylated polychlorinated diphenyl ether

OH-PCB – Hydroxylated polychlorinated biphenyl
OH-PHDE – Hydroxylated polyhalogenated diphenyl ether
PAWP – Palo Alto Regional Water Quality Control Plant
PBDE – Polybrominated diphenyl ether
PBDD – Polybrominated dibenzo-*p*-dioxin
PCB – Polychlorinated byphenyl
PCDD – Polychlorinated dibenzo-*p*-dioxin
PHDD – Polyhalogenated dibenzo-*p*-dioxin
RC – Reduced chlorination
SJWP – St. John's University Wastewater Treatment Plant
SPE – Solid phase extraction
SRM – Single reaction monitoring
TBDD – Tetrabrominated dibenzo-*p*-dioxin
TCDD – Tetrachlorinated dibenzo-*p*-dioxin
TEF – Toxic equivalency factor
TEQ – Toxic equivalency quotient
THM – Trihalogenated methane
TrBDD – Tribrominated dibenzo-*p*-dioxin
TrCDD – Trichlorinated dibenzo-*p*-dioxin
UV – Ultraviolet
WLSSD – Western Lake Superior Sanitation District
WSD – Waste disposal site
w.w. – Wet weight
WWTP – Wastewater treatment plant

1. Introduction and Literature Review

1.1 Anthropogenic Chemicals in the Environment

Every year industries around the world introduce thousands of new chemicals into the environment. As of June 2012, almost 68 million commercially available small chemicals have been registered with the Chemical Abstract Service (CAS) [1]. As the CAS registry continues to grow weekly, many of these chemicals make their way into the air, water, and food supplies, eventually partitioning into humans [2-4]. Major routes of anthropogenic chemicals into the environment include product off-gasing, landfills, incineration facilities, municipal wastewater treatment plants (WWTPs), stormwater runoff, application of sewage sludge as fertilizer, and manufacturing waste streams.

At the time this thesis was written, 295,096 chemicals were considered inventoried/regulated substances [1]. This number will continue to grow. The resources to track these chemicals and adhere to regulations are lacking. Thus, prioritizing which chemicals should be monitored is of utmost importance [5]. Many chemicals have been frequently detected in the environment, but do not have enough toxicological information to determine their effect on humans and animals. Meanwhile, toxicologists have tested other chemicals that may not be well documented in the environment [5, 6]. These chemicals include both natural and synthetic organic chemicals which have the potential to bioaccumulate up the food chain [7-9].

Bioaccumulation occurs when plants and animals ingest more of a chemical than they expel through waste or equilibrium processes. Depending on the toxicity of the

chemical to an organism, bioaccumulation can play an important role in determining the overall effects that an organism experiences. Frequent measuring of concentrations in wildlife must be made because levels depend on various dynamic processes such as consumption, excretion, exposure levels, etc. Because monitoring thousands of individual chemicals in various environmental matrices (water, soil, sediment, animals, humans) is not practical, environmental scientists are forced to monitor selected chemicals likely to have the greatest effect on the health of humans and the environment [5].

Many chemicals that are monitored continuously flow into the environment through municipal and industrial waste streams [10-12]. One example is triclosan, an antibacterial agent used in many handsoaps. Most of the triclosan present in personal care products eventually enters municipal WWTPs [13]. While a large percentage of the triclosan is removed, a substantial amount is still present in the final effluent and enters the environment. Flame retardants represent another group of chemicals that have been widely detected in the environment. Polybrominated diphenyl ethers (PBDEs), a class of flame retardants, are present in many products from electronics to furniture and end up in the air through incineration processes. PBDEs also off gas from their products and aggregate with other particles and chemicals to form dust [14]. As the dust makes its way into and through the lungs, the flame retardants partition into humans and have been found in breast milk of pregnant women and the blood serum of children and adults [15]. These flame retardants may also be metabolized into more toxic chemicals [16]. While many chemicals allow people to live long, healthy lives, others, like the metabolized

flame retardants, pose a variety of health risks and disrupt the endocrine system even at very low levels [6, 16]. Hundreds of studies in the past two decades have raised concern over the fate of these chemicals in the environment and adverse effects on animals and humans [6].

Not only do many anthropogenic compounds present environmental hazards, but their transformation products pose risks as well. As chemicals are released into the environment, many undergo various transformations into different chemicals. These reactions may be biotic or abiotic and occur under a variety of conditions. The products of these reactions, while not directly produced by human beings, are still anthropogenic in origin. Examples include metabolites of synthetic compounds and chemicals that undergo electrophilic substitution reactions with hypochlorite during wastewater disinfection [17, 18]. While some chemicals are broken down into harmless chemicals during transformation processes, other chemicals may form more dangerous products, like polyhalogenated dibenzo-*p*-dioxins (PHDDs) [17]. Two such chemicals, triclosan and a metabolite of a PBDE, are the focus of this thesis.

The adverse environmental effects of dioxins on humans and animals have been well documented and are generally accepted by the public. Research has found that combustion is the leading contributor to anthropogenic dioxin discharge. Consequently, the majority of effort in reducing dioxins has effectively been focused towards cleaner combustion techniques [19]. The photolysis of hydroxylated polyhalogenated diphenyl ethers (OH-PHDEs), however, can yield specific dioxin congeners [20, 21]. Thus loading

of OH-PHDEs directly into water through WWTPs may contribute significantly to total dioxin discharge. This thesis aims at elucidating the levels of five OH-PHDEs being discharged by various WWTPs and the effect of wastewater disinfection on these compounds.

1.2 *Overview of Wastewater Treatment Operations*

About 74% of residents, or 226.4 million people, in the United States depend on WWTPs to treat and dispose of wastewater in an environmentally sound fashion [22]. Based on the Environmental Protection Agency's (EPA) Clean Watersheds Needs Survey, 31,206 million gallons are treated every day by 15,609 WWTPs [23]. Most WWTPs are designed similarly and generally have at least two steps. Pre-treatment of wastewater consists of bar racks to remove larger objects and debris followed by a grit chamber which allows large particles to settle out. Wastewater then undergoes primary treatment, flowing into the primary clarifier which may remove solids that are more dense (via settling) or less dense (via skimming) than water. Roughly 30 WWTPs only have primary treatment serving approximately 3.9 million people [23]. These plants have special permits because they discharge directly into the ocean [23].

The next step, secondary treatment, uses microbial activity to degrade dissolved solids. Trickling filters, rotating biological contactors, and biotowers are fixed-film systems generally used by smaller communities and depend on biofilms grown on media or large rotating wheels to degrade dissolved solids. Larger WWTPs must use suspended-film systems. Many large plants employ activated sludge removal consisting of an

aeration chamber and a secondary clarifier. The aeration chamber provides the suspended microbes with oxygen so they can quickly degrade pollutants. The solids from the primary and secondary clarifier are treated and either disposed of in landfills, incinerated, or applied to agricultural fields as fertilizer. WWTPs that have secondary treatment systems with no additional advanced treatment technologies serve 30.2% of the US population.

Approximately 37% of the population is served by WWTPs with one or more advanced treatments in addition to the aforementioned steps [23]. These treatments include filtration, coagulation/flocculation, carbon adsorption, phosphorus removal, and/or nitrogen control. Some WWTPs, serving 5.5% of the population, do not discharge directly into surface waters, but use their effluent for agricultural purposes or groundwater recharge and likely use advanced treatment technologies to meet the stringent requirements placed on these types of facilities. The total discharge by plant type [23] is summarized in Table 1.1.

Table 1.1.

Summary of Wastewater Treatment Plants in the United States

Treatment level	Number of facilities	Average flow (MGD)	Number of people served	Percent of US population
Primary only	30	422	3,751,787	1.2
Secondary	7,302	13,142	92,650,605	30.2
Advanced	5,071	16,776	112,947,134	36.8
No Discharge	2,251	1,815	16,946,528	5.5
Total	14,654	32,155	226,296,054	73.7

With this knowledge, loading of pollutants into United States surface waters may be estimated with higher confidence than assuming everyone uses the same treatment system. The rest of the population uses septic systems, which may or may not affect surface waters. The final step for wastewater treatment is disinfection, which deactivates pathogenic organisms with the goal of keeping surface waters safe for human contact.

1.2.1 *Chlorination*

As of 2003, approximately 75% of all major WWTPs used chlorination technology for final effluent disinfection [24]. Chlorination has been used for over a century to treat drinking water and wastewater, thus the technology is convenient, relatively cheap, and effective. Chlorination is performed with either hypochlorite or chlorine gas, and both result in effective inactivation of pathogens. Due to increased awareness of risks involved with handling, transportation, and storage of chlorine gas, many WWTPs have switched from chlorine gas to hypochlorite over the past 30 years [24]. The formation of disinfection byproducts (DBPs) in both drinking water and wastewater is another disadvantage of chlorination [25]. Chlorine reacts with natural organic matter and other chemicals to form various trihalogenated methanes (THMs), halogenated acetic acids (HAAs), and many other DBPs [25]. THMs and HAAs are toxic to humans and to organisms in receiving waters [25]. Wastewater disinfection chambers using chlorination are designed with residence times ranging from 15 – 90 minutes depending on the permits obtained [24]. Chlorination is followed by dechlorination to quench residual chlorine and protect the wastewater impacted ecosystems.

1.2.2 *Ultraviolet Irradiation*

A steadily increasing number of WWTPs are shifting from chlorination to ultraviolet (UV) irradiation for disinfection of final effluent. UV irradiation successfully deactivates pathogens, while producing little or no DBPs [26]. A survey in 2003 estimated that 21% of major WWTPs in the US used UV disinfection and indicated that many chlorination WWTPs will be switching to UV in the future [24].

1.2.3 *Ozonation*

Very few plants in the US use ozone to treat wastewater. Ozone may be created on site by pumping oxygen or air through an electric field [27]. Oxygen molecules collide with each other in the electric field and form ozone. When ozonated air is pumped through a diffuser into the wastewater, some of the ozone dissolves in the water. Aqueous ozone degrades contaminants, inactivates pathogenic organisms, and reacts with hydroxide ions to form hydroxyl radicals [28]. Hydroxyl radicals also react quickly with wastewater substituents. Although more expensive than chlorination due to electrical costs of another aeration basin and the generation of ozone itself, some advantages to ozonation include removal of odorous compounds, effluent oxygenation, and destruction of endocrine disruptors and personal care products [29-32]. Like chlorination, ozonation of wastewater can produce significant levels of unwanted DBPs like bromates, but only when ozone dosages exceed the ozone demand of the wastewater [33]. Another study found that ozonated wastewater negatively affects trout when compared with conventionally treated wastewater [34]. A final sand filtration step helped eliminate the

disinfection byproducts of ozonation and the effect on trout [34]. Ozonation may also reduce the mutagenic and estrogenic activity of the wastewater [34, 35]. Many DBPs from the ozonation of wastewater have yet to be discovered or studied for negative affects to ecosystems or humans [36].

1.3 *Triclosan*

The antimicrobial agent, triclosan (Figure 1.1), is used in products ranging from hand soaps and toothpaste to plastic containers and socks. First patented in 1964, triclosan was first used in deodorants [37]. In 1972, hospitals and clinics began using surgical handsoaps with 1% triclosan to successfully reduce incidences of infection during surgeries [38]. Triclosan has since been infused into various medical supplies such as IV tubing, syringes, sutures, and other medical supplies [38]. Beginning in 1985, European toothpastes began including triclosan to help fight gum disease after experiments showed favorable results [38, 39]. In 1987, triclosan started being added to commercial handsoaps at lower percentages, 0.1 - 0.45%, than in surgical scrub soaps [40, 41].

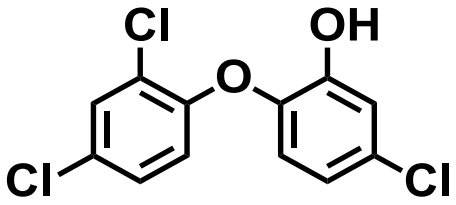


Figure 1.1 Structure of Triclosan.

A study of commercial hand soaps from 2001 found triclosan in 76% of liquid handsoaps and 29% of bar soaps [42]. As of Spring 2010, the US Food and Drug Administration has received no evidence that commercial handsoaps containing triclosan are more effective than ordinary handsoaps [43]. Triclosan has also been included in a variety of consumer products such as bicycle shorts, shoe insoles, socks, cutting boards,

sponges, and children's toys [4]. Evidence suggests incorporation of triclosan into plastics used to wrap raw meat provide no extra benefits [44]. Some common names that refer to products infused with triclosan include Irgasan® DP 300, Irgacare® MP, Ultra-Fresh®, Amicor®, Microban®, Monolith®, Bactonix®, and Sanitized® [4].

1.3.1 *Detection in WWTPs*

About 96% of triclosan in personal care products is flushed into sewer systems and enters WWTPs [13]. In WWTPs with activated sludge systems, 90-98% of the incoming triclosan sorbs to sludge or biodegrades from the influent to the effluent [45, 46]. Removal of triclosan from WWTPs using trickling filters was lower, ranging from 58-86% [46]. Aerobic biodegradation of triclosan accounts for 50-70% removal in the activated sludge, while the rest is removed via sorption to solids [47-50]. Triclosan has been detected in biosolids from WWTPs and the agricultural soils where those biosolids are applied [45, 46, 48, 51-53]. Chenxi et al. [54] reported on the persistence of triclosan in biosolids, observing no aerobic degradation of triclosan over a period of 77 days. Other studies have observed aerobic biodegradation of triclosan with a half-life from 18 – 58 days in soils and biosolids-amended soils [55, 56].

Triclosan concentrations have been detected in the effluent of WWTPs around the world ranging from 0.04 – 18.6 nM (0.011-5.4 µg/L) [11, 18, 45, 46, 57-59]. From measurements of wastewater effluent, Buth et al. [18] estimated that 11 metric tons of triclosan per year flows into the surface waters of the US. Halden and Paull [57] estimated similar mass loadings for triclosan. Depending on the time of year, regional

climate, and drought conditions, wastewater can contribute substantially to stream and river flow. Some contaminants may adversely affect the stability of the ecosystems in which wastewater flow dominates stream flow [60]. The contribution of WWTP effluent to triclosan concentrations in surface waters will vary substantially depending on the contributing factors for a given area. Nevertheless, wastewater streams are continuous sources of triclosan into the environment, and this author is not aware of any studies that have not detected triclosan in WWTP effluent.

1.3.2 *Detection in the Environment and Relevance*

Wastewater is the major contributing factor when determining amounts of triclosan in the aquatic environment. A box model accounting for wastewater inputs, dilution effects, photolysis, and adsorption was effective at predicting levels of triclosan in the water column and sediments of the Hudson River Estuary [61]. This study and others have detected triclosan in many surface waters around the world at concentrations up to 3.5 nM (1.1 µg/L) [48, 57, 62-66]. A US Geological Survey study in 2000 reported triclosan in 57.6% of the 139 streams across 30 states [64]. The highest concentration was 7 nM (2.3 µg/L), and the median concentration was 0.5 nM (140 ng/L) [64].

In 1970, Schulze et al. [67] found 17-62 nM (4.9-17.9 µg/L) of triclosan in human blood after handwashing with triclosan-containing soap. Since then, triclosan has been detected in various environmental matrices including surface water, algae, snails, fish, and breast milk [4, 56-58]. With an octanol-water partition coefficient (K_{ow}) of ~$10^{4.8}$, triclosan has the potential to bioaccumulate and bioconcentrate [57]. Coogan and La

Point reported bioaccumulation factors of 500 and 1400 for snails and algae, respectively [68]. Ricart et al. [69] suggested that bacterial community composition of river ecosystems may change when exposed to environmentally relevant triclosan concentrations and determined the no effect concentration for bacteria and algae, respectively, to be 730 pM (211 ng/L) and 1460 pM (422 ng/L). These concentrations are within the range reported from the effluent of WWTPs around the world, although the risk to aquatic species in most US surface waters seems to be minimal [13, 70, 71].

Another common route of environmental triclosan exposure occurs after the application of biosolids which generally contain high amounts of triclosan (500-15600 µg/kg) [45, 58]. Triclosan leaches into the soils below resulting in levels ranging from 20 – 833 µg/kg soil [72]. Kinney et al. [72] calculated bioaccumulation factors of 11 and 27 in earthworms. Earthworms and turnips are also some of the most triclosan-sensitive terrestrial organisms with predicted no effect concentrations ranging from 1-200 µg/kg soil [73].

Recently, triclosan has been found to have endocrine disrupting effects in frogs, fish, mammalian cultures, and rats [74-77]. These studies lead to questions about endocrine disruption in humans, because triclosan has a half-life in human blood of 9-14 hours after intravenous injection or brushing teeth with triclosan containing toothpaste [78]. If a person brushes their teeth twice a day with such toothpaste, triclosan may never fully dissipate in the body leading to chronic exposure. Another concern with triclosan is the potential development of antibiotic resistance. High levels of triclosan mixed with the

numerous bacterial communities present in activated sludge give bacteria the opportunity to evolve and adjust to efflux triclosan more effectively [62]. The bacteria that efflux triclosan more effectively can also efflux other drugs more effectively, an effect known as cross-resistance [62]. Triclosan can also be metabolized in activated sludge into methyl triclosan, which has been found in WWTP effluent, surface water, and fish tissue [47, 65, 79, 80]. Toxicological data on methyl triclosan, which is more lipophilic and more stable than triclosan, is lacking for a definitive risk assessment [2, 65]. Triclosan is clearly persistent in the environment and originates from the use of antibacterial handsoaps and other triclosan-containing products.

1.3.3 *Disinfection and Photolytic Byproducts*

An overview of triclosan's degradation pathways, relevant to this thesis, is shown in Figure 1.2. As soon as triclosan contacts chlorinated tap water, DBPs begin forming. The residual free chlorine reacts with triclosan to form byproducts such as chloroform, chlorophenols, and higher chlorinated triclosan derivatives (CTDs), which are detected during all stages of wastewater treatment [46, 81-83]. Chloroform and the chlorophenols formed from triclosan are toxic to humans and the latter are known to be endocrine disruptors [81]. Three CTDs known to form from the chlorination of triclosan: 4,5-chloro-2-(2,4-dichlorophenoxy)phenol (4-Cl-TCS), 5,6-chloro-2-(2,4-dichlorophenoxy)phenol (6-Cl-TCS), and 4,5,6-chloro-2-(2,4-dichlorophenoxy)phenol (4,6-Cl-TCS). Biomethylated analogues of CTDs have been detected in a wastewater impacted stream and in carp from a bay receiving wastewater [84]. Chlorination of

wastewater can increase concentrations of total CTDs in WWTP effluent up to 30% of the concentration of triclosan [18]. WWTPs with UV disinfection will not see this effect, although CTDs may still be present at low concentrations from reactions with bleach or residual chlorine in tap water on the way to the WWTPs. Toxicological information on CTDs is lacking, and whether CTDs have similar effects to triclosan has not been studied. CTDs have, however, recently become available for purchase through Wellington Laboratories [85].

Triclosan is included in a chemical class known since 1974 to be precursors to dioxins [86]. Degradation of triclosan and CTDs via solar photolysis in water samples has been confirmed to produce dioxins with a yield of 0.5 - 2.5% with a potential upper limit

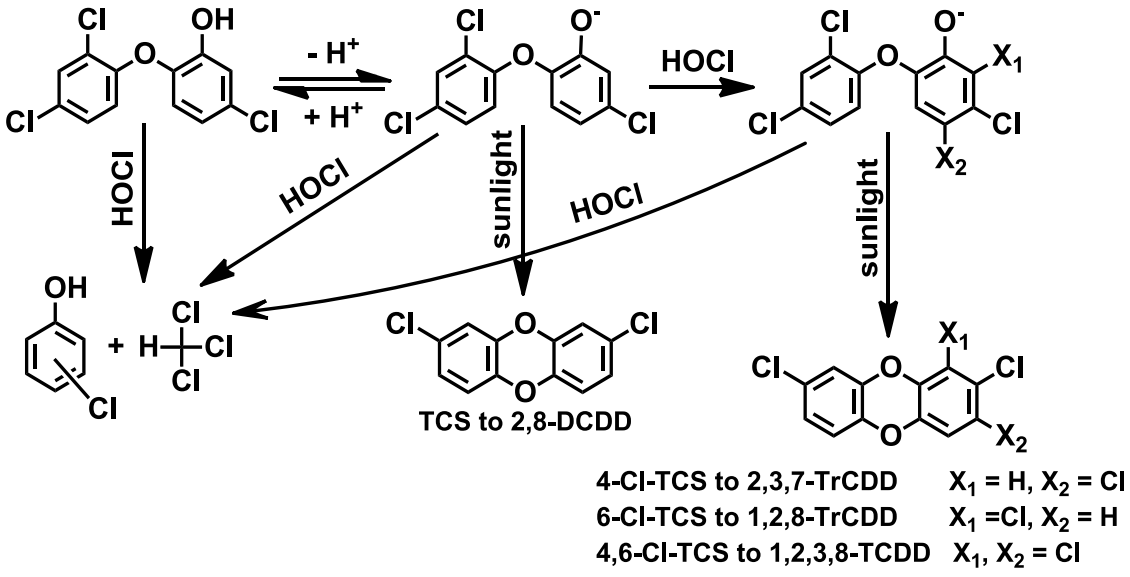


Figure 1.2 Chlorination and Photolysis of Triclosan.
Ionized triclosan undergoes photolysis at higher rates than its protonated form. Triclosan can be chlorinated forming chloroform, chlorophenols, and three CTDs. Triclosan and the CTDs may undergo photolysis to form dioxins. Dioxins formed from triclosan and CTDs have 2 or 3 laterally positioned chlorines and may be more toxic than other di- and tri-chlorinated dioxins.

of 3% [17, 87]. Photolytic degradation of triclosan and subsequent production of 2,8-dichloro-dibenzo-*p*-dioxin (DCDD) has been observed in both fresh water and sea water [88]. Friedman et al. [89] measured an efflux of 2,7/8-DCDD from Newark Bay to the surrounding atmosphere, which was attributed to the photolysis of triclosan. These experiments raise concerns about releasing triclosan and CTDs into sunlit environments, such as surface waters, and how to minimize the release of dioxins into the environment.

The toxicity of other dioxin congeners are referenced to the most toxic congener, 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD). A dioxin or dioxin-like compound is assigned a toxic equivalency factor (TEF) [90, 91]. By convention the TEF of 2,3,7,8-TCDD is equal to one. More toxic compounds have TEFs higher than one, while less toxic compounds have TEFs less than one. The toxic equivalency quotient (TEQ) of a compound can then be assessed by multiplying its concentration by its TEF. Summing the TEQs of individual compounds in mixture yields the total TEQ [90]. This type of metric may be used to compare estimates of triclosan-derived dioxins with dioxins from other sources, which is one purpose of this thesis.

Recently other tests have been developed to determine the relative toxicity of other dioxins and dioxin-like compounds [92]. The chemically-activated luciferace gene expression cell bioassay system (CALUX) by Xenobiotic Detection Systems is one such test [92]. CALUX relative potency values (REPs), similar to TEFs, were determined for a broad range of PHDDs and dibenzofurans [92]. The REPs and TEQs of some relevant dioxins are given in Table 1.2. Two of the triclosan-derived dioxins, 2,8-DCDD and

Table 1.2.

Methods of Comparing Congener Specific Dioxin Toxicity*

Method	CALUX	WHO	Ontario MoE
Year	2009	2005	1987
Reference	[92]	[90]	[91]
Toxicity Unit	REP	TEF	TEF
1-MCDD	0.00001	NA	0.0001
2,8-DCDD	0.0001	NA	0.001
2,8-DBDD	0.0002	NA	NA
2,3,7-TrCDD	0.0025	NA	0.01
2,3,7-TrBDD	0.0017	NA	NA
2,3,7,8-TCDD	1	1	1
2,3,7,8-TBDD	0.78	NA	NA
1,2,3,7,8-PeCDD	1.38	1	0.1

*The dioxin abbreviations are as follows: M – mono; D – di; Tr – Tri; T – Tetra; Pe – Penta; B – Brominated; C – Chlorinated; DD – dibenzo-*p*-dioxin.
NA = Not Available.

2,3,7-trichlorinated dibenzo-*p*-dioxin (TrCDD), have been assigned REPs by experimental means, while the other two dioxin REPs must be estimated.

The REP value for 1,2,3,7,8-pentachlorinated dibenzo-*p*-dioxin (PeCDD) is 1.38, which is greater than the REP of 2,3,7,8-TCDD. This comparison suggests that adding a chlorine substituent to the 1 position may increase toxicity. Two dioxins formed from CTDs, 1,2,8-TrCDD and 1,2,3,8-TCDD, have substituents in the 1 position with respect to 2,8-DCDD and 2,3,7-TrCDD (2,3,7- is the same dioxin as 2,3,8-), which may increase their toxicity. REPs for 1,2,8-TrCDD and 1,2,3,8-TCDD may be estimated according to this principle.

Dioxin toxicity also tends to increase with the number of lateral chlorine substituents [93]. 2,3,7,8-TCDD has all four lateral positions filled. The dioxins from triclosan chlorination and/or direct photolysis have two or three of the four lateral positions filled. Thus, the production of CTD's by chlorination of wastewater and

concurrent photochemical transformation in surface waters could unintentionally be raising levels of more toxic dioxins. Without congener specific tests of the lower chlorinated dioxins, analysis of the dioxin toxicity produced from triclosan and CTD photolysis is difficult to estimate. Despite this lack of knowledge, the type of wastewater disinfection used will play an integral role in estimating triclosan-derived dioxin loading to surface waters.

1.4 *Polybrominated Diphenyl Ethers*

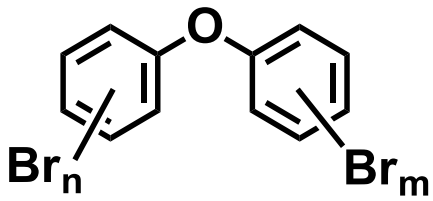


Figure 1.3 Structure of PBDEs.

Since the 1970's brominated flame retardants have been used in polyurethane foams, textiles, carpets, and electronics to prevent fires and the spread of fire [14, 94]. PBDEs (Figure 1.3) are compounds in one common class of flame retardants. Three different commercial mixtures exist: penta-, octa-, and deca- [95]. The mixtures contain 39 of the 209 possible PBDE congeners [96]. While the deca-mixture is still in production, the penta- and octa-mixtures have been phased out of the market due to health concerns [97]. The deca-mixture contains mostly BDE-209 (n = m = 5) and was thought to be less harmful than other mixtures. Evidence of debromination of the decaBDE, as well as new toxicological data on BDE-209, has guided the EPA to encourage the voluntary phase-out of manufacturing and importing of the deca mixture [97-101].

1.4.1 *Detection and Relevance in the Environment*

PBDEs, like the notorious polychlorinated biphenyls (PCBs), have been detected all over the world, even in remote regions in the Arctic [102, 103]. This pervasive chemical class has been detected in fish, birds, and lynx, moose, reindeer, and polar bears [104-107]. The EPA reviewed available literature on PBDE toxicology and concluded that current levels of exposure for wildlife are near adverse effect levels [97]. Neurotoxicity during developmental stages in mice have been observed at concentrations similar to those found in highly exposed infants and toddlers [108]. PBDEs may also disrupt the endocrine system and cause cancer [109, 110].

Human exposure to PBDEs occurs via ingestion of foods and inhalation of air and dust [14, 106, 111-124]. Many products containing PBDEs are disposed of in landfills, while electronics may be recycled to extract valuable metals for reuse [125]. Household and industrial wastes containing various congeners of PBDEs are commonly incinerated and represent an important source of PBDEs into the atmosphere and surrounding environment [111]. Humans breathe PBDEs while sitting on their couch, driving to work, taking a hike in the forest, or riding a bike in the city [114, 126-131]. Levels of PBDEs in North American and British dust tends to be higher than in the rest of the world due to more widespread use of PBDEs in these countries [122]. In some cases, the passage of stringent state flammability laws, like in California, have led to disproportionately large exposure to PBDEs [132].

Perhaps the most notable risk is how PBDEs affect babies and children. BDE-47, a tetraBDE, dominates the congeners of PBDEs present in human breast milk and the blood serum of children [15, 127]. A few studies found higher concentrations of PBDEs in the blood serum of children compared to adults [15, 133, 134]. Children subjected to higher prenatal concentrations of BDE-47, -99, or -100 scored significantly lower on tests of mental and physical development [135]. A follow-up study has concluded that postnatal exposure positively correlated to ADHD symptoms and poor social competence of the examined children [136]. Other studies have found PBDEs exert other toxic effects on animals and humans [16, 110, 128, 137]. More extensive reviews of PBDEs in the environment are available [103, 110, 114].

1.4.2 *Detection in WWTPs*

Although the most common route of exposure to PBDEs to humans and animals is through household dust and atmospheric concentrations, these chemicals enter aquatic and terrestrial environments via wastewater as well. PBDEs have been detected in biosolids and wastewater effluents around the world [10, 138-140]. The presence of PBDEs in domestic wastewater (as opposed to industry wastewater) suggests that humans constantly ingest and excrete them [138]. The PBDEs present in biosolids may end up in agricultural fields and accumulate in soils with little, if any, degradation [139].

1.4.3 *Degradation Pathways*

Although degradation was not observed in soils, metabolic debromination of the decaBDE, BDE-209, was observed in biota downstream of a WWTP [98]. The metabolic

pathways that seem to be most apparent include cleavage of the ether bond to produce brominated phenols and hydroxylation/debromination to form hydroxylated PBDEs (OH-PBDEs) [141-147]. Abiotic photolytic debromination and degradation can occur as well [148-151]. When incinerated or heated to high temperatures, PBDEs can form polybrominated dibenzo-*p*-dioxins (PBDDs) [152, 153]. With 209 possible congeners of PBDEs, determination of every degradation pathway is difficult and confined by the limits of analytical chemistry and research budgets. Despite the troubles encountered, scientists continue to work diligently to determine the most important transformation pathways of PBDEs.

1.5 *Hydroxylated PBDEs*

Due to the extent that PBDEs are found in environmental matrices, transformation products of PBDEs have also become a concern. While anthropogenically derived OH-PBDEs represent some of these PBDE transformation products, natural sources of OH-PBDEs exist as well. Whether natural or anthropogenic sources of OH-PBDEs play more of a role in sustaining environmental levels continues to be the foundation of many scientific investigations [154, 155]. Suggested formation pathways include metabolism of PBDEs by animals, oxidation of PBDEs by hydroxyl radicals in the atmosphere, and natural production from marine organisms [142, 143, 147, 156-159].

1.5.1 *A Variety of OH-PBDEs*

Natural sources of simple to complex organobrominated compounds have been known for years [160, 161]. OH-PBDEs with the hydroxyl group in the ortho- position,

as opposed to meta- and para-, with respect to the ether are thought to be naturally produced by sponges, cyanobacteria, and red algae (see Table 1.3) due to the high concentrations observed in these organisms compared to other organisms, as well as seasonal fluctuations [156, 161-165]. Despite this generalization, a few ortho-OH-PBDEs are thought to be metabolic byproducts of BDEs in rats, chicken, and humans [15, 142, 143, 147, 158, 159].

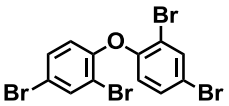
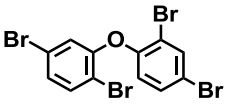
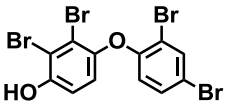
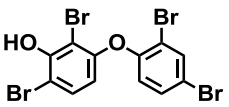
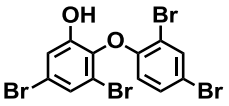
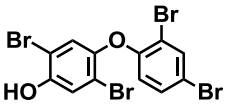
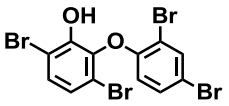
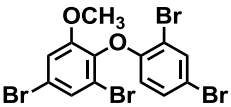
A comparison of the concentrations of individual compounds in humans in Table 1.3 gives evidence of 6-OH-BDE-47 being a metabolic byproduct [15]. Blood serum was analyzed from people living in a waste disposal site (WSD) with nearby incinerators and people living in a nearby city but much farther from the WSD [15]. Higher exposure to BDE-47 seems to correlate well with higher levels of 6-OH-BDE-47 in the body as well as some other congeners. Methylated OH-PBDEs (methoxylated-PBDEs or MeO-PBDEs) are also present in cyanobacteria, blue mussels, fish, and sea lions [155, 162, 166, 167]. This triad of compound classes - PBDEs, OH-PBDEs, and MeO-PBDEs - presents a challenge for future studies due to the large number of compounds involved and lack of pure standards to identify all relevant compounds.

1.5.2 *Detection and Relevance in the Environment*

Many studies have administered PBDEs to animals and detected OH-PBDEs as metabolites [142, 143, 158, 168]. As shown in Table 1.3, 4'-OH-BDE-49 and 4'-OH-BDE-101 were detected as metabolites in fish fed food spiked with PBDEs [145]. 6-OH-BDE-47, 6-MeO-BDE-47, and 2'-MeO-BDE-68 were also detected in the fish, but their

Table 1.3.

BDE-47 and Relevant Byproducts in Various Environmental Matrices in pg/g *

Compound	Red Algae (f.w.) [156]	Fish fed PBDEs (w.w.) [145]	Gulls (w.w.) [169]	FW (ng/L) [157]	FW Fish (w.w.) [170]	Dolphin (w.w.) [171]	Humans by WDS (l.w.) [15]	Humans in city (l.w.) [15]
 BDE-47	---	1000 - 9000	4430 - 24300	---	94 - 11480	340 - 15000	330480	14580
 BDE-49	---	200 - 3800 (A)	---	---	ND	ND	---	---
 4-OH-BDE-42	---	ND	ND	< 0.01 - 1	< 0.01 - 1.2	2.4 - 23 (A)	4954 (A)	165 (A)
 3-OH-BDE-47	---	ND	< 70 - 500	< 0.01 - 1.1	< 0.01	2.1 - 15	3354 (A)	165 (A)
 6-OH-BDE-47	1900 (Na)	9 - 95 (Na)	< 70 - 260	0.85 - 8.2	2.8 - 20.5	238 - 425 (Na)	6708 (A)	1032 (A)
 4'-OH-BDE-49	---	6 - 30 (A)	< 80 - 540	< 0.01 - 3.9	< 0.01 - 170.5	8.1 - 51 (A)	9288 (A)	495 (A)
 6'-OH-BDE-49	---	---	< 90 - 340	< 0.01 - 0.3	ND	< 2 - 4.3	---	---
 6-MeO-BDE-47	190 (Na)	116 - 270 (Na)	< 20 - 170	---	---	---	---	---

* f.w. – fresh weight; w.w. – wet weight; l.w. – lipid weight; "(Na)" under a concentration denotes a naturally occurring compound, while "(A)" denotes anthropogenic in origin.
ND = not detected. FW = Freshwater. WDS = Waste disposal site.

presence originated from the seawater suggesting a natural source [145]. A few studies have determined that 6-OH-BDE-47 may be methylated metabolically to form 6-MeOH-BDE-47 [155]. In return, 6-MeOH-BDE-47 may also be metabolically demethylated to form 6-OH-BDE-47 [172]. The same study found that BDE-47 was not metabolized into either compound by a Japanese medeka, a fish [172]. Other evidence suggests that 6-OH-BDE-47 is metabolized from PBDEs by human liver cells and specific OH-PBDEs congeners have been detected in human blood samples (see Table 1.3) [15, 147, 159]. These studies reported low rates of hydroxylation from PBDEs to OH-PBDEs [155], although oxidative metabolism of BDE-47 to 6-OH-BDE-47 adds to this congener's neurotoxic potential [16, 173]. OH-PBDEs are also endocrine disruptors and may affect animals and humans even at the low levels produced metabolically [16, 144, 174-179].

A summary of some OH-PBDEs known to occur as natural compounds, marked with "(Na)", or anthropogenic byproducts, marked with "(A)", in the environment is shown in Table 1.3. The OH-PBDEs in Table 1.3 are structurally closest to BDE-47. BDE-49 is also thought to be a metabolite of BDE-47 [145]. 6-OH-BDE-47 is the most relevant to this thesis. More complete studies are needed to fully assess the origin of OH-PBDEs in the environment and humans, as well as the significance of anthropogenic OH-PBDEs.

1.5.3 *Detection in Wastewater Effluent*

Only two studies have found OH-PBDEs in wastewater effluent. While looking for triclosan in wastewater from a WWTP on the Detroit River, one study reported other

peaks near the internal standard, 2'-OH-BDE-28, with the same mass fragmentation pattern, but the compounds were not identified [63]. 6-OH-BDE-47 and 5-OH-BDE-47 were recently detected at ~1 pg/L in wastewater effluent [180]. Other OH-PBDEs may also be present in wastewater effluent, but scientific evidence is lacking.

1.5.4 *Photolytic and Disinfection Byproducts*

OH-PBDEs, in addition to triclosan and CTDs, undergo photolysis to form PBDDs [21]. Chlorination of OH-PBDEs, like triclosan, produces chlorinated OH-PBDEs [21]. These chlorinated versions may also form PHDDs [21]. Brominated dioxins are found in marine wildlife including sponges, red algae, and cyanobacteria [156, 165, 181-183]. PHDDs may also be produced anthropogenically through incineration of various products [184]. PBDEs may transform into OH-PBDEs, be released into surface waters, and undergo photolysis to form PHDDs. PBDDs are thought to be as potent as PCDDs and the various congeners follow the same pattern of toxicity as described previously [92, 185]. The toxicity of PBDDs relative to 2,3,7,8-TCDD is shown in Table 1.2. The release of OH-PBDEs into surface waters from WWTPs may lead to increased levels of PBDDs. PBDDs are hydrophobic and likely bioaccumulate in fish leading to increased human exposure to these toxic compounds.

2. Effect of Wastewater Disinfection on Five Dioxin Precursors

2.1 *Introduction*

Much of the triclosan used in personal care products ends up at wastewater treatment plants. Kumar et al. [11] detected up to 86 µg/L of triclosan in wastewater influent. If 99% of incoming triclosan is removed during secondary treatment, the secondary effluent would contain 860 ng/L in the effluent. As triclosan reacts with hypochlorite, three CTDs will form. Buth et al. [18] found that CTDs increased in a WWTP due to the final chlorination step. In the same study, a WWTP using UV irradiation had only small amounts of CTDs relative to the chlorinating WWTP [18].

The focus of this research was to further elucidate how disinfection practices affects levels of CTDs in effluents. Three methods of disinfection (chlorination, UV irradiation, and ozonation) were investigated. Four WWTPs were selected and each sampled on two dates: a large plant that chlorinates from May to October, a smaller plant that chlorinates at lower levels or not at all, and two plants that disinfect with UV irradiation (one small, one large). A wastewater sample was collected from the large chlorinating plant during the non-chlorinating period (winter). Finally, a grab sample from one of the large plants was obtained and ozonated in the laboratory. These samples were extracted and analyzed to determine the effect of disinfection on triclosan and CTDs in wastewater.

PBDEs can be metabolized by humans or bacteria into products similar to the brominated version of triclosan, OH-PBDEs. One of these compounds, 6-OH-BDE-47,

was added to the list of analytes. Only one study has identified this compound from a single WWTP in China at very low levels. A more comprehensive analysis of 6-OH-BDE-47 in various wastewater effluents is needed to determine whether wastewater is a significant source of OH-PBDEs to the environment.

2.2 *Experimental Methods*

2.2.1 *Chemicals*

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol; >97%) was purchased from Sigma Aldrich. A methanolic solution of high purity (>99%) isotopically labeled triclosan ($^{13}C_{12}$-triclosan) was purchased from Wellington Laboratories. Three CTDs (4-Cl-TCS, 6-Cl-TCS, and 4,6-Cl-TCS) and 6-hydroxy-2,2',4,4'-tetrabromodiphenyl ether (6-OH-BDE-47) were synthesized for previous studies [17, 21]. Stock solutions of each compound were made gravimetrically in methanol. A final combined stock solution with all the compounds was created containing 50 µM triclosan and 10 µM of CTDs and 6-OH-BDE-47. Sulfuric acid (ACS grade) and commercial grade silica gel (60 Å) were purchased from BDH. Ammonium acetate was purchased from Mallinckrodt AR. Ultrapure water (18.2 MΩ-cm) was obtained from a Millipore Simplicity UV purification system. HPLC grade (>99.9%) acetonitrile was purchased from J.T. Baker. HPLC grade (>99.9%) methanol and acetone were purchased from Sigma-Aldrich. Methyl *t*-butyl ether (MTBE) was obtained from Sigma-Aldrich (>99.0%). Ethyl acetate was obtained from Macron Chemicals (>99.5%). Industrial grade nitrogen was used for the blowing down of eluents. Potassium indigotrisulfonate was obtained from Sigma-Aldrich to

measure the dose of ozone to a batch reactor. A Thermo-Orion Ross Ultra Semi-Micro pH meter was used to make pH measurements.

2.2.2 *Cleaning Procedure*

Triclosan is ubiquitous in laboratories, bathrooms, dust, and populated areas. Thus contamination of samples at all stages of experimentation is of concern. Powder-free nitrile gloves were used while conducting extractions and changed frequently. The plastic syringes and all glassware, except disposable pipets, were brush-scrubbed with triclosan-free soap, and rinsed 3 times with tap water then DI water. The glassware was then ashed at 550 °C for 4 hours or rinsed 3 times with both methanol and ethyl acetate. Disposable glassware, including centrifuge tubes and glass pipets, were also ashed. Silica gel, glass wool, and sand used in the silica column were ashed prior to use. Foil was used to cover glassware when appropriate and laid out to serve as a protective layer from any contamination on lab benches and in fume hoods. Before each transfer, glass, gas-tight syringes were flushed five times consecutively with acetone, methanol, and acetonitrile. After each transfer, the outside of the syringe needle/tip was rinsed with ~1 mL of acetone to minimize cross contamination. Separate syringes were used for spiking $^{13}C_{12}$-triclosan, spiking the analytes of interest, and resuspending the final extracts in acetonitrile and water. The pH meter was also rinsed with ultrapure water before and after each use.

2.2.3 *Collection and Preparation of Samples*

Composite samples (24-hour) from three WWTPs were analyzed in addition to grab samples from a fourth WWTP. WWTP operators or technicians collected pre- and post-disinfection effluent, offset to represent the same wastewater stream, in solvent rinsed glass containers. Samples were filtered within a day through 0.7 µm glass fiber filters (47 mm; Fisher Scientific) into other solvent rinsed glass bottles. The pH of each sample was recorded and then adjusted to 3-4 with sulfuric acid. The pK_as of the compounds of interest range from 5.9 – 7.6 [17, 186]. At pH < 4, all of the analytes will be > 98% in their hydrophobic, neutral forms allowing high recovery from solid-phase extraction. Samples were then stored in the dark at 4 °C until further processing, which was usually carried out within 72 hours.

Metropolitan Wastewater Treatment Plant (MWP) in St. Paul, MN has a capacity of 251 million gallons/day (MGD) serving 1.8 million people. MWP chlorinates their effluent from April through October with a dosage of 1.25 µg/L Cl as Cl_2 for > 30 min, aiming for a residual of 0.20 µg/L Cl as Cl_2. Effluent is dechlorinated with sodium bisulfite at 0.95 µg/L. MWP discharges directly into the Mississippi River. Composite samples from MWP were obtained on three separate dates. On two dates in the fall, pre- and post-chlorination samples were obtained, and one non-chlorinated effluent sample was obtained during the winter. A pre-chlorination grab sample was also obtained to determine the effects of ozonation on the analytes.

Two activated sludge WWTPs, Palo Alto Regional Water Quality Control Plant (PAWP) and Saint John's University Wastewater Treatment Plant (SJWP), were each sampled (24-hour composite, pre- and post-UV) during two dates to measure the effect of UV disinfection on triclosan and CTDs in wastewater. PAWP treats on average 21.8 MGD serving 220,000 people with ~5% industry wastewater. PAWP disinfects year round using a system of Trojan UV 3000 Plus assemblies with an energy output of 35 mW-s cm^{-2}. The average ultraviolet transmittance of the wastewater is 62% with a contact time of 3.8 seconds. PAWP discharges effluent directly into the southern San Francisco Bay.

St. John's University uses groundwater for their potable water supply and does not chlorinate prior to use. The SJWP treats the used water serving a population of 2600 during the academic year and 1200 in summer. SJWP is licensed to process a maximum flow of 0.23 MGD and treats about 0.16 MGD on an average day. After filtration through sand, secondary effluent is disinfected using a Package Treatment UV-3000 system containing six modules. Each module has four 162.6-cm lamps which provide 190 µW cm^{-2} at 1 m with radiation centered at 254 nm. The contact time in the disinfection tank is between 2 and 4 minutes depending on flow conditions. SJWP discharges into East Lake Gemini which then drains into the North Fork of the Watab River and eventually into the Mississippi River.

Effluent grab samples from Western Lake Superior Sanitation District (WLSSD) were collected by boat on two occasions. Approximately half of the wastewater that

WLSSD treats originates from industries. WLSSD filters the secondary effluent through mixed media beds and disinfects with chlorine, but is only required to chlorinate when a fecal coliform analysis of treatment plant intake exceeds 100 MPN/100 mL. The chlorine dosages were not available for the dates that sampling took place. WLSSD discharges into the St. Louis Bay which flows into Lake Superior.

2.2.4 *Solid Phase Extraction*

A method developed by Buth et al. [18] was slightly modified for analysis of the five compounds of interest. Three or four 500 mL replicates were prepared in Erlenmeyer flasks by spiking 0.5 nM $^{13}C_{12}$-triclosan as a surrogate for the compounds of interest. This type of isotope dilution methodology is described in detail elsewhere and commonly used to determine concentrations of analytes in complex matrices [182, 183]. Another 500 mL sample was prepared in the same manner, but was also spiked with 1.5 nM triclosan and 0.3 nM of the other analytes (3 CTDs and 6-OH-BDE-47) to verify that the other compounds partition as triclosan does throughout the extraction method. All the flasks were then shaken and stored overnight in the dark to allow for equilibration of the analytes into the wastewater matrix.

On the next day, Oasis HLB solid phase extraction (SPE) cartridges were loaded on to a vacuum manifold and preconditioned with consecutive 5 mL aliquots of MTBE, methanol, and pH 3 ultrapure water. A thin layer of liquid/solvent was always left above the SPE cartridges from this point onward to keep the cartridge from drying out. Teflon transfer lines with attached rubber stoppers were connected to the SPE manifold with 6

mL plastic syringe barrels and vacuum-flushed consecutively with ~5 mL of MTBE, methanol, and pH 3 ultrapure water before extraction. The tips of the transfer lines that came into contact with the samples were also rinsed with acetone. Wastewater replicates were loaded onto the SPE cartridges at a flow rate of 15 g/min. Samples spiked with all analytes were processed after the replicates to minimize cross contamination. A balance and stop watch were used to measure the flow rate from the sample containers. The flow rate tended to slow down near the end of the extraction due to increased head loss from organics on the cartridge. Pressure was adjusted to maintain a rate close to 15 g/min.

After loading the samples, cartridges were flushed with 3 consecutive aliquots of 50:50 methanol:H_2O (v/v) under slight vacuum (~5 g/min) and dried under vacuum for at least 15 minutes. This step removes the more polar organic matter from the cartridge while leaving the analytes of interest on the cartridge. Cartridges were secured with tape above glass centrifuge tubes and eluted with 10 mL of methanol and 5 mL of 90:10 MTBE:methanol (v/v). Eluents were then blown down with a gentle stream of nitrogen to ~500 µL for silica column clean up.

2.2.5 *Silica Column Clean-up*

Silica columns were prepared in washed and rinsed 6 mL plastic Luer tip syringes. A small plug of glass wool was placed in the bottom of the column to keep sand and silica from escaping. A thin layer of sand, followed by 2 g of silica gel, and another thin layer of sand completed the silica column. Each column was tapped to settle the silica and sand into the column and flushed with 10 mL of ethyl acetate to rinse the

column of any contamination. The eluent from the SPE step was immediately loaded onto the column using an ashed, disposable glass pipet. Three 1 mL aliquots of ethyl acetate were used to triple rinse the centrifuge tube containing the SPE eluent. As soon as the eluent passed the top layer of sand, each rinse was consecutively loaded on to the silica column with a glass pipet. The column was not allowed to dry out during the run. After the rinses were loaded, the column was eluted with ~11 mL of ethyl acetate. The collected ethyl acetate (~14 mL) was blown down with a gentle stream of nitrogen to ~300 µL. This final extract was transferred using a glass pipet to amber glass vials with 350 µL conical inserts. The extract was allowed to dry overnight in the vial and resuspended in 40-50 µL of 50:50 acetonitrile:H_2O (v/v). Spiked samples were diluted 5-10 times to lessen the effects of triclosan suppressing $^{13}C_{12}$-triclosan.

2.2.6 *Mass Spectrometry*

Extracts were analyzed by high pressure liquid chromatography and tandem mass spectrometry (LC-MS/MS; Agilent 1100 Series HPLC and a Thermo Scientific TSQ Vantage triple quadrupole mass spectrometer (MS-Q^3)). A previously published method, described here, was used for analysis of processed samples [18]. Sample injections of 8 µL were made onto a Phenomenex Synergi RP-Max column (150 × 0.5 mm, 4 µm, 80 Å) using a binary gradient of 10 mM ammonium acetate buffer in ultrapure water (A) and acetonitrile (B) at a constant flow rate of 10 µL/min. The method began by starting at 50% A for ten minutes, then ramping up to 100% B by 20 minutes and back down to

50% A from 23-35 minutes for column reequilibration. The first and last ten minutes of flow during each run were diverted to waste to prevent contamination of the ion source.

Negative mode electrospray ionization (ESI) provided sufficient signal for all the compounds of interest. The single reaction monitoring (SRM) transitions used to detect the analytes are summarized in Table 2.1. A second SRM transitions was also selected for each compound of interest, except $^{13}C_{12}$-triclosan. Before each LC-MS/MS run, $^{13}C_{12}$-triclosan was used to tune the ESI-MS-Q^3 parameters and make adjustments to the ion source. The parameters were set to the following values (dependent on the tuning): spray voltage 2800-3500 V, nitrogen sheath gas pressure 17-45, capillary temperature 250 ˚C, declustering voltage 0-8, collision energy 8-13, dwell time 0.15 s, collision pressure 0.8, quad MS/MS bias 1.5-4.1, and S-lens 65-84. Instrument blanks (50:50 acetonitrile:H_2O (v/v)) were analyzed approximately every 6-8 samples to ensure no carry-over of contamination occurred between samples.

Table 2.1.

SRM Transitions for Analyte Detection

Analyte	Precursor ion m/z	Product ion m/z	Purpose
Triclosan	287	35.1	Quantification
	289	37.1	Confirmation
4-Cl-TCS	321	35.1	Quantification
	323	37.1	Confirmation
6-Cl-TCS	321	35.1	Quantification
	323	37.1	Confirmation
4,6-Cl-TCS	355	35.1	Quantification
	357	37.1	Confirmation
6-OH-BDE-47	500.7	79	Quantification
	498.7	79	Confirmation
$^{13}C_{12}$-Triclosan	299	35.1	Quantification

Calibration curves using more than five points were constructed by plotting the analyte peak area to internal standard peak area ratio (y-axis) versus the analyte concentration (x-axis). Triclosan concentrations in standards ranged from 0.005 – 15 µM (0.001 – 4.3 mg/L), while the concentrations of CTDs and 6-OH-BDE-47 ranged from .001 – 3 µM (0.0003 – 1.5 mg/L). In most cases, two calibrations curves were plotted for each analyte, one for low ranges and one for high ranges. The concentrations of the spiked samples determined the endpoints of the high range calibration curve, while the concentration of the unspiked and blank samples determined the endpoints of the low range calibration curve. At higher concentrations, the $^{13}C_{12}$-triclosan signal became suppressed by triclosan, thus changing the slope of the calibration curve. The slope of an analyte calibration curve and the concentration of $^{13}C_{12}$-triclosan, 1.42 µM (424 µg/L) in the standards were multiplied together. The inverse of this number is sometimes referred to as a response factor. This response factor multiplied by the ratio of analyte peak area to internal standard peak area in the sample equals the concentration of analyte without correcting for recovery.

For analyses with only one method blank, the limit of quantitation (LOQ) for each analyte was defined as 10 times the analyte concentration determined in the method blank. For analyses with multiple method blanks, the LOQ was the concentration determined in the method blank plus 10 times the standard deviation of the method blanks. The limit of detection (LOD) was calculated as 3 times the method blank or the average method blank plus 3 times the standard deviation of the method blanks. Using

multiple method blanks allowed for lower LODs and LOQs as the standard deviation of the analyte concentrations in the method blanks were much lower than the analyte concentrations in the method blanks. The limits with only one method blank are, therefore, conservative. A chromatogram of a representative method blank is given in the Appendix.

2.2.7 *Ozonation of MWP Grab Sample*

A 4-L grab sample of secondary effluent (pre-chlorination) from MWP was collected in April 2012. A 2-L subsample was ozonated with an Aqua-6 Ozone Generator manufactured by A2Z Ozone Systems, Inc. To prevent overheating of the unit, the maximum run time of the generator is 20 minutes. The 2-L subsample was ozonated for three 20 minute periods with 20 minutes in between to let the ozonator cool down.

Although the specifications indicate the generator produces 600 mg of O_3 h^{-1}, the ozone dose to a 2-L batch reactor was determined by the indigo blue method [187]. A 4-L Erlenmeyer flask was filled with 2-L of pH 2 water containing 100 uM of potassium indigotrisulfonate. Ozone degrades indigo blue at a 1:1 molar ratio, thus allowing the ozone dose to be measured. At this pH decomposition of ozone in water is minimized and allows for an estimate of ozone dose to the batch reactor. The diffuser was placed about 1 inch above the bottom of the container, while a stir bar and stir plate were used to continuously mix the sample. Samples were transferred to quartz cuvettes using a glass pipet every four minutes for 20 minutes. A spectrophotometer was used to determine the change in absorbance before and after a twenty minute exposure. In three separate trials,

the concentration of indigo blue decreased from 100 to ~3.6 µM in 20 minutes indicating an ozone dose of 4.63 ± 0.07 mg/L. Having been ozonated for three 20 minute runs, the wastewater received an ozone dose of 13.9 ± 0.2 mg/L.

2.3 *Results and Discussion*

2.3.1 *Method Validation*

The LC method effectively separated the analytes and provided satisfactory peak shapes without processing through peak fitting. Peaks were integrated manually using the Qual Browser of Excalibur 2.1 software from Thermo Scientific. Example chromatograms with the peaks of each analyte labeled are shown in Figures 2.1 – 2.4 from a representative standard, MWP (grab sample), WLSSD (grab sample), and SJWP (composite sample), respectively. Various unidentified compounds were detected and will be discussed later. The calibration curves were fit to a linear function. Most curves were of high quality ($R^2 > 0.99$). Four fits had $R^2 = 0.98$ and three had $R^2 > 0.93$. A summary of LOQ and LOD information for each sample is located in Table A.1 in the Appendix. The LOQs ranged from 7.9 – 100 pM (2.3 – 29 ng/L) for triclosan, 0.01 – 7.9 pM (0.003 – 2.8 ng/L) for the CTDs, and 0.43 – 6.1 pM (0.22 – 3 ng/L) for 6-OH-BDE-47. The LODs are 0.3 × LOQs. The chromatographic peak area for every reported concentration was greater than 10 times the peak area of the corresponding instrument and method blanks. If an analyte response was between the LOD and LOQ, the concentration is stated (in Table 2.3) as less than (<) the LOQ. If the analyte response was less than the LOD, "ND" (not detected) is indicated.

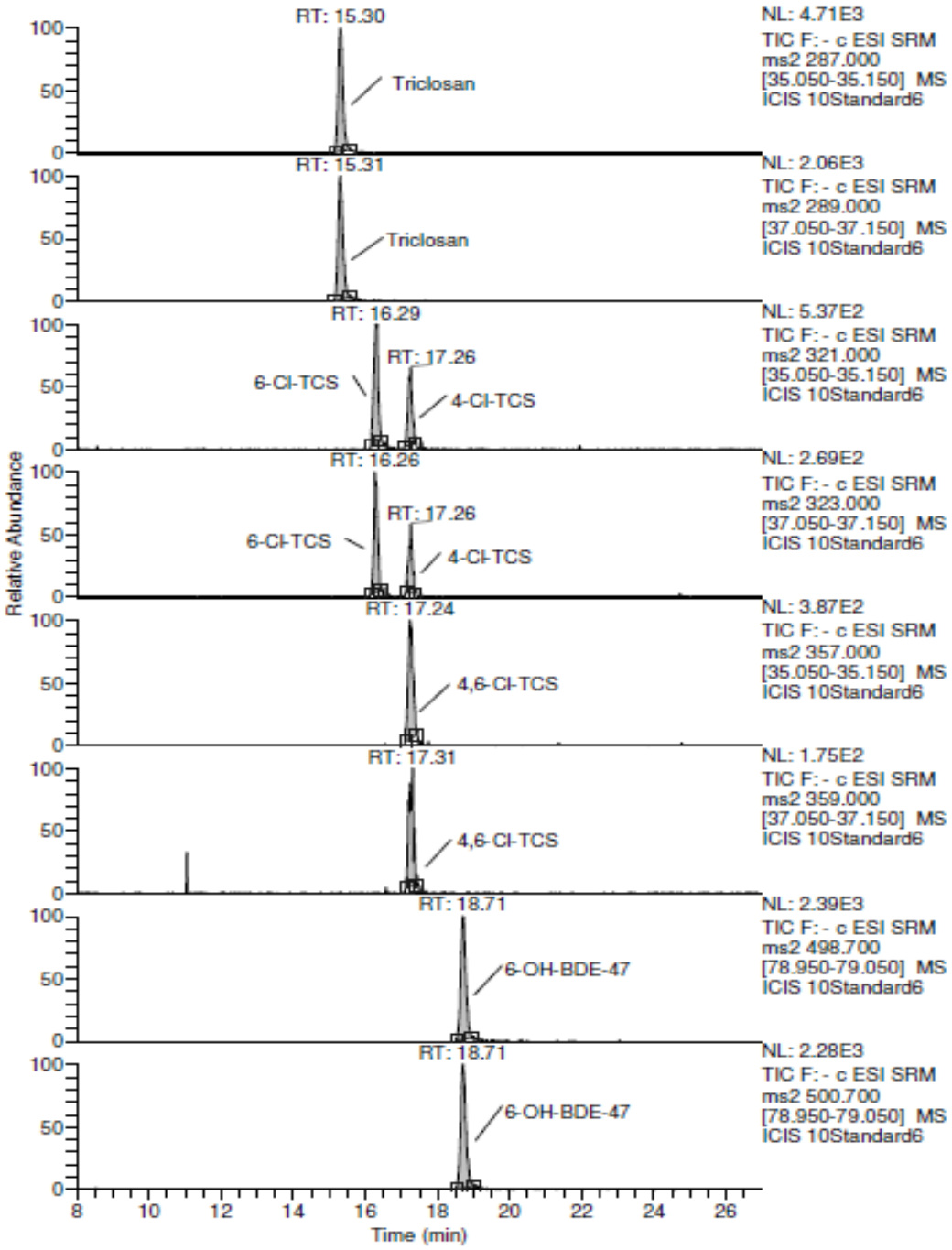


Figure 2.1 Standard Chromatograms.
These chromatograms are representative of a standard response (normalized to the highest peak) of the LC-MS/MS used in this experiment and include all the analytes analyzed, except for $^{13}C_{12}$-triclosan (nearly identical to the triclosan chromatogram). Retention times (RT) are noted for known analytes. The SRM transitions are indicated to the right of each chromatogram. The parent *m/z* is located after "ms2", while the product *m/z* range is given in brackets.

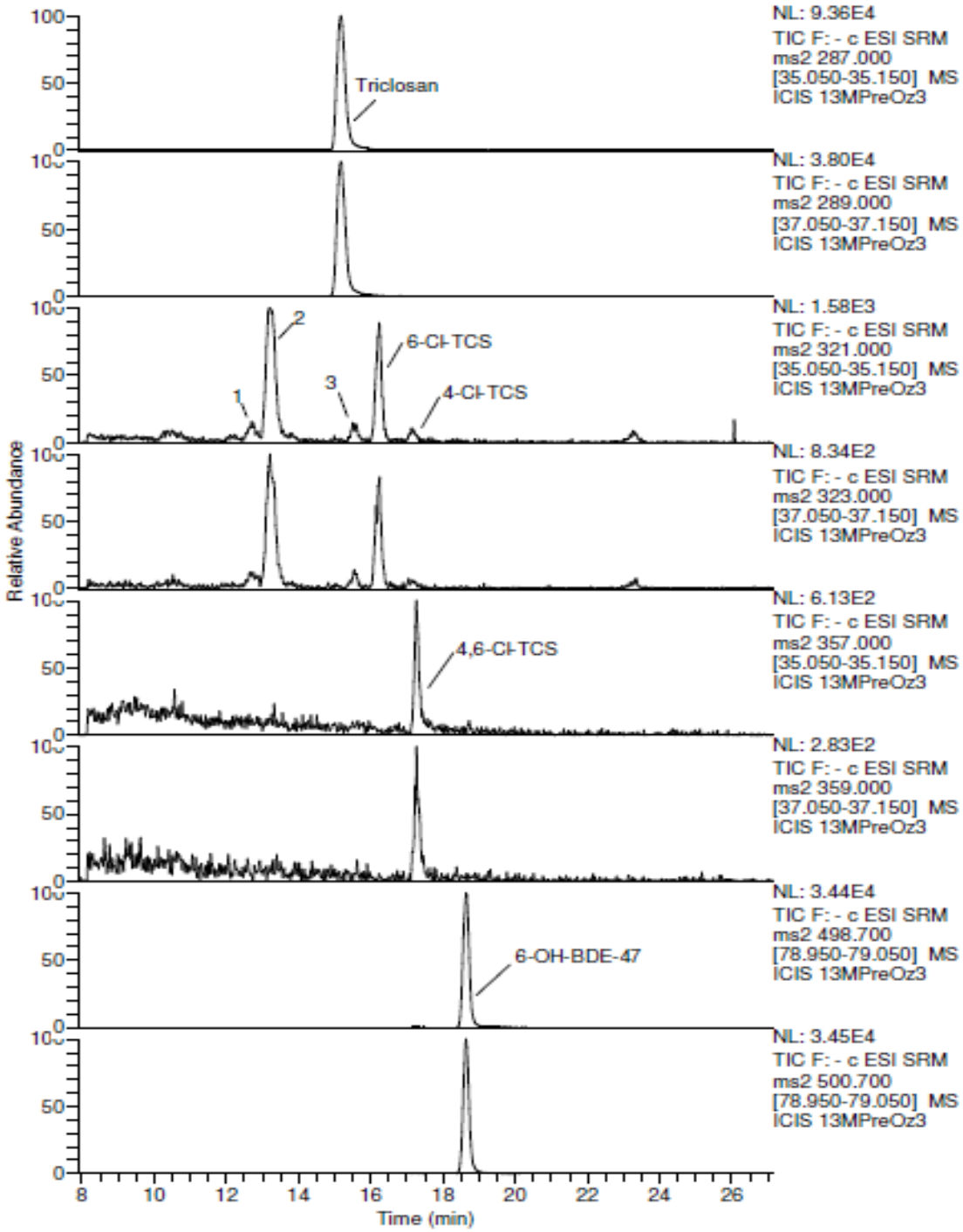


Figure 2.2 MWP Grab Sample Chromatograms.
These chromatograms are from a MWP grab sample of secondary effluent (before chlorination) taken in April 2012. Retention times for all known analytes were generally within 0.15 min of the standards. The peak area ratios for the peaks of three unknown compounds (1, 2, and 3) were greater than 3 times the peak area ratio (or noise) from the method/instrument blank. The unknown peaks were not detected in the highest standard.

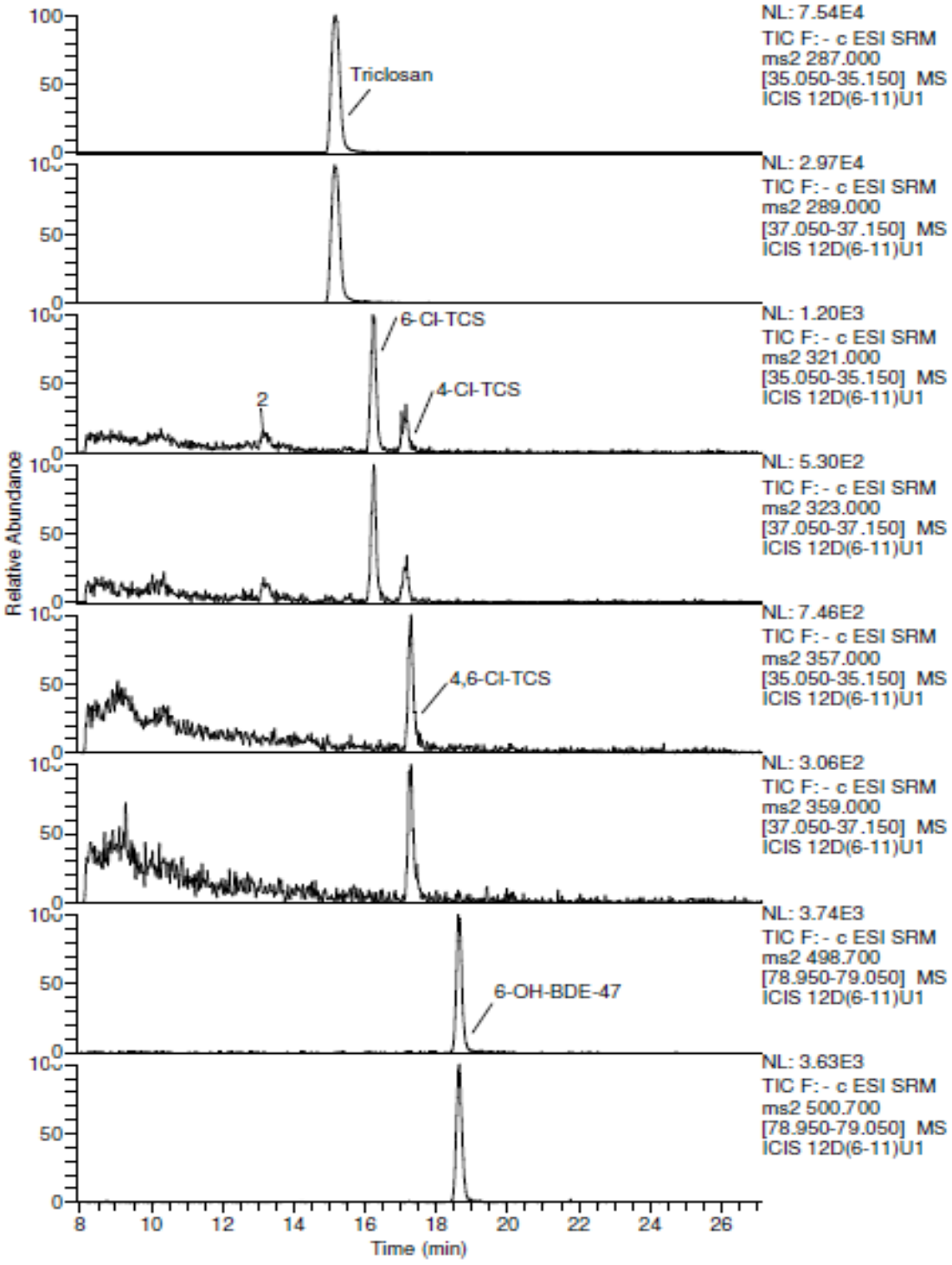


Figure 2.3 WLSSD Chromatograms.
Representative chromatograms from the extract of a WLSSD grab sample. The peak area ratio for peak 2 is greater than 3 times that detected in the method/instrument blank. The concentration of 6-OH-BDE-47 in this sample was 2.4 pM (1.2 ng/L) without recovery correction and 3.5 pM (1.8 ng/L) with correction.

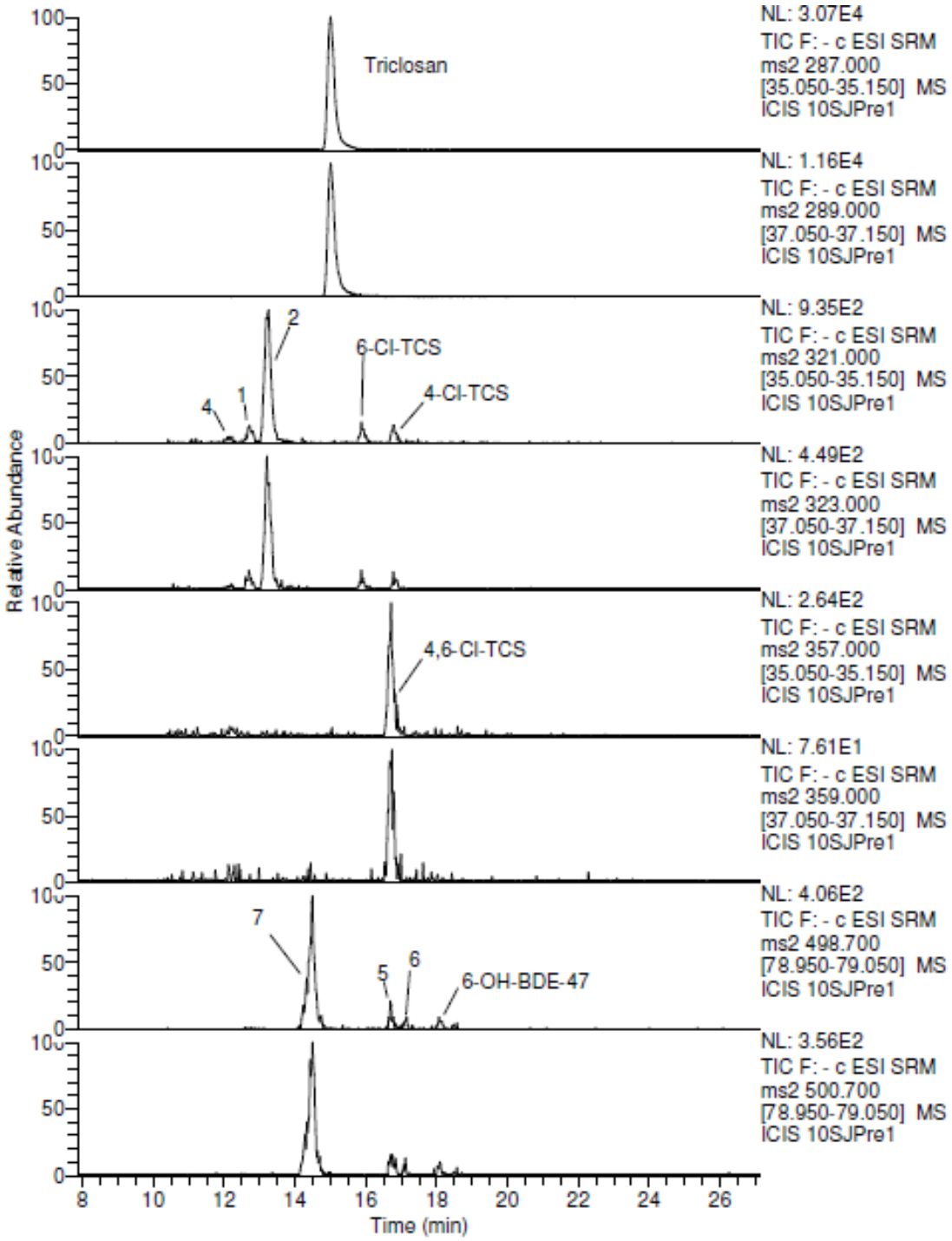


Figure 2.4 SJWP Chromatograms.
These chromatograms are from a pre-UV SJWP composite sample. The peak area ratios for peaks 1, 2, 4, 5, 6, and 7 are greater than 3 times that detected in the blanks. Interestingly peak 7 disappears after UV disinfection. The calculated concentration for 6-OH-BDE-47 was less than LOD in this extract; however, a few compounds elute before with peak area ratios above the LOD (5,6, and 7).

The absolute recovery of $^{13}C_{12}$-triclosan was 59 ± 31 % (average ± standard deviation). Accurate results are confirmed by the relative recovery of each analyte, rather than the absolute recovery, based on isotope dilution methodology [182, 183]. Spiked samples were used to determine the relative recovery as compared with triclosan. The average relative recoveries of all spiked samples for each analyte are shown in Table 2.2. Concentrations shown in Table 2.3 are recovery corrected values using the relative recoveries for wastewater samples processed at the same time. The recoveries used for correction in each matrix are given in Table A.2 in the Appendix. Error was propagated accordingly.

Table 2.2.

Relative Recovery of Analytes

Compound	Recovery
Triclosan	93 ± 18
4-Cl-TCS	84 ± 18
6-Cl-TCS	75 ± 31
4,6-Cl-TCS	59 ± 15
6-OH-BDE-47	54 ± 15

2.3.2 *Disinfection of Triclosan in Wastewater*

Triclosan and the CTDs were detected in all wastewater samples analyzed, except for 4-Cl-TCS in the SJWP samples in April 2012. Table 2.3 shows the results of all the wastewater samples analyzed. Units are given in both picomolar (pM), for direct comparisons with related analytes, and nanograms per liter (ng/L), for easy comparison with other published material. Triclosan concentrations varied from < 14 – 1609 pM (36 – 465 ng/L). Concentrations of total CTDs ranged from below the LOD to 79.8 pM (27.2 ng/L). 6-OH-BDE-47 concentrations ranged from below the LOD to 33.6 pM (1.8 ng/L).

Table 2.3.

PHDDs in Wastewater Before and After Disinfection in pM (ng/L)*

Disinfection	WWTP	Date (Type[f])	Triclosan (289)**	4-Cl-TCS (324)**	6-Cl-TCS (324)**	4,6-Cl-TCS (358)**	Sum of CTDs	6-OH-BDE-47 (502)**
Pre Chlorination	MWP (n=3)	09/11 (Co)	828 ± 316 (239 ± 91)	1.6 ± 0.8 (0.5 ± 0.3)	5.8 ± 3.5 (1.9 ± 1.1)	5.4 ± 2.4 (1.9 ± 0.9)	12.8 ± 6.7 (4.3 ± 2.3)	< 3.6[a] (< 1.8)
Post Chlorination	MWP (n=4)	09/11 (Co)	1470 ± 531 (425 ± 153)	12.3 ± 5.7 (4 ± 1.8)	30.2 ± 18.5 (9.8 ± 6)	37.3 ± 16.9 (13.4 ± 6.1)	79.8 ± 41.1 (27.2 ± 13.9)	6.7 ± 4.4[b] (3.4 ± 2.2)
Pre Chlorination	MWP (n=4)	10/11 (Co)	388 ± 20 (112 ± 6)	2.3 ± 0.4 (0.7 ± 0.1)	26.1 ± 7.1 (8.5 ± 2.3)	31.5 ± 7.1 (11.3 ± 2.5)	59.9 ± 14.6 (20.5 ± 4.9)	ND
Post Chlorination	MWP (n=4)	10/11 (Co)	497 ± 47 (144 ± 14)	6 ± 1 (1.9 ± 0.3)	24.3 ± 6.3 (7.9 ± 2)	32.7 ± 7.9 (11.7 ± 2.8)	63.0 ± 15.3 (21.5 ± 5.1)	ND
No Chlorination	MWP (n=4)	11/11 (Co)	1609 ± 316 (465 ± 91)	< 2.9[c] (< 0.9)	< 7.5[c] (< 2.4)	< 7.9[c] (< 2.8)	---	< 0.7[a] (< 0.4)
Reduced Chlorination	WLSSD (n=3)	06/11 (G)	325 ± 70 (94 ± 20)	1 ± 0.3 (0.3 ± 0.1)	9.2 ± 2.1 (3.0 ± 0.7)	13.2 ± 2.7 (4.7 ± 1.0)	23.4 ± 5.1 (10 ± 1.8)	3.6 ± 0.4 (1.8 ± 0.2)
Reduced Chlorination	WLSSD (n=3)	04/12 (G)	374 ± 17 (157 ± 10)	0.5 ± 0.1 (0.16 ± 0.03)	5.9 ± 0.7 (1.9 ± 0.2)	11 ± 1.9 (3.9 ± 0.7)	17.4 ± 2.7 (6.0 ± 0.9)	ND
Pre UV Irradiation	PAWP (n=4)	07/11 (Co)	1349 ± 119 (390 ± 34)	3.8 ± 0.7[a] (1.2 ± 0.2)	13 ± 3 (4.2 ± 1)	21.8 ± 3.8 (7.8 ± 1.4)	38.6 ± 7.5 (13.2 ± 2.6)	2.8 ± 0.6 (1.4 ± 0.3)
Post UV Irradiation	PAWP (n=4)	07/11 (Co)	1083 ± 251 (313 ± 73)	3.6 ± 1[b] (1.2 ± 0.3)	9.7 ± 3[b] (3.1 ± 1)	16.1 ± 2.9 (5.8 ± 1)	29.4 ± 6.9 (10.1 ± 2.3)	< 0.7[c] (< 0.4)
Pre UV Irradiation	PAWP (n=4)	01/12 (Co)	178 ± 37 (51 ± 11)	1.0 ± 0.4 (0.3 ± 0.1)	6.3 ± 2.0 (2 ± 0.7)	11.5 ± 3.5 (4.1 ± 1.3)	18.8 ± 5.9 (6.4 ± 2.1)	ND
Post UV Irradiation	PAWP (n=4)	01/12 (Co)	202 ± 15 (58 ± 4)	1.3 ± 0.2 (0.4 ± 0.1)	7.4 ± 1.9 (2.4 ± 0.6)	11.9 ± 2.9 (4.3 ± 1)	20.5 ± 5 (7.1 ± 1.7)	ND
Pre UV Irradiation	SJWP (n=4)	01/12 (Co)	166 ± 11 (48 ± 3)	0.8 ± 0.2 (0.3 ± 0.1)	2.0 ± 0.6 (0.6 ± 0.2)	3.9 ± 1 (1.4 ± 0.4)	6.8 ± 1.8 (2.3 ± 0.7)	ND
Post UV Irradiation	SJWP (n=4)	01/12 (Co)	166 ± 25 (48 ± 7)	0.6 ± 0.2 (0.2 ± 0.1)	1.5 ± 0.5 (0.5 ± 0.2)	3.4 ± 1 (1.2 ± 0.4)	5.4 ± 1.7 (1.9 ± 0.7)	< 0.4[d] (< 0.2)
Pre UV Irradiation	SJWP (n=3)	02/12 (Co)	198 ± 10 (57 ± 3)	< 0.7[c] (< 0.2)	< 0.5[c] (< 0.2)	1.9 ± 0.3 (0.7 ± 0.1)	1.9 ± 0.3 (0.7 ± 0.1)	ND
Post UV Irradiation	SJWP (n=3)	02/12 (Co)	123 ± 6 (36 ± 2)	ND	< 0.5[c] (<0.2)	3.2 ± 0.6 (1.1 ± 0.2)	3.2 ± 0.6 (1.1 ± 0.2)	ND
Pre Ozonation[e]	MWP (n=3)	04/12 (G)	279 ± 5 (80.6 ± 1.4)	0.4 ± 0.1 (0.13 ± 0.03)	4.2 ± 1.3 (1.4 ± 0.4)	5.4 ± 1.4 (1.9 ± 0.5)	10 ± 2.8 (3.4 ± 0.9)	33.6 ± 6.1 (16.9 ± 3.1)
Post Ozonation[e]	MWP (n=3)	04/12 (G)	< 14[c] (< 4)	< 0.07[c] (< 0.02)	0.4 ± 0.1 (0.13 ± 0.03)	1.1 ± 0.3 (0.4 ± 0.1)	1.5 ± 0.4 (0.53 ± 0.13)	23.1 ± 5.4[a] (11.6 ± 2.7)

* LODs and LOQs of analytes for each sample analyzed are summarized in the Appendix; ND - not detected (< LOD)
If a replicate is > LOD but < LOQ, the LOQ is shown..

** Molecular weights shown in parenthesis under analyte name

[a] One replicate between LOD and LOQ, while other replicates above LOQ

[b] Two replicates between LOD and LOQ, while other replicate(s) above LOQ

[c] All replicates between LOD and LOQ

[d] One replicate between LOD and LOQ, while other replicates below LOD

[e] Ozone dosed in the laboratory.

[f] (G) – grab sample; (Co) – Composite (24-hour) sample

The concentrations of triclosan in wastewater samples are shown in Figure 2.5. Error bars on all graphs are one standard deviation. Samples that do not have error bars had detectable concentrations but not quantifiable, so instead of the calculated concentration, the LOQ is shown. The student T-test was used to assess differences between triclosan and CTDs before and after disinfection. Hypotheses are deemed significant if $p < 0.05$. Hypothesis tests were only used for samples that had more than one extract above the LOQ.

Chlorination of triclosan should result in some decrease as seen in June and Sept. 2009 MWP samples. The increase seen in Oct. 2011 MWP is questionable and may indicate that the samplers gathering wastewater did not pick up the same representative flow. Laboratory ozonation in the April 2012 MWP samples degraded triclosan to below the LOQ of 13 pM (3.7 ng/L). Triclosan in the Feb. 2012 SJWP sample may have decreased due to photolysis, although the effect is not seen for Jan 2012 at SJWP. Fluctuations in the flow could explain this situation. During low flow periods, contact time in the disinfection basin can reach up to four minutes, potentially allowing for the degradation of more contaminants, like triclosan. During high flow times, the contact time may be as low as two minutes. Triclosan was not significantly degraded in the PAWP samples, which may be attributed to the short UV contact time – only a few seconds.

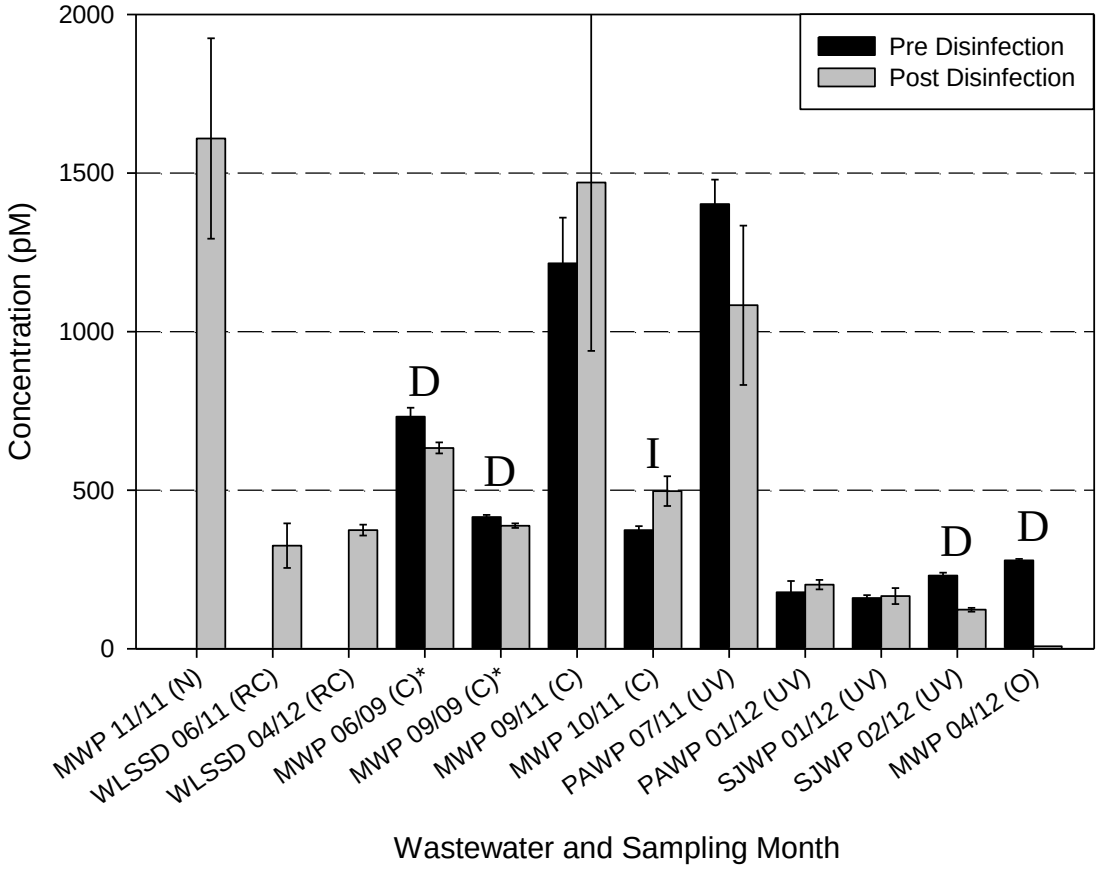


Figure 2.5 Triclosan in Wastewater.
Triclosan was detected in all wastewater samples analyzed. Sampling was performed in such a way that concentrations of triclosan from before and after disinfection should either decrease or remain the constant. The first three samples on the left did not have pre-disinfection samples. Statistical differences ($p < 0.05$) from pre to post are marked with a "D" for decrease or an "I" for increase. Error bars are 1 σ.
(N) – No disinfection; (RC) – Reduced Chlorination; (C) – Chlorination; (UV) – UV Irradiation; O – Ozonation.
*Data from Buth et al. [18]

Comparing CTDs in samples with different concentrations of triclosan may not accurately portray differences between samples. Kinetically speaking, the rate of total CTD formation is directly proportional to the concentration of triclosan. Another way to compare the data is to normalize the concentration of CTDs by the concentration of triclosan. By comparing both graphs of CTDs concentrations and [CTD]/[triclosan], insights into the reaction of triclosan and CTDs with hypochlorite may be gained.

2.3.3 *Chlorination of Triclosan in Wastewater*

Buth et al. [18] had previously determined that CTDs increase following chlorination of MWP wastewater. Figure 2.6 shows the concentrations of individual CTDs from in wastewater from Buth et al. [18] and the samples collected in this study at the MWP. Concentrations of CTDs increase after chlorination for three of the four samples.

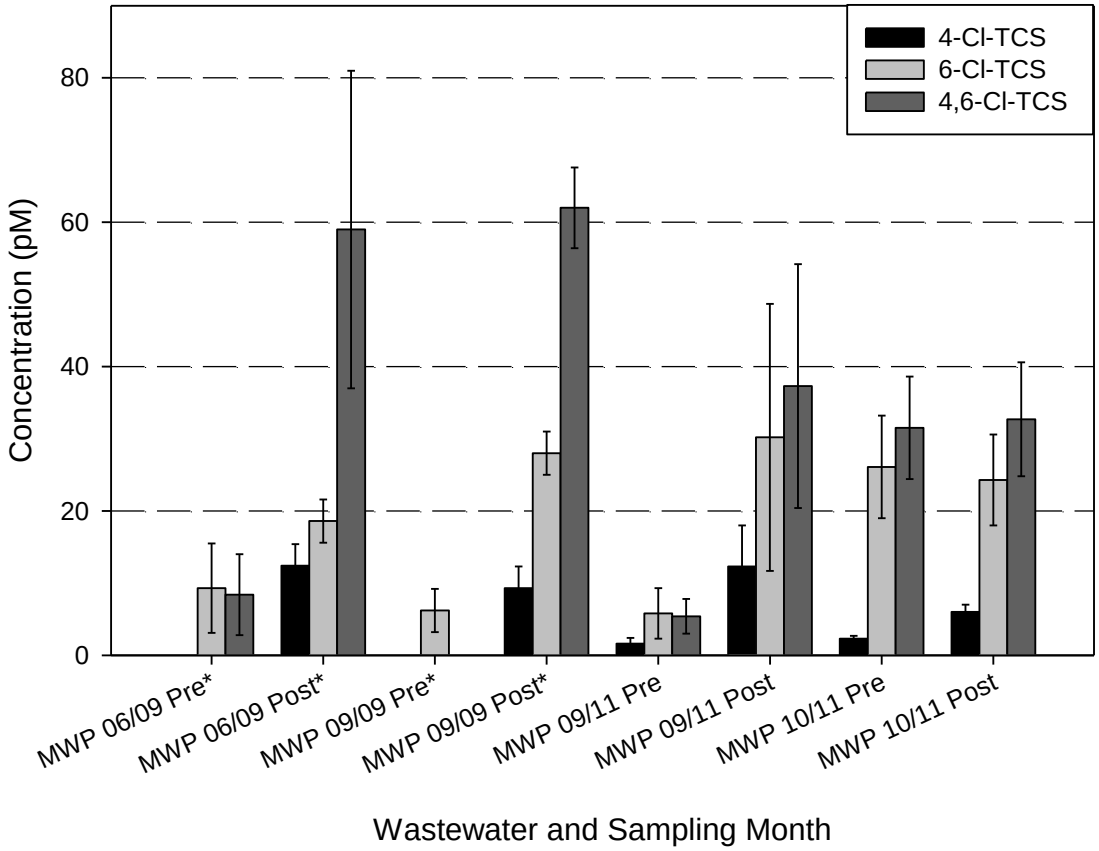


Figure 2.6 Chlorination of CTDs in Wastewater.
Chlorination of wastewater generally increases CTD concentrations. However, if the rate of formation and degradation of CTDs are equal, no change in levels may be seen as in the Oct. 2011 samples.
*From Buth et al. [18]

In Figure 2.6, chlorination of wastewater increases most of the individual CTD concentrations in samples from June and Sept. 2009 and Sept. 2011. In the Oct. 2011 sample, only 4-Cl-TCS increases, while the other CTDs change insignificantly during chlorination. CTDs in the Oct. 2011 pre-chlorination sample are higher than the other pre-chlorination samples. This may explain the stagnant levels of CTDs from Oct. 2011. Canosa et al. [81] determined that the concentration of CTDs reaches a maximum and begins to decline when chlorinating triclosan in ultrapure and tap water. At the maximum, the rate of formation equals the rate of degradation of CTDs. Thus the lack of change of CTDs from pre to post-chlorination in Oct. 2011 MWP samples may still be of concern. CTDs were probably still forming during chlorination, only being converted to other toxic chloroform and chlorophenols at similar rates to their formation.

When comparing CTD to triclosan ratios for different sampling dates in Figure 2.7, similar trends appear. In the Oct. 2011, the apparent decrease in 6-Cl-TCS and 4,6-Cl-TCS ratio is not significant. Most of the CTD ratios significantly increase for the other samples. CTDs in the Sept. 2011 sample increased less than the samples obtained in June and Sept. 2009. The Sept. 2011 wastewater may have had higher concentrations of other particles and chemicals that competed for the hypochlorite resulting in less conversion of triclosan to CTDs.

Overall, CTDs were detected at similar concentrations to other studies before and after disinfection [18, 46]. Triclosan first converts to 4-Cl-TCS or 6-Cl-TCS, then undergoing further chlorination to 4,6-Cl-TCS [81]. 4-Cl-TCS had the fastest reaction

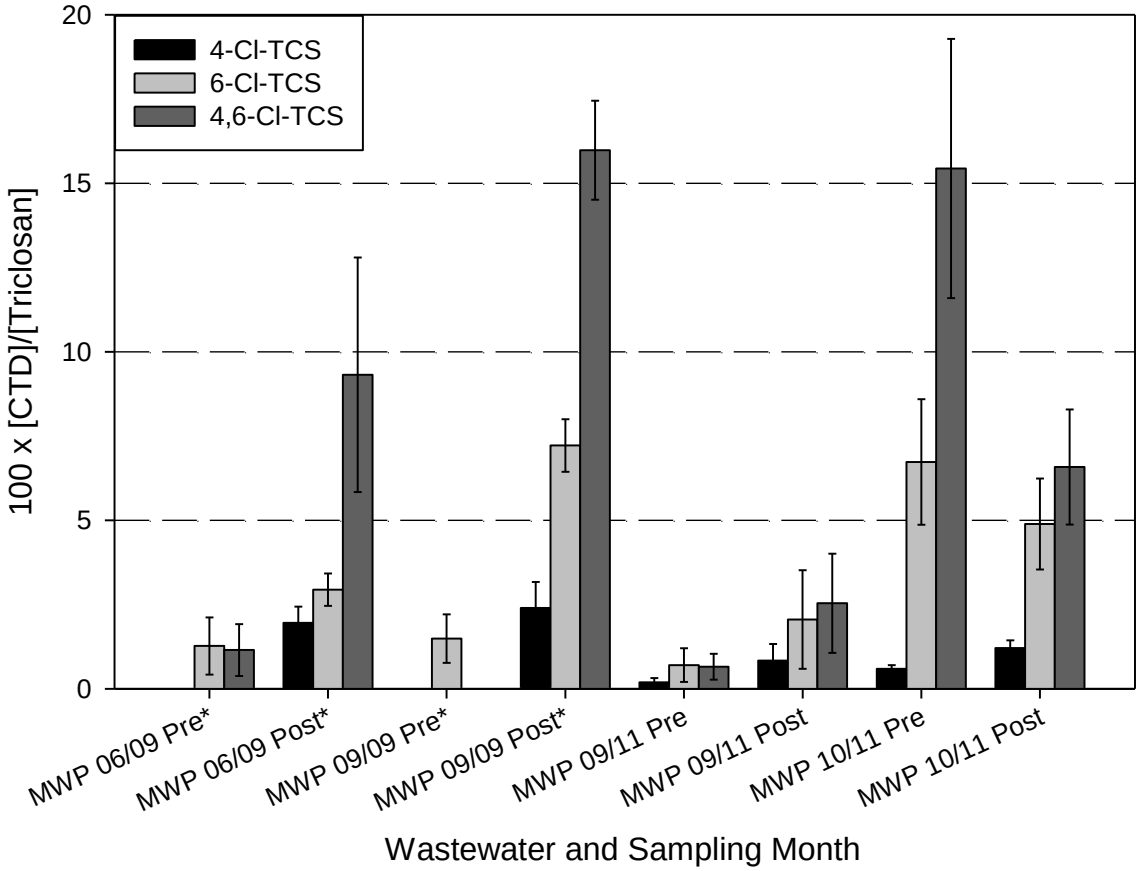


Figure 2.7 CTD:Triclosan Ratios in Wastewater: Chlorination.
This [CTD]/[Triclosan] graph shows similar trends of increasing CTD levels. The only difference is a larger decrease in the Oct. 2011 samples. This decrease may be explained by greater rates of CTD degradation than formation.
* From Buth et al. [18]

kinetics with hypochlorite, followed by 6-Cl-TCS [81]. 4,6-Cl-TCS was the slowest to degrade [81]. CTDs in both pre and post chlorination wastewater seem to obey these same kinetics based on relative levels. 4-Cl-TCS is detected at much lower levels than the others, potentially confirming its faster kinetics in wastewater. Like the results of Buth et al. [18], 4,6-Cl-TCS is detected at higher concentrations in chlorinated effluent than the other CTDs. This CTD also forms the highest chlorinated dioxin which is likely the most toxic, or at least as toxic as the dioxin formed from 4-Cl-TCS.

2.3.4 *Reduced and Non-disinfected Wastewater Effluent*

Lower levels of CTDs were found in WLSSD effluent grab samples than chlorinated MWP effluent. WLSSD only chlorinates when high coliform counts deem it necessary. Because of this "reduced" chlorination, CTDs were expected to be at lower concentrations than MWP. MWP does not chlorinate its wastewater from November to March. A composite sample (24-hour) from MWP was obtained in November for the final effluent only. Levels in this sample should be similar or lower than WLSSD samples, although WLSSD serves 50% industry and may have less triclosan and CTDs even when chlorinating. A comparison of CTDs in WLSSD effluent to the non-chlorinated MWP effluent is also shown in Figure 2.9. Concentrations of CTDs in the Nov. 2011 MWP samples were above the LOD but below the LOQ for all replicates. The concentrations of individual CTDs are similar between this MWP sample and WLSSD grab samples. After normalizing by the concentration of triclosan, the Nov. 2011 MWP wastewater seems to have much lower levels than WLSSD in Figure 2.8. This indicates that WLSSD may have been chlorinating when samples were obtained, but this cannot be verified.

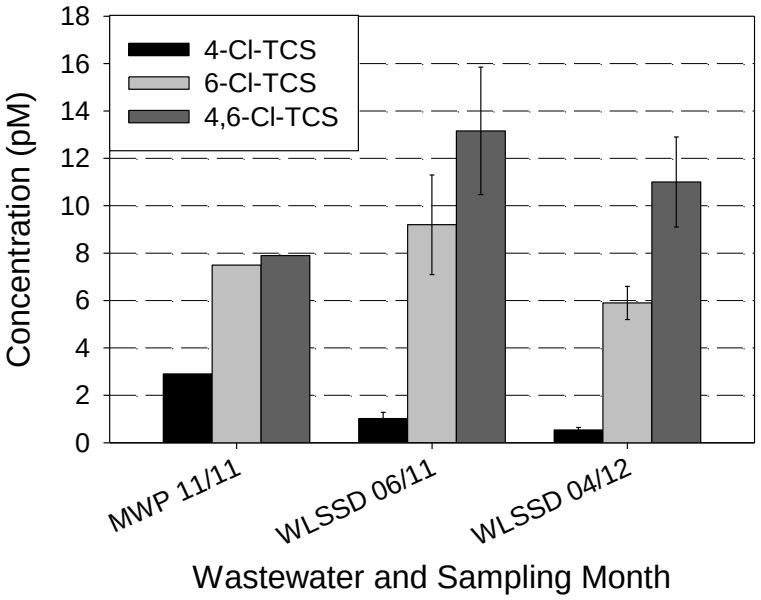


Figure 2.9 CTDs in Wastewater Effluent with Reduced or No Chlorination.
Concentration of CTDs are similar in WLSSD and non-chlorinated MWP effluent. All CTDs in the MWP extracts were detected, but not above the LOQ (shown).

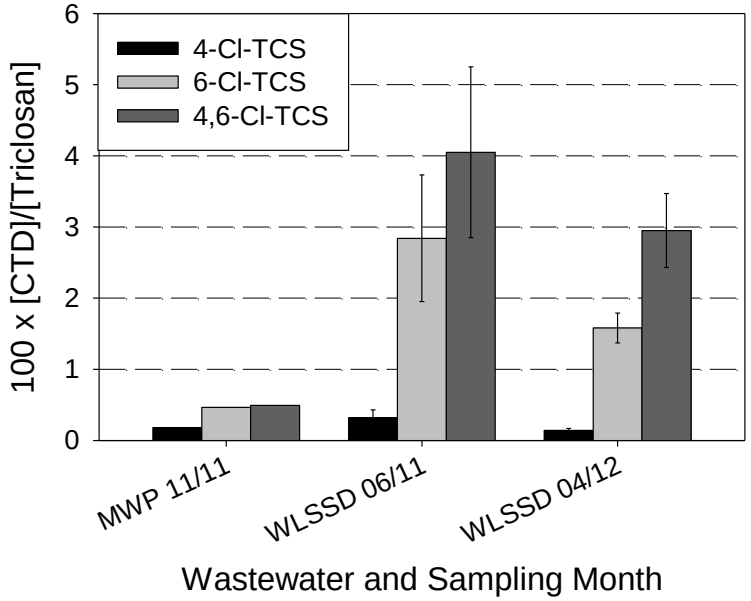


Figure 2.8 CTD:Triclosan Ratios in Wastewater: No/Reduced Disinfection.
By normalizing CTD concentrations by triclosan a more pronounced difference can be seen between non-chlorinated effluent and WLSSD effluent. WLSSD has a special permit for reduced chlorination, but it could not be verified if they were chlorinating when these samples were obtained.

2.3.5 *UV Irradiation of Triclosan and CTDs in Wastewater Effluent*

PAWP and SJWP are two WWTPs that disinfect with UV systems. The levels of CTDs remain unchanged from pre to post disinfection when comparing both concentrations and ratios of CTDs to triclosan, as seen in Figure 2.10 and Figure 2.11. Levels of individual CTDS in the PAWP and SJWP wastewater extracts follow the trend: 4-Cl-TCS < 6-Cl-TCS ≤ 4,6-Cl-TCS.

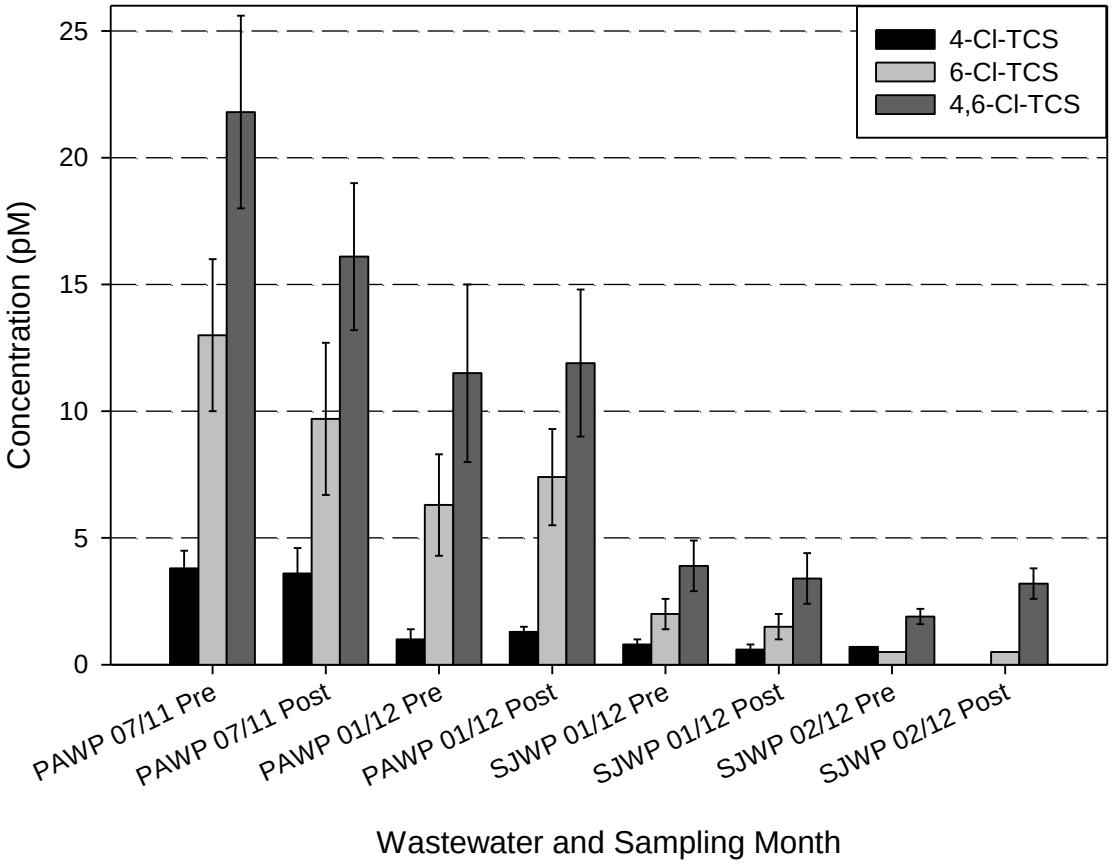


Figure 2.10 Effect of UV Irradiation on CTDs in Wastewater.
CTD levels were relatively constant from before and after UV irradiation. PAWP levels were higher than SJWP, which may be explained by the lack of residual chlorine in water that SJWP treats.

Concentrations of CTDs in the final effluent of both PAWP samples are higher than either SJWP final effluents. PAWP serves a community that has residual chlorine in their tap water, while SJWP serves a community that does not. Any CTDs in SJWP must be formed by bleach or other disinfectants reacting with triclosan in wastewater. The concentrations of CTDs in PAWP final effluent are still less than chlorinated MWP effluent and similar to WLSSD and non-chlorinated MWP effluents.

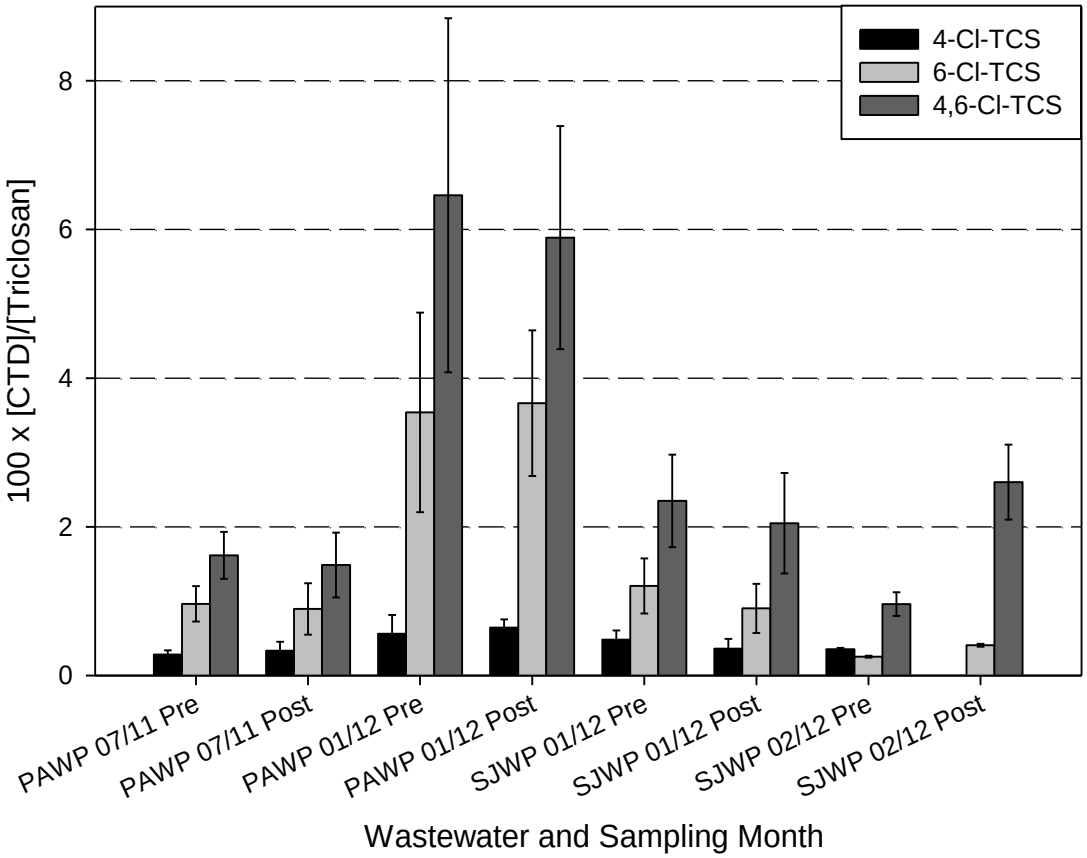


Figure 2.11 CTD:Triclosan Ratios in Wastewater: UV Irradiation.
Normalized CTD levels were similar from pre to post-UV irradiation.

2.3.6 *Ozonation of Triclosan and CTDs in MWP Grab Sample*

The ozonation of the April 2012 grab sample from MWP resulted in the significant decrease of triclosan and all the CTDs. Triclosan and 4-Cl-TCS degraded from 278 pM and 0.4 pM, respectively, to below the LOQ (12 pM and 0.1 pM, respectively). Removal efficiencies for 6-Cl-TCS and 4,6-Cl-TCS were 91% and 80%.

2.3.7 *Effect of Disinfection on Individual and Total CTDs*

These data may be used to more accurately predict the levels of CTDs entering surface waters and thus the consequent dioxin formation. Figures 2.12 – 2.14

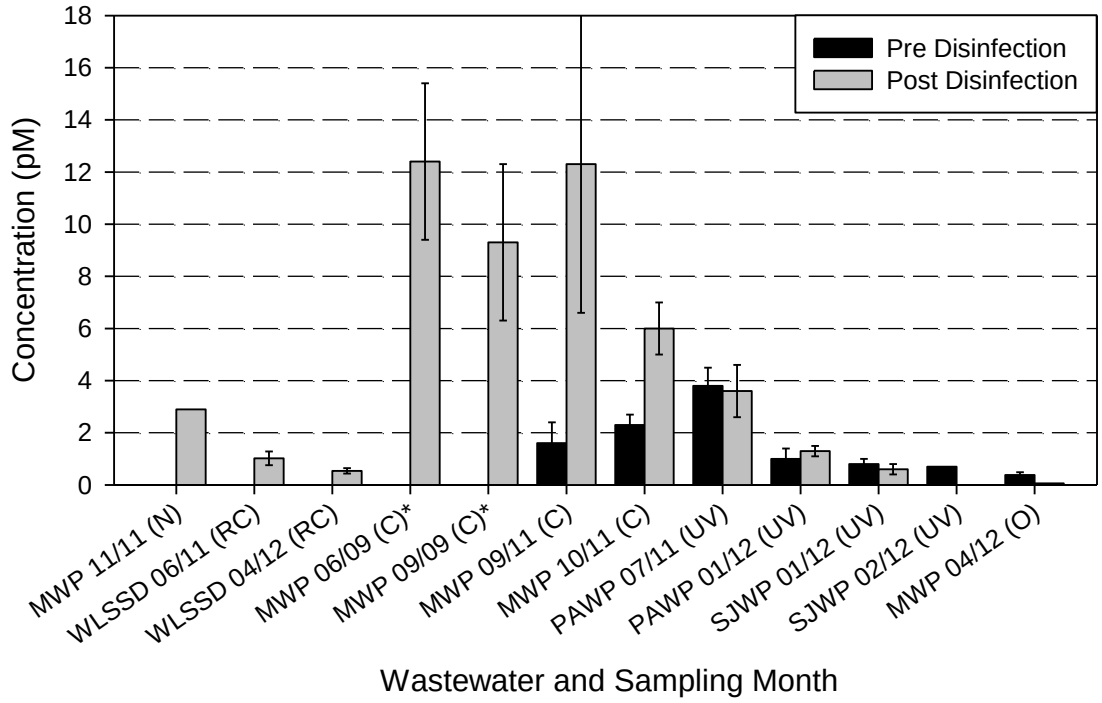


Figure 2.12 4-Cl-TCS in Wastewater by Disinfection Method.
Chlorination increased the amount of 4-Cl-TCS in wastewater effluent. N – no disinfection; RC – reduced chlorination; C – chlorination; UV – ultraviolet irradiation; and O – ozonation.
*denotes data from Buth et al. [18]

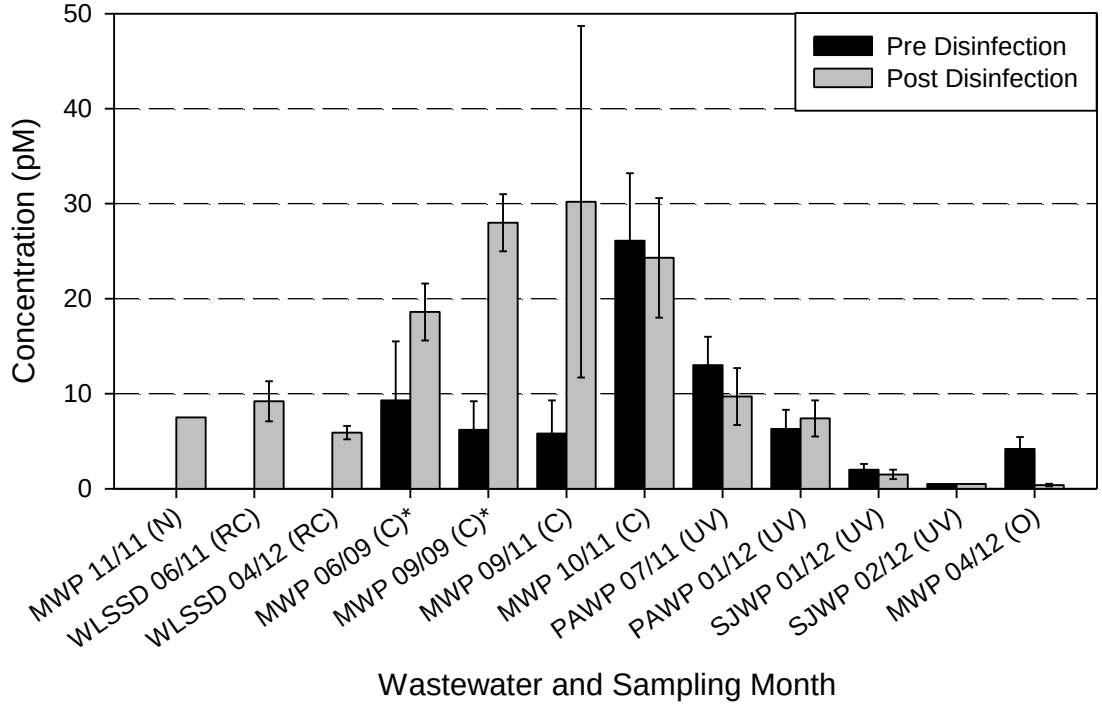


Figure 2.13 6-Cl-TCS in Wastewater by Disinfection Method.
6-Cl-TCS is detected in the highest amounts after chlorination.
*denotes data from Buth et al. [18]

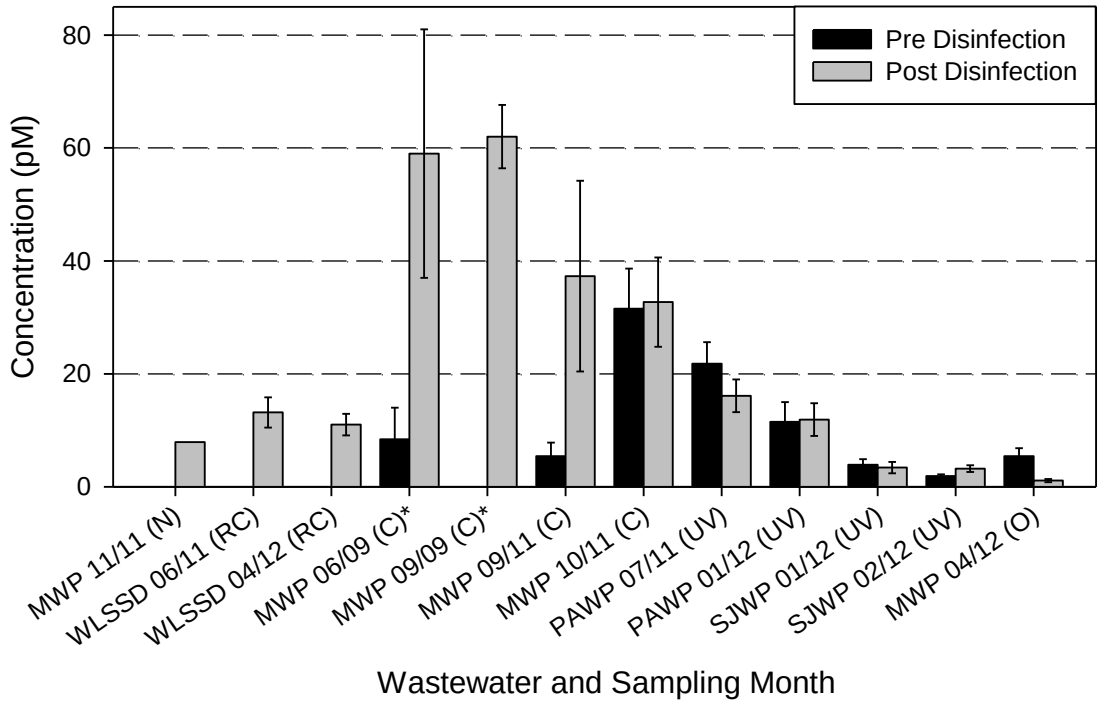


Figure 2.14 4,6-Cl-TCS in Wastewater by Disinfection Method.
4,6-Cl-TCS is detected at higher levels in chlorinated effluent than other effluent.
*denotes data from Buth et al. [18]

compare the individual CTD concentrations from each method of disinfection. The figures clearly show chlorinated effluent has higher amounts of all of the CTDs than any other disinfection method. UV treatment did not affect levels of CTDs, although PAWP had the highest amount of CTDs of any non-chlorinated effluent. Ozonation decreased the concentrations of all CTDs. CTDs are detected in most wastewater regardless of the method of disinfection used.

As expected chlorinated effluent has the largest concentrations of CTDs and will be the major source of CTDs to surface waters. UV irradiated wastewater will contribute less, but still a substantial amount of CTDs to surface waters. Figure 2.13 shows the concentrations of total CTDs in wastewater before and after disinfection. Large WWTPs like PAWP and MWP have the highest levels of CTDs, while the smaller treatment plant SJWP has lower amounts. Data from WLSSD grab samples may be compared to the composite samples, but the comparisons are less informative. The sampling method is important as composite samples account for the daily fluctuations of chemicals unlike like grab samples. Composite samples will be more reliable for estimating triclosan and CTD loading.

2.3.8 *6-OH-BDE-47 in Wastewater Effluent*

One other study has published proof of 6-OH-BDE-47 in wastewater [180]. Ueno et al. [157] detected elevated levels of OH-PBDEs near a WWTP. Figure 2.14 highlights the samples from this study that had detectable amounts of 6-OH-BDE-47 in at least one replicate extract. A grab sample from MWP had

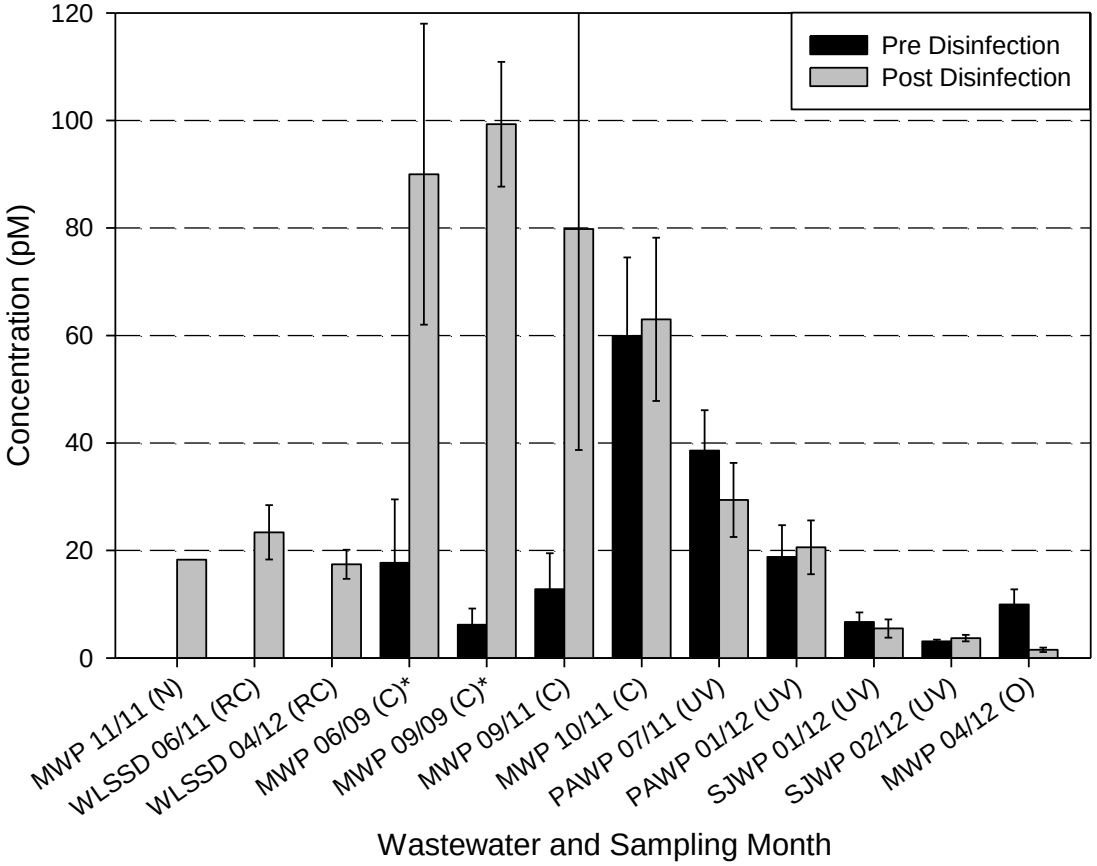


Figure 2.13 ΣCTDs in Wastewater by Disinfection Method.
The concentrations of total CTDs generally increased for chlorination and decreased after ozonation. PAWP had the highest amounts of CTDs of the non-chlorination plants. *denotes data from Buth et al. [18]

by far the highest amounts of 6-OH-BDE-47, while lower levels were detected in the composite samples. The data from Figure 2.14 displays evidence of the highest amounts of 6-OH-BDE-47 in wastewater to date (~33 pM or 16 ng/L). From comparison of pre and post disinfection extracts, 6-OH-BDE-47 is susceptible to UV light (>71% removal) and ozone (32% removal).

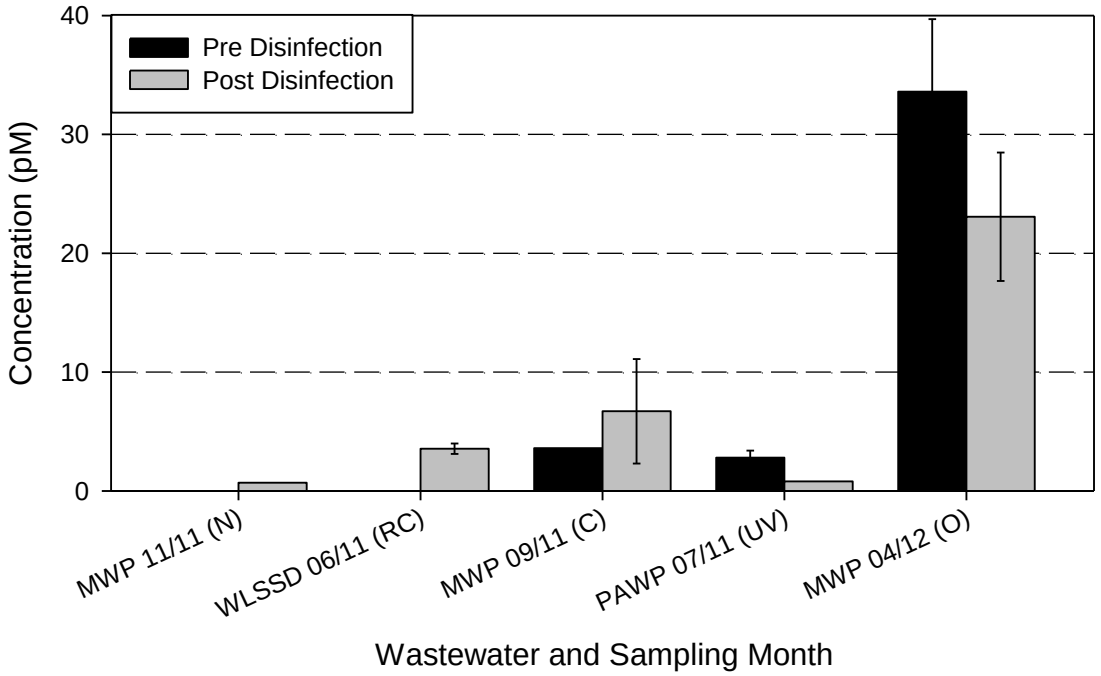


Figure 2.14 6-OH-BDE-47 in Wastewater.
6-OH-BDE-47 was detected in some of the wastewater analyzed. Significant decreases occurred after UV irradiation and ozonation. Bars without standard deviation represent samples that were above the LOD but below the LOQ, in those cases the LOQ is shown. The largest amount of 6-OH-BDE-47 was found in a MWP grab sample collected before chlorination.

2.3.9 *Unidentified Compounds in Wastewater Extracts*

Other compounds were also detected in wastewater extracts. Unidentified peaks eluted at 12.2, 12.8, and 13.3 minutes in the April 2012 grab sample at MWP (see chromatograms in Figure 2.15). Peaks with similar retention times and SRM transitions were also present in other samples (see peaks 1, 2, and 4 in Figure 2.4). Each peak was detected for three SRM transitions, including 319 → 35, which was only monitored in a few samples (mainly to look for ozonation byproducts).

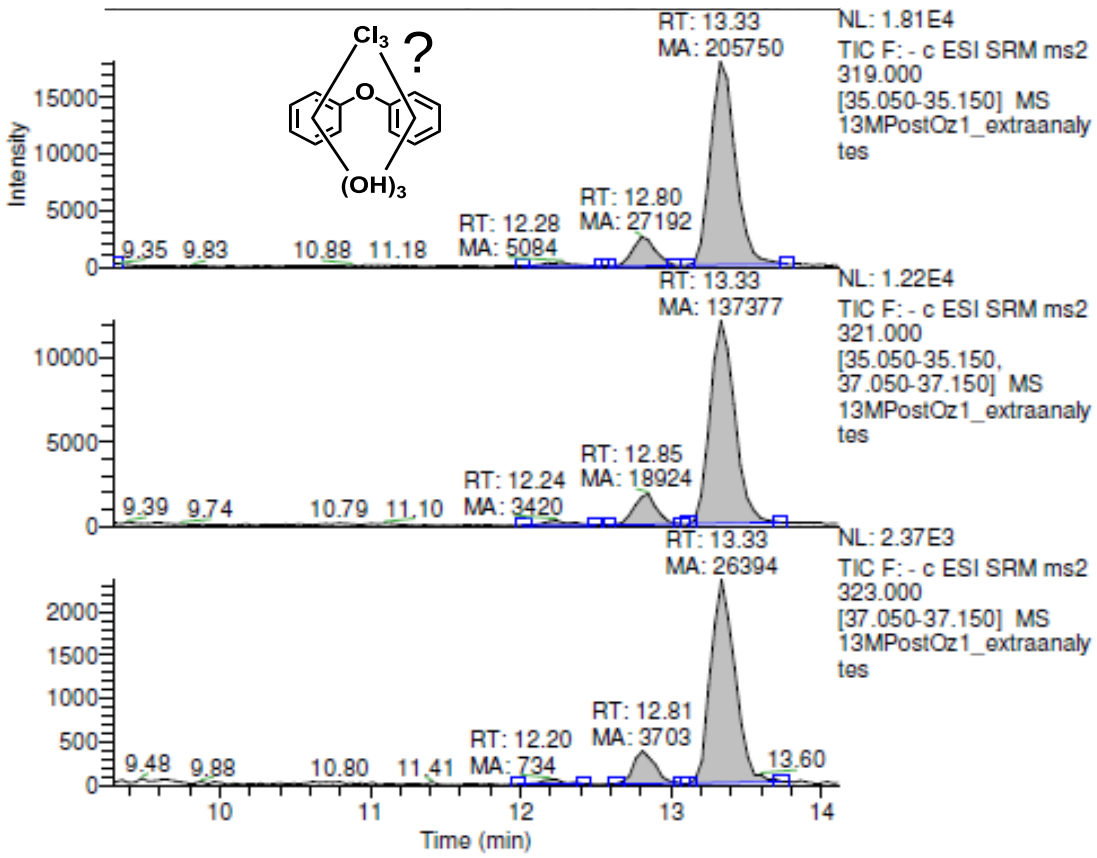


Figure 2.15 Chromatograms of Unidentified Compounds.
Three unknown peaks eluted with the following SRM transitions in order of decreasing peak area: 319 → 35.1, 321 → 35.1/37.1, and 323 → 37.1. One molecular formula that fits this isotope abundance pattern would be $C_{12}H_6Cl_3O_4$, potentially corresponding to deprotonated dihydroxy-triclosan. These peaks were detected in other samples as well.

While the three peaks are not ozonation byproducts, the retention times, relative peak areas, and SRM transitions shed light on what the compounds could be. The isotopes of a deprotonated dihydroxy-triclosan ($C_{12}H_6Cl_3O_4$), pictured in Figure 2.15, would produce signals similar to those seen in these chromatograms (see Figure A.1 in the Appendix). Kim et al. [188] found bacteria can metabolize triclosan to form dihydroxy-triclosan, which matches the proposed formula. The large amounts of bacteria present in activated sludge could be transforming triclosan into this compound.

Dihydroxy-triclosan was also found to be a product of ozonation [189], although none of these peaks were significantly higher in the April 2012 MWP grab sample after ozonation. The compounds elute just before triclosan, suggesting these compounds are more polar, providing more evidence for this compound as a dihydroxy-triclosan. The three peaks could correspond to triclosan that has been twice hydroxylated in different positions. A CTD could also be hydroxylated and dechlorinated to form similar compounds to di-hydroxy-triclosan. Another possibility would be compounds like chlorophenols and chlorocatechols dimerizing in the mass spectrometer and producing signals similar to those seen here.

One ozonation byproduct was observed. The peak eluted at 10.23 minutes with the same SRM transitions (287 → 35.1 and 289 → 37.1) as triclosan. Based on the chromatography in Figure 2.16, the compound is more hydrophilic/polar than triclosan (eluting while the mobile phase still had close to 50% water). In a previous study, ozonation byproducts included mono- and di-hydroxy-triclosan, which are both more hydrophilic than triclosan [189]. The SRM transitions for these compounds are different than triclosan and could not be the source of this peak. Possible reaction pathways from ozonation involve electrophilic substitution of hydroxyl radicals with a chlorine or hydrogen substituent and further hydroxylation to form a trihydroxy-dichloro-diphenyl ether (tri-OH-PCDE). The SRM transitions for triclosan would correspond to the second and third most abundant isotopes of this compound (see Figure A.2 in Appendix), which is the molecule pictured on the left in Figure 2.16. The most abundant transition for

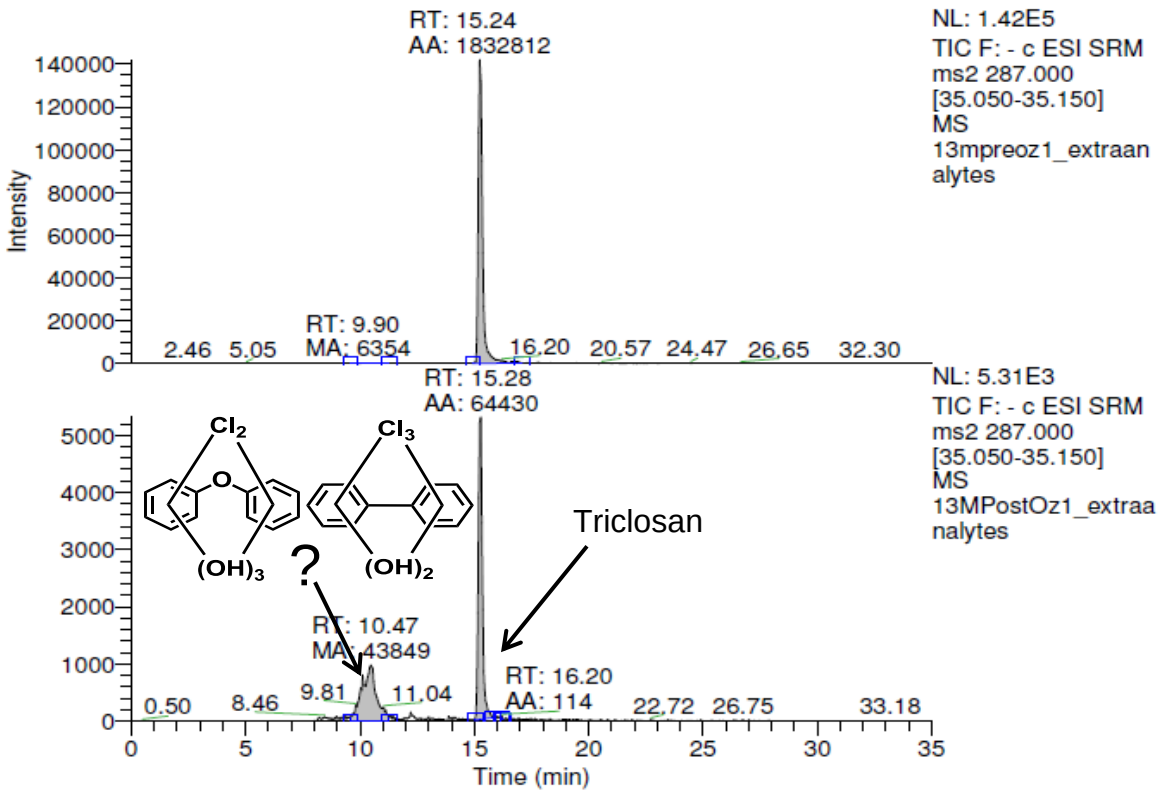


Figure 2.16 Chromatogram of Ozonation Byproduct Formation.
The top and bottom chromatograms are from pre and post ozonation extracts, respectively. One peak eluted at 10.47 minutes in the post ozonation extract with the same SRM transitions as triclosan. The "peak" labeled on the top chromatogram with a retention time of 9.9 min is a conservative representation of the background from the pre ozonation extract. The compound could be a diphenyl ether with three hydroxyl groups and two chlorines (left) or a dihydroxy trichlorinated biphenyl (right).

this compound corresponds to 295 → 35.1 which was not monitored. Another possible molecular formula is $C_{12}H_7Cl_3O_2$ corresponding to a dihydroxy-trichloro-biphenyl (di-OH-PCB) (pictured on the right in Figure 2.16). The isotope abundance for this compound is the same as triclosan (see Figure A.3 in Appendix). Without more experimentation, however, the identity of these compounds cannot be confirmed.

2.4 *Environmental Significance*

The estimated yearly loading from the WWTPs from this study are shown in Table 2.4. The goal of this representation is not to see which WWTPs discharge more pollutants, but rather only to compare disinfection. All of the WWTPs in this study treat wastewater to high standards using some of the best technology available. With almost 2 million people to serve, MWP discharges by far the most triclosan and CTDs. Dioxins derived from triclosan and CTDs were found in sediments of Lake Pepin, which is located about 30 miles downstream of MWP and is a catchment basin for the Mississippi River [20]. Triclosan and CTDs discharging from MWP is likely the largest contributing source of these dioxins, although other WWTPs discharge into the Mississippi River downstream of MWP and upstream of Lake Pepin.

Two different methods were used to calculate low, medium, and high estimates of CTD loadings to US surface waters. The first method assumes that concentrations of CTDs at other chlorination and UV irradiation plants around the US are similar to samples collected in this study (see Table 2.5). In the second method, CTDs are estimated

Table 2.4.

Mass Loadings of Triclosan and CTDs from Individual WWTPs

WWTP	Population	Triclosan (kg/year)	4-Cl-TCS (g/year)	6-Cl-TCS (g/year)	4,6-Cl-TCS (g/year)
MWP	(1,800,000)	50 – 150	660 – 1400	2100 – 3400	4000 – 7600
WLSSD	(85,500)	5.2 – 8.7	9 – 17	100 – 170	220 – 260
PAWP	(220,000)	1.8 – 9.5	12 – 37	73 – 94	130 – 180
SJWP	(2,600)	0.011 – 0.015	< 0.06	< 0.16	< 0.38

by assuming the [CTD]/[Triclosan] levels in other US WWTPs are the same as MWP, SJWP, and PAWP (see Table 2.6). In both cases, eight studies analyzing a total of 19 WWTP effluents were used to calculate the average triclosan concentration in US WWTP effluent [11, 18, 45, 46, 57-59]. The average of the base ten logarithms of the triclosan concentrations for these studies was 2.27, which corresponds to a concentration of $10^{2.27}$ ng/L or 186 ng/L. The average from the logarithms was almost equal to the median concentration of 180 ng/L. The standard deviation of the base ten logarithms was 0.63. Using this number, the high and low estimates of triclosan concentrations were 801 ng/L ($10^{2.27 + 0.63}$ ng/L) and 43 ng/L ($10^{2.27 - 0.63}$ ng/L).

With the concentrations of triclosan in wastewater estimated, only an estimate of how much wastewater is discharged into US surface waters is needed to calculate mass loadings of triclosan and CTDs. Information available from Table 1.1 was used for this purpose. Plants that only use primary treatment were ignored as they made up only a small portion of total WWTP effluent and they discharge to the ocean. Wastewater treated by septic systems was excluded, as was effluent from WWTPs that had no effective discharge. The final estimate of wastewater discharge was 29,918 MGD for the US.

Disinfection of wastewater was also taken into account. In 2003 about 75% of WWTPs chlorinated their effluent [24]. This percentage was reduced to 50% to account for WWTPs switching to UV since 2003 and those that do not chlorinate in the winter. Using the data from Table 2.3 and Buth et al. [18], the estimates for total discharges of

Table 2.5

Estimate of Triclosan, CTD, and Dioxin Loading By Concentration

	Compound →	Triclosan	4-Cl-TCS	6-Cl-TCS	4,6-Cl-TCS
	(Dioxin) →	(2,8-DCDD)	(2,3,7-TrCDD)	(1,2,8-TrCDD)	(1,2,3,8-TCDD)
Compound	Low	4342	6.2	26.8	49.5
Loading	Medium	7677	55.7	144	312
(kg/year)	High	20754	107	270	574
Dioxin	Low	4342	3.1	42.9	28.7
Loading	Medium	23033	89.2	578	467
(g/year)	High	187790	966	2433	5164
	REP →	0.0001	0.0025	0.0002	0.005
TEQ	Low	0.4	0.007	0.009	0.14
Loading	Medium	2.3	0.22	0.12	2.3
(g/year)	High	18.7	2.4	0.49	25.8
		TEQ Estimates	Low	Medium	High
		(g TEQ/year)	0.59	4.98	47.4

Table 2.6

Estimate of Triclosan, CTD, and Dioxin Loading By [CTD]/[Triclosan]

	Compound →	Triclosan	4-Cl-TCS	6-Cl-TCS	4,6-Cl-TCS
	(Dioxin) →	(2,8-DCDD)	(2,3,7-TrCDD)	(1,2,8-TrCDD)	(1,2,3,8-TCDD)
Compound	Low	1775	10.4	21.4	35.3
Loading	Medium	7678	80.6	220	497
(kg/year)	High	33063	503	1805	3619
Dioxin	Low	1775	5.2	34	20
Loading	Medium	23033	129	881	745
(g/year)	High	297572	4523	16247	32569
	REP →	0.0001	0.0025	0.0002	0.005
TEQ	Low	0.18	0.013	0.007	0.10
Loading	Medium	2.3	0.32	0.18	3.7
(g/year)	High	29.8	11.3	3.2	162.8
		TEQ Estimates	Low	Medium	High
		(g TEQ/year)	0.30	6.53	207

triclosan and CTDs to US surface waters (using both methods) are displayed in Table 2.5 and Table 2.6.

Once triclosan or a CTD enters a water body, only a certain percentage undergoes photolysis. After correcting for pH and photic zone, a box model for a river that accounts for photolysis and sorption to particles as the major loss processes estimated photolysis accounted for 10% of degradation of triclosan [17]. Photolysis of triclosan in a lake system was estimated to account for up to 80% of triclosan degradation [190]. Values of 10%, 20%, and 30% were used for low, medium, and high estimates of triclosan and CTD photolysis rates. The estimate of 30% would assume that about 29% of WWTPs discharge into lakes (80% photolysis) and the rest discharge into rivers (10% photolysis). CTDs are assumed to have the same photolysis rates as triclosan. Of the triclosan and CTDs that undergo photolysis, only a small percentage converts to dioxins. Low and high estimates of dioxin yields for triclosan and the three CTDs were obtained from Buth et al. [17], while the average of the low and high estimate was used for the medium estimate. Dioxin loading estimates are shown for each method in Table 2.5 and Table 2.6.

Dioxin loadings are generally converted to a final TEQ. The REPs, similar to TEFs, for each dioxin are based on values given in Table 1.2. Values of REPs used are shown in Tables 2.5 and 2.6. Estimates range from 0.3 – 207 g TEQ/year, the later would be a worst case scenario. The estimated US air emissions inventory of dioxins is 2500 g TEQ/year [191, 192]. The use of triclosan in personal care products generates a PCDD load equivalent to 0.012 – 8.3% of air emissions. Similar dioxin loadings were estimated

by Buth et al. [17]. The lowest estimate may be a small fraction of air emissions; however, it represents a direct discharge into the aquatic environment. Dioxins from air emissions will fallout mostly on land as opposed to inland waters, which only make up a small percentage total US land area. Being hydrophobic, the dioxins will stay attached to soil particles and not make their way into aquatic environments. Sediment records have also confirmed that dioxins derived from triclosan have increased over the past 40 years and contribute up to 31% of total dioxins in the sediment [20]. Any risk assessment or governmental review of triclosan should, therefore, take into account the formation of dioxins from triclosan.

The confirmation of 6-OH-BDE-47 in WWTP effluent is of concern for the same reasons as triclosan and CTDs. PBDDs may form from OH-PBDEs that undergo photolysis. The presence of 6-OH-BDE-47, which is not manufactured directly by humans, in wastewater provides evidence that this compound is a metabolite of PBDEs, which are also present in wastewater. Whether 6-OH-BDE-47 was produced by human metabolism or bacterial metabolism from the activated sludge is unknown.

3. Conclusions and Recommendations

The method of wastewater disinfection affects levels of CTDs in the final effluent. Chlorination can significantly increase all three CTDs, although not always. Even in the case where CTDs did not increase after chlorination, CTDs are still detected in higher amounts than other non-chlorinating plants.

UV disinfection has little, if any, effect on triclosan and CTDs in wastewater. In one case from SJWP, concentrations of triclosan decreased, suggesting triclosan may have degraded potentially forming more dioxins than would otherwise be produced from interaction with sunlight in surface waters. PAWP samples, however, showed no degradation of triclosan from pre to post UV irradiation. The difference may be explained by significantly shorter contact times of wastewater with the UV lamps in PAWP.

Ozonation significantly reduced levels of all the analytes. One ozonation byproduct was detected but not identified. The product could be a tri-OH-PCDE or a di-OH-PCB based on retention time and relative isotope abundance. Other compounds that were not ozonation byproducts were also detected. These compounds are hypothesized to be di-hydroxy-triclosans. Without further experimentation, the compounds' identities cannot be confirmed.

The pattern of CTD levels observed (4-Cl-TCS < 6-Cl-TCS ≤ 4,6-Cl-TCS) is important when determining total dioxin formation potential in surface waters. Higher amounts of TCDDs may be formed because 4,6-Cl-TCS is generally detected at higher concentrations than the other CTDs. 1,2,3,8-TCDD (the dioxin produced from 4,6-Cl-

TCS) may be more toxic than 2,3,7- or 1,2,8-TrCDDs (the dioxins produced from the other CTDs) due to the extra chlorine substituent, however, only 2,3,7-TrCDD has been evaluated for toxicity.

Loadings of triclosan derived dioxins were estimated to be 0.012 – 8.3% of dioxin air emissions. Although comparatively low, this dioxin estimate is unique in the sense that the dioxins are discharged directly into surface waters. In some cases, 2,8-DCDD may be present at high enough concentrations (from the photolysis of triclosan) in the water to partition into the air, leading to atmospheric emissions from surface waters [89]. Reduction in the use of triclosan in personal care products would lead to reduced dioxin exposure for people who consume fish or live nearby wastewater impacted streams and rivers.

This study is the first to confirm wastewater as a source of 6-OH-BDE-47 to fresh water bodies. Whether wastewater is the most important source of OH-PBDEs, and consequently PBDDs via photolysis, in freshwater environments remains to be seen. Natural production of OH-PBDEs in freshwater is unlikely, owing to lack of bromide ions available needed for construction of these compounds. Low pg/L levels of OH-PBDEs in Lake Ontario is, therefore, likely anthropogenic in origin [157]. Contributions from rain and snow may be a significant source [157]. Photolysis of brominated phenols could be contributing to sustained levels of OH-PBDEs in the fresh water as well. Sustained levels of 2'-OH-BDE-68 were formed from photolysis of 2,4-dibromophenol [193]. Like PBDEs, brominated phenols are present in dust [194] and may also be present

in wastewater. Photolysis experiments with combinations of different brominated/halogenated phenols may lead to the production of other OH-PBDEs/OH-PHDEs. The detection of brominated phenols in river water has been achieved at the low ng/L level [195]. If brominated phenols are present in wastewater, they could be contributing to levels of OH-PBDEs and PBDDs in fresh water environments.

Despite the presence of 6-OH-BDE-47 in wastewater, the exact origins of this compound are still unclear. A mass balance of 6-OH-BDE-47 together with BDE-47 and 6-MeO-BDE-47 (and other compounds from Table 1.3) through a WWTP might provide sufficient evidence to determine whether OH-PBDEs are derived from metabolism by humans or bacteria in activated sludge. Experiments spiking PBDEs into bioreactors created from activated sludge would help determine if PBDEs were being transformed into OH-PBDEs or MeO-PBDEs by the sludge bacteria. Accelerated solvent extraction could be used on freeze dried activated sludge to extract these compounds. Chlorinated derivatives of OH-PBDEs, OH-BHDEs, may also be present in wastewater and undergo photolysis to form PHDDs. Methods with low detection limits (pg/L), however, are needed for these types of analyses.

Well-designed experiments would separate the phenolic (OH-PBDEs) and the hydrophobic (PBDEs and MeO-PBDEs) fractions. The relatively simple extraction method used in this research was able to detect 6-OH-BDE-47 in only some of the wastewater effluents analyzed. Other procedures, like derivatization with dansyl chloride [180], must be used to achieve lower detection limits, especially to detect other OH-

PBDEs present in various matrices. Since this study began, a growing number of OH-PBDEs have been synthesized in labs as experimental demand increases. As these compounds become more available, a comprehensive analysis of OH-PBDEs and their precursors and byproducts in a variety of environmental matrices would provide valuable insights into these complex transformations.

Bibliography

1. Chemical Abstract Service. CAS Database Counter. **June 8, 2012.** [Accessed June 8, 2012], Available from: www.cas.org/cgi-bin/cas/regreport.pl?l=en.
2. Brausch, J.M. and Rand, G.M. A review of personal care products in the aquatic environment: Environmental concentrations and toxicity. *Chemosphere*. **2011,** 82, 1518-1532.
3. Hites, R.A. Critical Review Polybrominated Diphenyl Ethers in the Environment and in People : A Meta-Analysis of Concentrations. *Environmental Science & Technology*. **2004,** 38, 945-956.
4. Adolfsson-Erici, M., Pettersson, M., Parkkonen, J., and Sturve, J. Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden. *Chemosphere*. **2002,** 46, 1485-1489.
5. Murray, K.E., Thomas, S.M., and Bodour, A.A. Prioritizing research for trace pollutants and emerging contaminants in the freshwater environment. *Environmental Pollution*. **2010,** 158, 3462-3471.
6. Diamanti-Kandarakis, E., Bourguignon, J.P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., and Gore, A.C. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocrine Reviews*. **2009,** 30, 293-342.
7. Palenske, N.M., Nallani, G.C., and Dzialowski, E.M. Physiological effects and bioconcentration of triclosan on amphibian larvae. *Comparative Biochemistry and Physiology - Part C*. **2010,** 152, 232-240.
8. Chalew, T.E. and Halden, R.U. Environmental exposure of aquatic and terrestrial biota to triclosan and triclocarban. *Journal - American Water Works Association*. **2009,** 45, 4-13.
9. Wu, J., Luo, X., Zhang, Y., Luo, Y., Chen, S., Mai, B., and Yang, Z. Bioaccumulation of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in wild aquatic species from an electronic waste (e-waste) recycling site in South China. *Environment International*. **2008,** 34, 1109-13.
10. North, K.D. Tracking polybrominated diphenyl ether releases in a wastewater treatment plant effluent, Palo Alto, California. *Environmental Science and Technology*. **2004,** 38, 4484-8.
11. Kumar, K.S., Priya, S.M., Peck, A.M., and Sajwan, K.S. Mass loadings of triclosan and triclocarbon from four wastewater treatment plants to three rivers and landfill in Savannah, Georgia, USA. *Archives of Environmental Contamination and Toxicology*. **2010,** 58, 275-85.
12. Vanderford, B.J. and Snyder, S.A. Analysis of pharmaceuticals in water by isotope dilution liquid chromatography/tandem mass spectrometry. *Environmental Science and Technology*. **2006,** 40, 7312-7320.
13. Reiss, R., Mackay, N., Habig, C., and Griffin, J. An ecological risk assessment for triclosan in lotic systems following discharge from wastewater treatment plants in

the United States. *Environmental Toxicology and Chemistry*. **2002,** 21, 2483-2492.
14. Wu, N., Herrmann, T., Paepke, O., Tickner, J., Hale, R., Harvey, L.E., La Guardia, M., McClean, M.D., and Webster, T.F. Human exposure to PBDEs: associations of PBDE body burdens with food consumption and house dust concentrations. *Environmental Science and Technology*. **2007,** 41, 1584-1589.
15. Athanasiadou, M., Cuadra, S.N., Marsh, G., Bergman, A., and Jakobsson, K. Polybrominated diphenyl ethers (PBDEs) and bioaccumulative hydroxylated PBDE metabolites in young humans from Managua, Nicaragua. *Environmental Health Perspectives*. **2007,** 116, 400-408.
16. Ji, K., Choi, K., Giesy, J.P., Musarrat, J., and Takeda, S. Genotoxicity of several polybrominated diphenyl ethers (PBDEs) and hydroxylated PBDEs, and their mechanisms of toxicity. *Environmental Science and Technology*. **2011,** 45, 5003-5008.
17. Buth, J.M., Grandbois, M., Vikesland, P.J., McNeill, K., and Arnold, W.A. Aquatic photochemistry of chlorinated triclosan derivatives: Potential source of polychlorodibenzo-p-dioxins. *Environmental Toxicology and Chemistry*. **2009,** 28, 2555-2563.
18. Buth, J.M., Ross, M.R., McNeill, K., and Arnold, W.A. Removal and formation of chlorinated triclosan derivatives in wastewater treatment plants using chlorine and UV disinfection. *Chemosphere*. **2011,** 84, 1238-1243.
19. Rappe, C., Andersson, R., Bergqvist, P., Brohede, C., Hansson, M., Kjeller, L., Lindstrom, G., Marklund, S., Nygren, M., Swanson, S.E., Tysklind, M., and Wiberg, K. Overview on environmental fate of chlorinated dioxins and dibenzofurans: Sources, levels and isomeric pattern in various matrices. *Chemosphere*. **1987,** 16, 1603-1618.
20. Buth, J.M., Steen, P.O., Sueper, C., Blumentritt, D., Vikesland, P.J., Arnold, W.A., and McNeill, K. Dioxin photoproducts of triclosan and its chlorinated derivatives in sediment cores. *Environmental Science and Technology*. **2010,** 44, 4545-4551.
21. Steen, P.O., Grandbois, M., McNeill, K., and Arnold, W.A. Photochemical formation of halogenated dioxins from hydroxylated polybrominated diphenyl ethers (OH-PBDEs) and chlorinated derivatives (OH-PBCDEs). *Environmental Science and Technology*. **2009,** 43, 4405-4411.
22. U.S. Wastewater Treatment Factsheet. Center for Sustainable Systems, University of Michigan. **2011.** Pub No. CSS04-14.
23. Environmental Protection Agency. Clean watersheds needs survey 2008 - Report to Congress. EPA-832-R-10-002. **2008,** Washington D.C.
24. Leong, L., Kuo, J., and Tang, C. Disinfection of wastewater effluent: Comparison of alternative technologies. Water Environment Research Foundation. **2008.**

25. Krasner, S.W., Westerhoff, P., Chen, B., Rittmann, B.E., and Amy, G. Occurrence of disinfection byproducts in United States wastewater treatment plant effluents. *Environmental Science and Technology*. **2009,** 43, 8320-8325.
26. Liberti, L., Notarnicola, M., and Petruzzelli, D. Advanced treatment for municipal wastewater reuse in agriculture. UV disinfection: parasite removal and by-product formation. *Desalination*. **2002,** 152, 315-324.
27. Roth, J.A. and Sullivan, D.E. Solubility of ozone in water. *Industrial & Engineering Chemistry Fundamentals*. **1981,** 20, 137-140.
28. Ku, Y., Su, W., and Shen, Y. Decomposition kinetics of ozone in aqueous solution. *Industrial & Engineering Chemistry Research*. **1996,** 35, 3369-3374.
29. Snyder, S.A., Wert, E.C., Rexing, D.J., Zegers, R.E., and Drury, D.D. Ozone oxidation of endocrine disruptors and pharmaceuticals in surface water and wastewater. *Ozone: Science and Engineering*. **2006,** 28, 445-460.
30. Huber, M.M., Göbel, A., Joss, A., Hermann, N., Löffler, D., McArdell, C.S., Ried, A., Siegrist, H., Ternes, T.A., and von Gunten, U. Oxidation of pharmaceuticals during ozonation of municipal wastewater effluents: A pilot study. *Environmental Science and Technology*. **2005,** 39, 4290-4299.
31. Ternes, T.A., Stüber, J., Herrmann, N., McDowell, D., Ried, A., Kampmann, M., and Teiser, B. Ozonation: a tool for removal of pharmaceuticals, contrast media and musk fragrances from wastewater? *Water Research*. **2003,** 37, 1976-1982.
32. Nakada, N., Shinohara, H., Murata, A., Kiri, K., Managaki, S., Sato, N., and Takada, H. Removal of selected pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) during sand filtration and ozonation at a municipal sewage treatment plant. *Water Research*. **2007,** 41, 4373-4382.
33. Wert, E.C., Rosario-Ortiz, F.L., Drury, D.D., and Snyder, S.A. Formation of oxidation byproducts from ozonation of wastewater. *Water Research*. **2007,** 41, 1481-1490.
34. Stalter, D., Magdeburg, A., Weil, M., Knacker, T., and Oehlmann, J. Toxication or detoxication? In vivo toxicity assessment of ozonation as advanced wastewater treatment with the rainbow trout. *Water Research*. **2010,** 44, 439-448.
35. Mišík, M., Knasmueller, S., Ferk, F., Cichna-Markl, M., Grummt, T., Schaar, H., and Kreuzinger, N. Impact of ozonation on the genotoxic activity of tertiary treated municipal wastewater. *Water Research*. **2011,** 45, 3681-3691.
36. Pereira, R.D., de Alda, M.L., Joglar, J., Daniel, L.A., and Barceló, D. Identification of new ozonation disinfection byproducts of 17β-estradiol and estrone in water. *Chemosphere*. **2011,** 84, 1535-1541.
37. Geigy. Preparation of halogenated 2-hydroxydiphenyl ethers. European Patent: NL6401526 (A). **Aug. 24, 1964.**
38. Jones, R.D., Jampani, H.B., Newman, J.L., and Lee, A.S. Triclosan: A review of effectiveness and safety in health care settings. *American Journal of Infection Control*. **2000,** 28, 184-196.

39. Lindhe, J., Rosling, B., Socransky, S.S., and Volpe, A.R. The effect of a triclosan-containing dentifrice on established plaque and gingivitis. *Journal of Clinical Periodontology*. **1993,** 20, 327-334.
40. Susman, E. Too clean for comfort. *Environmental Health Perspectives*. **2001,** 109, 16-19.
41. Aiello, A.E., Larson, E.L., and Levy, S.B. Consumer antibacterial soaps: Effective or just risky? *Clinical Infectious Diseases*. **2007,** 45, S137-S147.
42. Perencevich, E.N., Wong, M.T., and Harris, A.D. National and regional assessment of the antibacterial soap market: A step toward determining the impact of prevalent antibacterial soaps. *American Journal of Infection Control*. **2001,** 29, 281-283.
43. U.S. Food and Drug Administration. Triclosan - What consumers should know. **April 8, 2010.** [Accessed May 6, 2012], Available from: http://www.fda.gov/forconsumers/consumerupdates/ucm205999.htm.
44. Junker, L.M. and Hay, A.G. Effects of triclosan incorporation into ABS plastic on biofilm communities. *Journal of Antimicrobial Chemotherapy*. **2004,** 53, 989-996.
45. Heidler, J. and Halden, R.U. Mass balance assessment of triclosan removal during conventional sewage treatment. *Chemosphere*. **2007,** 66, 362-369.
46. McAvoy, D.C., Schatowitz, B., Jacob, M., Hauk, A., and Eckhoff, W.S. Measurement of triclosan in wastewater treatment systems. *Environmental Toxicology and Chemistry*. **2002,** 21, 1323-1329.
47. Chen, X., Nielsen, J.L., Furgal, K., Liu, Y., Lolas, I.B., and Bester, K. Biodegradation of triclosan and formation of methyl-triclosan in activated sludge under aerobic conditions. *Chemosphere*. **2011,** 84, 452-456.
48. Singer, H., Müller, S., Tixier, C., and Pillonel, L. Triclosan: occurrence and fate of a widely used biocide in the aquatic environment: field measurements in wastewater treatment plants, surface waters, and lake sediments. *Environmental Science and Technology*. **2002,** 36, 4998-5004.
49. Sabaliunas, D., Webb, S.F., Hauk, A., Jacob, M., and Eckhoff, W.S. Environmental fate of triclosan in the River Aire Basin, UK. *Water Research*. **2003,** 37, 3145-3154.
50. Thompson, A., Griffin, P., Stuetz, R., and Cartmell, E. The fate and removal of triclosan during wastewater treatment. *Water Environment Research*. **2005,** 77, 63-67.
51. Cha, J. and Cupples, A.M. Detection of the antimicrobials triclocarban and triclosan in agricultural soils following land application of municipal biosolids. *Water Research*. **2009,** 43, 2522-2530.
52. Bester, K. Triclosan in a sewage treatment process--balances and monitoring data. *Water Research*. **2003,** 37, 3891-3896.

53. Chu, S. and Metcalfe, C.D. Simultaneous determination of triclocarban and triclosan in municipal biosolids by liquid chromatography tandem mass spectrometry. *Journal of Chromatography A*. **2007,** 1164, 212-218.
54. Chenxi, W., Spongberg, A.L., and Witter, J.D. Determination of the persistence of pharmaceuticals in biosolids using liquid-chromatography tandem mass spectrometry. *Chemosphere*. **2008,** 73, 511-518.
55. Wu, C., Spongberg, A.L., and Witter, J.D. Adsorption and degradation of triclosan and triclocarban in soils and biosolids-amended soils. *Journal of Agricultural and Food Chemistry*. **2009,** 57, 4900-4905.
56. Ying, G., Yu, X., and Kookana, R.S. Biological degradation of triclocarban and triclosan in a soil under aerobic and anaerobic conditions and comparison with environmental fate modelling. *Environmental Pollution*. **2007,** 150, 300-305.
57. Halden, R.U. and Paull, D.H. Co-occurence of triclocarban and triclosan in U.S. water resources. *Environmental Science and Technology*. **2005,** 39, 1420-1426.
58. Thomas, P.M. and Foster, G.D. Tracking acidic pharmaceuticals, caffeine, and triclosan through the wastewater treatment process. *Environmental Toxicology and Chemistry*. **2005,** 24, 25-30.
59. Yu, C. and Chu, K. Occurence of pharmaceuticals and personal care products along the West Prong Little Pigeon River in east Tennessee, USA. *Chemosphere*. **2009,** 75, 1281-1286.
60. Brooks, B.W., Chambliss, C.K., Stanley, J.K., Ramirez, A., Banks, K.E., Johnson, R.D., and Lewis, R.J. Determination of select antidepressants in fish from an effluent-dominated stream. *Environmental Toxicology and Chemistry*. **2005,** 24, 464-469.
61. Wilson, B., Chen, R.F., Cantwell, M., Gontz, A., Zhu, J., and Olsen, C.R. The partitioning of triclosan between aqueous and particulate bound phases in the Hudson River Estuary. *Marine Pollution Bulletin*. **2009,** 59, 207-212.
62. Bedoux, G., Roig, B., Thomas, O., Dupont, V., and Le Bot, B. Occurrence and toxicity of antimicrobial triclosan and by-products in the environment. *Environmental Science and Pollution Research International*. **2011,** 35-43.
63. Hua, W., Bennett, E.R., and Letcher, R.J. Triclosan in waste and surface waters from the upper Detroit River by liquid chromatography-electrospray-tandem quadrupole mass spectrometry. *Environment International*. **2005,** 31, 621-630.
64. Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., and Buxton, H.T. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. *Environmental Science and Technology*. **2002,** 36, 1202-11.
65. Lindström, A., Buerge, I.J., Poiger, T., Bergqvist, P.A., Müller, M.D., and Buser, H.-R. Occurrence and environmental behavior of the bactericide triclosan and its methyl derivative in surface waters and in wastewater. *Environmental Science and Technology*. **2002,** 36, 2322-2329.

66. Peng, X., Yu, Y., Tang, C., Tan, J., Huang, Q., and Wang, Z. Occurrence of steroid estrogens, endocrine-disrupting phenols, and acid pharmaceutical residues in urban riverine water of the Pearl River Delta, South China. *Science of The Total Environment*. **2008,** 397, 158-166.
67. Schulze, J., Marquardt, F.H., Lyman, F., and Spitzer, C. Determination of free and conjugated triclosan in blood by electron capture gas liquid chromatography. *Journal of the American Oil Chemists' Society*. **1974,** 52, 215-218.
68. Coogan, M.A. and la Point, T.W. Snail bioaccumulation of triclocarban, triclosan, and methyltriclosan in a North Texas, USA, stream affected by wastewater treatment plant runoff. *Environmental Toxicology and Chemistry*. **2008,** 27, 1788-1793.
69. Ricart, M., Guasch, H., Alberch, M., Barcelo, D., Bonnineau, C., Geiszinger, A., Farre, M.l., Ferrer, J., Ricciardi, F., Romani, A.M., Morin, S., Proia, L., Sala, L., Sureda, D., and Sabater, S. Triclosan persistence through wastewater treatment plants and its potential toxic effects on river biofilms. *Aquatic Toxicology*. **2010,** 100, 346-353.
70. Capdevielle, M., Egmond, R.V., Whelan, M., Versteeg, D., Hofmann, M., Inauen, J., Cunningham, V., Woltering, D., Egmond, À.R.V., and Hofmann-kamensky, M. Consideration of exposure and species sensitivity of triclosan in the freshwater environment consideration of exposure and species sensitivity of triclosan in the freshwater environment. *Integrated Environmental Assessment and Management*. **2008,** 4, 15-23.
71. Orvos, D.R., Versteeg, D.J., Inauen, J., Capdevielle, M., Rothenstein, A., and Cunningham, V. Aquatic toxicity of triclosan. *Environmental Toxicology and Chemistry*. **2002,** 21, 1338-1349.
72. Kinney, C.A., Furlong, E.T., Kolpin, D.W., Burkhardt, M.R., Zaugg, S.D., Werner, S.L., Bossio, J.P., and Benotti, M.J. Bioaccumulation of pharmaceuticals and other anthropogenic waste indicators in earthworms from agricultural soil amended with biosolid or swine manure. *Environmental Science and Technology*. **2008,** 42, 1863-1870.
73. Amorim, M., Oliveira, E., Soares, A., and Scott-Fordsmand, J. Predicted no effect concentration (PNEC) for triclosan to terrestrial species (invertebrates and plants). *Environment International*. **2010,** 36, 338-343.
74. Raut, S.A. and Angus, R.A. Triclosan has endocrine-disrupting effects in male western mosquitofish, Gambusia affinis. *Environmental Toxicology and Chemistry*. **2010,** 29, 1287-1291.
75. Veldhoen, N., Skirrow, R.C., Osachoff, H., Wigmore, H., Clapson, D.J., Gunderson, M.P., Van Aggelen, G., and Helbing, C.C. The bactericidal agent triclosan modulates thyroid hormone-associated gene expression and disrupts postembryonic anuran development. *Aquatic Toxicology*. **2006,** 80, 217-227.

76. Zorrilla, L.M., Gibson, E.K., Jeffay, S.C., Crofton, K.M., Setzer, W.R., Cooper, R.L., and Stoker, T.E. The effects of triclosan on puberty and thyroid hormones in male wistar rats. *Toxicological Sciences*. **2009,** 107, 56-64.
77. Hinther, A., Bromba, C.M., Wulff, J.E., and Helbing, C.C. Effects of triclocarban, triclosan, and methyl triclosan on thyroid hormone action and stress in frog and mammalian culture systems. *Environmental Science and Technology*. **2011,** 45, 5395-5402.
78. United States Food and Drug Administration. Supporting information for toxicological evalutation by the national toxicology program. **December 08, 2011.** [Accessed April 22, 2012], Available from: http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/triclosan_508.pdf.
79. Balmer, M.E., Poiger, T., Droz, C., Romanin, K., Bergqvist, P.A., Müller, M.D., and Buser, H.-R. Occurrence of methyl triclosan, a transformation product of the bactericide triclosan, in fish from various lakes in Switzerland. *Environmental Science and Technology*. **2003,** 38, 390-395.
80. Bohmer, W., Ruedel, H., Wenzel, A., and Schroeter-Kermani, C., *Retrospective monitoring of triclosan and methyl-triclosan in fish: Results from the german environmental specimen bank.*, in *DIOXIN 2004 - 24th International Symposium on Halogenated Environmental Organic Pollutants and POPs*. 2004: Berlin.
81. Canosa, P., Morales, S., Rodríguez, I., Rubí, E., Cela, R., and Gómez, M. Aquatic degradation of triclosan and formation of toxic chlorophenols in presence of low concentrations of free chlorine. *Analytical and bioanalytical chemistry*. **2005,** 383, 1119-1126.
82. Fiss, E.M., Rule, K.L., and Vikesland, P.J. Formation of chloroform and other chlorinated byproducts by chlorination of triclosan-containing antibacterial products. *Environmental Science and Technology*. **2007,** 41, 2387-2394.
83. Rule, K.L., Ebbett, V.R., and Vikesland, P.J. Formation of chloroform and chlorinated organics by free-chlorine-mediated oxidation of triclosan. *Environmental Science and Technology*. **2005,** 39, 3176-3185.
84. Leiker, T.J., Abney, S.R., Goodbred, S.L., and Rosen, M.R. Identification of methyl triclosan and halogenated analogues in male common carp (Cyprinus carpio) from Las Vegas Bay and semipermeable membrane devices from Las Vegas Wash, Nevada. *Science of The Total Environment*. **2009,** 407, 2102-2114.
85. Wellington Laboratories. Standards for environmental testing and research. **2010.** [Accessed June 05, 2012], Available from: http://www.well-labs.com/wellingtoncatalogue0910.html.
86. Nilsson, C.-A., Andersson, K., Rappe, C., and Westermark, S. Chromatographic evidence for the formation of chloro-dioxins from chloro-2-phenoxyphenols. *Journal of Chromatography*. **1974,** 96, 137-147.

87. Latch, D.E., Packer, J.L., Arnold, W.A., and McNeill, K. Photochemical conversion of triclosan to 2,8-dichlorodibenzo-p-dioxin in aqueous solution. *Journal of Photochemistry and Photobiology A: Chemistry*. **2003,** 158, 63-66.
88. Aranami, K. and Readman, J.W. Photolytic degradation of triclosan in freshwater and seawater. *Chemosphere*. **2007,** 66, 1052-1056.
89. Friedman, C.L., Cantwell, M.G., and Lohmann, R. Passive sampling provides evidence for Newark Bay as a source of polychlorinated dibenzo-*p*-dioxins and furans to the New York/New Jersey, USA, atmosphere. *Environmental Toxicology and Chemistry*. **2011,** 31, 253-261.
90. Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., and Peterson, R.E. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicological Sciences*. **2006,** 93, 223-241.
91. Bradley, J. and McLeod, R. Ministry of the Environment. Scientific criteria document for standard development - polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). **1985,** Ontario, Canada.
92. Samara, F., Gullett, B.K., Harrison, R.O., Chu, A., and Clark, G.C. Determination of relative assay response factors for toxic chlorinated and brominated dioxins/furans using an enzyme immunoassay (EIA) and a chemically-activated luciferase gene expression cell bioassay (CALUX). *Environment International*. **2009,** 35, 588-593.
93. Safe, S.H. Comparative toxicology and mechanism of action of polychlorinated dibenzo-p-dioxins and dibenzofurans. *Annual Review of Pharmacology and Toxicology*. **1986,** 26, 371-399.
94. de Wit, C.A. An overview of brominated flame retardants in the environment. *Chemosphere*. **2002,** 46, 583-624.
95. Alaee, M., Arias, P., Sjödin, A., and Bergman, Ã.k. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environment International*. **2003,** 29, 683-689.
96. La Guardia, M.J., Hale, R.C., and Harvey, E. Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used penta-, octa-, and deca-PBDE technical flame-retardant mixtures. *Environmental Science and Technology*. **2006,** 40, 6247-6254.
97. US Environmental Protection Agency. Polybrominated diphenyl ethers (PBDEs) action plan summary. **May 10, 2012.** [Accessed May 13, 2012], Available from: http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/pbde.html.
98. La Guardia, M.J., Hale, R.C., and Harvey, E. Evidence of debromination of decabromodiphenyl ether (BDE-209) in biota from a wastewater receiving stream. *Environmental Science and Technology*. **2007,** 41, 6663-6670.

99. Stapleton, H.M., Brazil, B., Holbrook, R.D., Mitchelmore, C.L., Benedict, R., Konstantinov, A., and Potter, D. In vivo and in vitro debromination of decabromodiphenyl ether (BDE 209) by juvenile rainbow trout and common carp. *Environmental Science and Technology*. **2006,** 40, 4653-4658.
100. Van den Steen, E., Covaci, A., Jaspers, V.L., Dauwe, T., Voorspoels, S., Eens, M., and Pinxten, R. Accumulation, tissue-specific distribution and debromination of decabromodiphenyl ether (BDE 209) in European starlings (Sturnus vulgaris). *Environmental Pollution*. **2007,** 148, 648-653.
101. Tseng, L., Li, M., Tsai, S., Lee, C., Pan, M., Yao, W., and Hsu, P. Developmental exposure to decabromodiphenyl ether (PBDE 209): Effects on thyroid hormone and hepatic enzyme activity in male mouse offspring. *Chemosphere*. **2008,** 70, 640-647.
102. de Wit, C.A., Alaee, M., and Muir, D.C.G. Levels and trends of brominated flame retardants in the Arctic. *Chemosphere*. **2006,** 64, 209-233.
103. Law, R.J. and Herzke, D., *Current levels and trends of brominated flame retardants in the environment*, in *Handbook of Environmental Chemistry*. 2010, Springer.
104. Braune, B.M., Mallory, M.L., Grant Gilchrist, H., Letcher, R.J., and Drouillard, K.G. Levels and trends of organochlorines and brominated flame retardants in Ivory Gull eggs from the Canadian Arctic, 1976 to 2004. *Science of The Total Environment*. **2007,** 378, 403-417.
105. Fernie, K.J., Mayne, G., Shutt, J.L., Pekarik, C., Grasman, K.A., Letcher, R.J., and Drouillard, K. Evidence of immunomodulation in nestling American kestrels (Falco sparverius) exposed to environmentally relevant PBDEs. *Environmental Pollution*. **2005,** 138, 485-493.
106. Luo, X., Liu, J., Luo, Y., Zhang, X., Wu, J., Lin, Z., Chen, S., Mai, B., and Yang, Z. Polybrominated diphenyl ethers (PBDEs) in free-range domestic fowl from an e-waste recycling site in South China: Levels, profile and human dietary exposure. *Environment International*. **2009,** 35, 253-258.
107. She, J., Holden, A., Adelsbach, T.L., Tanner, M., Schwarzbach, S.E., Yee, J.L., and Hooper, K. Concentrations and time trends of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in aquatic bird eggs from San Francisco Bay, CA 2000-2003. *Chemosphere*. **2008,** 73, S201-S209.
108. Costa, L.G. and Giordano, G. Developmental neurotoxicity of polybrominated diphenyl ether (PBDE) flame retardants. *Neurotoxicology*. **2007,** 28, 1047-1067.
109. Abel, J., Fritsche, E., Gassmann, K., Gotz, C., Hubenthal, U., Krause, G., Merk, H.F., Moors, M., Nguyen, N.-H., Rose, C.R., Scanlan, T.S., and Schreiber, T. Polybrominated diphenyl ethers induce developmental neurotoxicity in a human in vitro model: Evidence for endocrine disruption. *Environmental Health Perspectives*. **2010,** 118, 572.
110. Vonderheide, A.P., Mueller, K.E., Meija, J., and Welsh, G.L. Polybrominated diphenyl ethers: Causes for concern and knowledge gaps regarding environmental

distribution, fate and toxicity. *Science of The Total Environment*. **2008,** 400, 425-436.
111. Agrell, C., ter Schure, A.F.H., Sveder, J., Bokenstrand, A., Larsson, P., and Zegers, B.N. Polybrominated diphenyl ethers (PBDES) at a solid waste incineration plant I: Atmospheric concentrations. *Atmospheric Environment*. **2004,** 38, 5139-5148.
112. Brown, F.R., Winkler, J., Visita, P., Dhaliwal, J., and Petreas, M. Levels of PBDEs, PCDDs, PCDFs, and coplanar PCBs in edible fish from California coastal waters. *Chemosphere*. **2006,** 64, 276-286.
113. Butt, C.M., Diamond, M.L., Truong, J., Ikonomou, M.G., and ter Schure, A.F.H. Spatial distribution of polybrominated diphenyl ethers in southern Ontario as measured in indoor and outdoor window organic films. *Environmental Science and Technology*. **2003,** 38, 724-731.
114. Frederiksen, M., Vorkamp, K., Thomsen, M., and Knudsen, L.E. Human internal and external exposure to PBDEs: A review of levels and sources. *International Journal of Hygiene and Environmental Health*. **2009,** 212, 109-134.
115. Guo, J., Wu, F., Mai, B., Luo, X., and Zeng, E.Y. Polybrominated diphenyl ethers in seafood products of south China. *Journal of Agricultural and Food Chemistry*. **2007,** 55, 9152-9158.
116. Harrad, S., de Wit, C.A., Abdallah, M.A.-E., Bergh, C., Björklund, J.A., Covaci, A., Darnerud, P.O., de Boer, J., Diamond, M., Huber, S., Leonards, P., Mandalakis, M., Ostman, C., Haug, L.S., Thomsen, C., and Webster, T.F. Indoor contamination with hexabromocyclododecanes, polybrominated diphenyl ethers, and perfluoroalkyl compounds: An important exposure pathway for people? *Environmental Science and Technology*. **2010,** 44, 3221-3231.
117. Harrad, S., Ibarra, C., Diamond, M., Melymuk, L., Robson, M., Douwes, J., Roosens, L., Dirtu, A.C., and Covaci, A. Polybrominated diphenyl ethers in domestic indoor dust from Canada, New Zealand, United Kingdom and United States. *Environment International*. **2008,** 34, 232-238.
118. Harrad, S., Wijesekera, R., Hunter, S., Halliwell, C., and Baker, R. Preliminary assessment of U.K. human dietary and inhalation exposure to polybrominated diphenyl ethers. *Environmental Science and Technology*. **2004,** 38, 2345-2350.
119. Karlsson, M., Julander, A., van Bavel, B., and Hardell, L. Levels of brominated flame retardants in blood in relation to levels in household air and dust. *Environment International*. **2007,** 33, 62-69.
120. Schecter, A., Harris, T.R., Shah, N., Musumba, A., and Päpke, O. Brominated flame retardants in US food. *Molecular Nutrition and Food Research*. **2008,** 52, 266-272.
121. Schecter, A., Päpke, O., Harris, T.R., Tung, K.C., Musumba, A., Olson, J., and Birnbaum, L. Polybrominated diphenyl ether (PBDE) levels in an expanded market basket survey of U.S. food and estimated PBDE dietary intake by age and sex. *Environmental Health Perspectives*. **2006,** 114, 1515-1520.

122. Sjödin, A., Päpke, O., McGahee, E., Focant, J.-F., Jones, R.S., Pless-Mulloli, T., Toms, L.-M.L., Herrmann, T., Müller, J., Needham, L.L., and Patterson Jr, D.G. Concentration of polybrominated diphenyl ethers (PBDEs) in household dust from various countries. *Chemosphere*. **2008,** 73, S131-S136.
123. Stapleton, H.M., Dodder, N.G., Offenberg, J.H., Schantz, M.M., and Wise, S.A. Polybrominated diphenyl ethers in house dust and clothes dryer lint. *Environmental Science and Technology*. **2004,** 39, 925-931.
124. ter Schure, A.F.H., Larsson, P., Agrell, C., and Boon, J.P. Atmospheric transport of polybrominated diphenyl ethers and polychlorinated biphenyls to the Baltic Sea. *Environmental Science and Technology*. **2004,** 38, 1282-1287.
125. Cui, J. and Forssberg, E. Mechanical recycling of waste electric and electronic equipment: a review. *Journal of Hazardous Materials*. **2003,** 99, 243-263.
126. Covaci, A., Voorspoels, S., Roosens, L., Jacobs, W., Blust, R., and Neels, H. Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in human liver and adipose tissue samples from Belgium. *Chemosphere*. **2008,** 73, 170-175.
127. Fängström, B., Athanassiadis, I., Odsjö, T., Norén, K., and Bergman, Å. Temporal trends of polybrominated diphenyl ethers and hexabromocyclododecane in milk from Stockholm mothers, 1980–2004. *Molecular Nutrition and Food Research*. **2008,** 52, 187-193.
128. Gill, U., Chu, I., Ryan, J.J., Feeley, M., Ware, G.W., and Gunther, F.A. Polybrominated diphenyl Ethers: Human Tissue Levels and Toxicology *Reviews of Environmental Contamination and Toxicology*. **2004,** 183, 55-97.
129. Harrad, S., Ibarra, C., Abdallah, M.A.-E., Boon, R., Neels, H., and Covaci, A. Concentrations of brominated flame retardants in dust from United Kingdom cars, homes, and offices: Causes of variability and implications for human exposure. *Environment International*. **2008,** 34, 1170-1175.
130. Moon, H., Kannan, K., Lee, S., and Choi, M. Atmospheric deposition of polybrominated diphenyl ethers (PBDEs) in coastal areas in Korea. *Chemosphere*. **2007,** 66, 585-593.
131. Sjödin, A., Wong, L.-Y., Jones, R.S., Park, A., Zhang, Y., Hodge, C., DiPietro, E., McClure, C., Turner, W., Needham, L.L., and Patterson, D.G. Serum concentrations of polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyl (PBB) in the United States population: 2003-2004. *Environmental Science and Technology*. **2008,** 42, 1377-1384.
132. Zota, A.R., Rudel, R.A., Morello-Frosch, R.A., and Brody, J.G. Elevated house dust and serum concentrations of PBDEs in California: Unintended consequences of furniture flammability standards? *Environmental Science and Technology*. **2008,** 42, 8158-8164.
133. Rose, M., Bennett, D.H., Bergman, A., Fängström, B., Pessah, I.N., and Hertz-Picciotto, I. PBDEs in 2-5 year-old children from California and associations with

diet and indoor environment. *Environmental Science and Technology*. **2010,** 44, 2648-2653.
134. Fischer, D., Hooper, K., Athanasiadou, M., Athanassiadis, I., and Bergman, A. Children show highest levels of polybrominated diphenyl ethers in a California family of four: a case study. *Environmental Health Perspectives*. **2006,** 114, 1581-1584.
135. Herbstman, J.B., Sjodin, A., Kurzon, M., Lederman, S.A., Jones, R.S., Rauh, V., Needham, L.L., Tang, D., Niedzwiecki, M., Wang, R.Y., and Perera, F. Prenatal exposure to PBDEs and neurodevelopment. *Environmental Health Perspectives*. **2010,** 1180, 712-719.
136. Gascon, M., Vrijheid, M., Martinez, D., Forns, J., Grimalt, J.O., Torrent, M., and Sunyer, J. Effects of pre and postnatal exposure to low levels of polybromodiphenyl ethers on neurodevelopment and thyroid hormone levels at 4 years of age. *Environment International*. **2011,** 37, 605-611.
137. Jin, S., Yang, F., Hui, Y., Xu, Y., Lu, Y., and Liu, J. Cytotoxicity and apoptosis induction on RTG-2 cells of 2,2,4,4-tetrabromodiphenyl ether (BDE-47) and decabrominated diphenyl ether (BDE-209). *Toxicology in Vitro*. **2010,** 24, 1190-1196.
138. Clarke, B.O., Porter, N.A., Symons, R.K., Marriott, P.J., Stevenson, G.J., and Blackbeard, J.R. Investigating the distribution of polybrominated diphenyl ethers through an Australian wastewater treatment plant. *Science of The Total Environment*. **2010,** 408, 1604-1611.
139. Andrade, N.A., McConnell, L.L., Torrents, A., and Ramirez, M. Persistence of Polybrominated Diphenyl Ethers in Agricultural Soils after Biosolids Applications. *Journal of Agricultural and Food Chemistry*. **2010,** 58, 3077-3084.
140. Peng, X., Tang, C., Yu, Y., Tan, J., Huang, Q., Wu, J., Chen, S., and Mai, B. Concentrations, transport, fate, and releases of polybrominated diphenyl ethers in sewage treatment plants in the Pearl River Delta, South China. *Environment International*. **2009,** 35, 303-309.
141. Cheng, J., Mao, L., Zhao, Z., Shen, M., Zhang, S., Huang, Q., and Gao, S. Bioaccumulation, depuration and biotransformation of 4,4'-dibromodiphenyl ether in crucian carp (Carassius auratus). *Chemosphere*. **2012,** 86, 446-453.
142. Erratico, C.A., Moffatt, S.C., and Bandiera, S.M. Comparative oxidative metabolism of BDE-47 and BDE-99 by rat hepatic microsomes. *Toxicological Sciences*. **2011,** 123, 37-47.
143. Hakk, H., Huwe, J.K., Murphy, K., and Rutherford, D. Metabolism of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) in chickens. *Journal of Agricultural and Food Chemistry*. **2010,** 58, 8757-8762.
144. Hamers, T., Kamstra, J.H., Sonneveld, E., Murk, A.J., Visser, T.J., Van Velzen, M.J.M., Brouwer, A., and Bergman, Å. Biotransformation of brominated flame retardants into potentially endocrine-disrupting metabolites, with special attention

to 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47). *Molecular Nutrition and Food Research*. **2008,** 52, 284-298.
145. Munschy, C., Haas-Moisan, K., Tixier, C., Pacepavicius, G., and Alaee, M. Dietary exposure of juvenile common sole (Solea solea L.) to polybrominated diphenyl ethers (PBDEs): Part 2. Formation, bioaccumulation and elimination of hydroxylated metabolites. *Environmental Pollution*. **2010,** 158, 3527-3533.
146. Qiu, X., Mercado-Feliciano, M., Bigsby, R.M., and Hites, R.A. Measurement of polybrominated diphenyl ethers and metabolites in mouse plasma after exposure to a commercial pentabromodiphenyl ether mixture. *Environmental Health Perspectives*. **2007,** 115, 1052-1058.
147. Ryden, A., Nestor, G., Jakobsson, K., and Marsh, G. Synthesis and tentative identification of novel polybrominated diphenyl ether metabolites in human blood. *Chemosphere*. **2012,**
148. Bendig, P. and Vetter, W. Photolytical transformation rates of individual polybrominated diphenyl ethers in technical octabromo diphenyl ether (DE-79). *Environmental Science and Technology*. **2010,** 44, 1650-1655.
149. Soderstrom, G., Sellstrom, U., de Wit, C.A., and Tysklind, M. Photolytic debromination of decabromodiphenyl ether (BDE 209). *Environmental Science and Technology*. **2003,** 38, 127-132.
150. Raff, J.D. and Hites, R.A. Gas-phase reactions of brominated diphenyl ethers with OH radicals. *The Journal of Physical Chemistry A*. **2006,** 110, 10783-10792.
151. Watanabe, I. and Tatsukawa, R. Formation of brominated dibenzofurans from the photolysis of flame retardant decabromobiphenyl ether in hexane solution by UV and sun light. *Bulletin of Environmental Contamination and Toxicology*. **1987,** 39, 953-959.
152. Buser, H.R. Polybrominated dibenzofurans and dibenzo-Para-dioxins—Thermal-reaction products of polybrominated diphenyl ether flame retardants. *Environmental Science and Technology*. **1986,** 20, 404-408.
153. Thoma, H., Hauschulz, G., Knorr, E., and Hutzinger, O. Polybrominated dibenzofurans (Pbdf) and dibenzodioxins (Pbdd) from the pyrolysis of neat brominated diphenylethers, biphenyls and plastic mixtures of these compounds. *Chemosphere*. **1987,** 16, 277-285.
154. Wan, Y., Wiseman, S., Chang, H., Zhang, X., Jones, P.D., Hecker, M., Kannan, K., Tanabe, S., Hu, J., Lam, M.H.W., and Giesy, J.P. Origin of hydroxylated brominated diphenyl ethers: natural compounds or man-made flame retardants? *Environmental Science and Technology*. **2009,** 43, 7536-7542.
155. Wiseman, S.B., Wan, Y., Chang, H., Zhang, X., Hecker, M., Jones, P.D., and Giesy, J.P. Polybrominated diphenyl ethers and their hydroxylated/methoxylated analogs: Environmental sources, metabolic relationships, and relative toxicities. *Marine Pollution Bulletin*. **2011,** 63, 179-188.
156. Malmvärn, A., Zebühr, Y., Kautsky, L., Bergman, A., and Asplund, L. Hydroxylated and methoxylated polybrominated diphenyl ethers and

polybrominated dibenzo-p-dioxins in red alga and cyanobacteria living in the Baltic Sea. *Chemosphere*. **2008,** 72, 910-916.
157. Ueno, D., Darling, C., Alaee, M., Pacepavicius, G., Teixeira, C., Campbell, L., Letcher, R.J., Bergman, A., Marsh, G., and Muir, D. Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) in the abiotic environment: surface water and precipitation from Ontario, Canada. *Environmental Science and Technology*. **2008,** 42, 1657-64.
158. Marsh, G., Athanasiadou, M., Athanassiadis, I., and Sandholm, A. Identification of hydroxylated metabolites in 2,2',4,4'-tetrabromodiphenyl ether exposed rats. *Chemosphere*. **2006,** 63, 690-697.
159. Stapleton, H.M., Kelly, S.M., Pei, R., Letcher, R.J., and Gunsch, C. Metabolism of polybrominated diphenyl ethers (PBDEs) by human hepatocytes in vitro. *Environmental Health Perspectives*. **2009,** 117, 197-202.
160. Gribble, G.W. The diversity of naturally occurring organobromine compounds. *Chemical Society Reviews*. **1999,** 28, 335-346.
161. Handayani, D., Edrada, R.A., Proksch, P., Wray, V., Witte, L., Van Soest, R.W.M., Kunzmann, A., and Soedarsono. Four new bioactive polybrominated diphenyl ethers of the sponge Dysidea herbacea from West Sumatra, Indonesia. *Journal of Natural Products*. **1997,** 60, 1313-1316.
162. Löfstrand, K., Liu, X., Lindqvist, D., Jensen, S., and Asplund, L. Seasonal variations of hydroxylated and methoxylated brominated diphenyl ethers in blue mussels from the Baltic Sea. *Chemosphere*. **2011,** 84, 527-532.
163. Malmvärn, A., Marsh, G., Kautsky, L., Athanasiadou, M., Bergman, A., and Asplund, L. Hydroxylated and methoxylated brominated diphenyl ethers in the red algae Ceramium tenuicorne and blue mussels from the Baltic Sea. *Environmental Science and Technology*. **2005,** 39, 2990-2997.
164. Marsh, G., Athanasiadou, M., Bergman, Ã.k., and Asplund, L. Identification of hydroxylated and methoxylated polybrominated diphenyl ethers in Baltic Sea Salmon (Salmo salar) blood. *Environmental Science and Technology*. **2004,** 38, 10-18.
165. Malmvärn, A., Eriksson, U., Kautsky, L., and Asplund, L. Cyanobacteria from the Baltic Sea − A producer of hydroxylated polybrominated diphenyl ethers (HO-PBDEs), methoxylated polybrominated diphenyl ethers (MeO-PBDEs) and polybrominated-dibenzo-*p*-dioxin (PBDD)? *Environmental Science and Technology*. **2005,** 67, 1380-1383.
166. Liu, F., Wiseman, S., Wan, Y., Doering, J.A., Hecker, M., Lam, M.H.W., and Giesy, J.P. Multi-species comparison of the mechanism of biotransformation of MeO-BDEs to OH-BDEs in fish. *Aquatic Toxicology*. **2012,** 114â€“115, 182-188.
167. Stapleton, H.M., Dodder, N.G., Kucklick, J.R., Reddy, C.M., Schantz, M.M., Becker, P.R., Gulland, F., Porter, B.J., and Wise, S.A. Determination of HBCD, PBDEs and MeO-BDEs in California sea lions (Zalophus californianus) stranded between 1993 and 2003. *Mar Pollut Bull*. **2006,** 52, 522-531.

168. Hakk, H. and Letcher, R.J. Metabolism in the toxicokinetics and fate of brominated flame retardants: a review. *Environment International*. **2003,** 29, 801-828.
169. Verreault, J., Gabrielsen, G.V., Chu, S.G., Muir, D.C.G., Andersen, M., Hamaed, A., and Letcher, R.J. Flame retardants and methoxylated and hydroxylated polybrominated diphenyl ethers in two Norwegian Arctic top predators: Glaucous gulls and polar bears. *Environmental Science and Technology*. **2005,** 39, 6021-6028.
170. Valters, K., Li, H., Alaee, M., D'Sa, I., Marsh, G., Bergman, A.k., and Letcher, R.J. Polybrominated diphenyl ethers and hydroxylated and methoxylated brominated and chlorinated analogues in the plasma of fish from the detroit river. *Environmental Science and Technology*. **2005,** 39, 5612-5619.
171. Houde, M., Pacepavicius, G., Darling, C., Fair, P.A., Alaee, M., Bossart, G.D., Solomon, K.R., Letcher, R.J., Bergman, Å., Marsh, G., and Muir, D.C.G. Polybrominated diphenyl ethers and their hydroxylated analogs in plasma of bottlenose dolphins (Tursiops truncatus) from the United States east coast. *Environmental Toxicology and Chemistry*. **2009,** 28, 2061-2068.
172. Wan, Y., Liu, F.Y., Wiseman, S., Zhang, X.W., Chang, H., Hecker, M., Jones, P.D., Lam, M.H.W., and Giesy, J.P. Interconversion of hydroxylated and methoxylated polybrominated diphenyl ethers in Japanese medaka. *Environmental Science and Technology*. **2010,** 44, 8729-8735.
173. Dingemans, M., Groot, A.D., Kleef, R.V., Bergman, A., van den Berg, M., Vijverberg, H., and Westerink, R. Hydroxylation increases the neurotoxic potential of BDE-47 to affect exocytosis and calcium homeostasis in PC12 cells. *Environmental Health Perspectives*. **2008,** 116, 637-643.
174. Canton, R.F., Scholten, D.E., Marsh, G., De Jong, P.C., and Van den Berg, M. Inhibition of human placental aromatase activity by hydroxylated polybrominated diphenyl ethers (OH-PBDEs). *Toxicology and Applied Pharmacology*. **2008,** 227, 68-75.
175. Dingemans, M., Berg, M., and Westerink, R. Neurotoxicity of brominated flame retardants: (In)direct effects of parent and hydroxylated polybrominated diphenyl ethers on the (developing) nervous system. *Environmental Health Perspectives*. **2011,** 119, 900-907.
176. Kitamura, S., Shinohara, S., Iwase, E., Sugihara, K., Uramaru, N., Shigematsu, H., Fujimoto, N., and Ohta, S. Affinity for thyroid hormone and estrogen receptors of hydroxylated polybrominated diphenyl ethers. *Journal of Health Science*. **2008,** 54, 607-614.
177. Meerts, I., Letcher, R.J., Hoving, S., Marsh, G., Bergman, A., Lemmen, J.G., van der Burg, B., and Brouwer, A. In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds. *Environmental Health Perspectives*. **2001,** 109, 399-407.

178. Mercado-Feliciano, M. and Bigsby, R.M. Hydroxylated metabolites of the polybrominated diphenyl ether mixture DE-71 are weak estrogen receptor-alpha ligands. *Environmental Health Perspectives*. **2008,** 116, 1315-1321.
179. Usenko, C.Y., Hopkins, D.C., Trumble, S.J., and Bruce, E.D. Hydroxylated PBDEs induce developmental arrest in zebrafish. *Toxicology and Applied Pharmacology*. **2012,** 262, 43-51.
180. Chang, H., Wu, F., Jin, F., Feng, C., Zhao, X., and Liao, H. Picogram per liter level determination of hydroxylated polybrominated diphenyl ethers in water by liquid chromatography-electrospray tandem mass spectrometry. *Journal of Chromatography A*. **2012,** 1223, 131-135.
181. Malmvärn, A., Zebühr, Y., Jensen, S., Kautsky, L., Greyerz, E., Nakano, T., and Asplund, L. Identification of polybrominated dibenzo-*p*-dioxins in blue mussels (Mytillus edulis) from the Baltic Sea. *Environmental Science and Technology*. **2005,** 39, 8235-8242.
182. Unger, M., Asplund, L., Haglund, P., Malmvärn, A., Arnoldsson, K., and Gustafsson, O. Polybrominated and mixed brominated/chlorinated dibenzo-*p*-dioxins in sponge (Ephydatia fluviatilis) from the Baltic Sea. *Environmental Science and Technology*. **2009,** 43, 8245-8250.
183. Utkina, N.K., Denisenko, V.A., Scholokova, O.V., Virovaya, M.V., Gerasimenko, A.V., Popov, D.Y., Krasokhin, V.B., and Popov, A.M. Spongiadioxins A and B, Two new polybrominated dibenzo-*p*-dioxins from an Australian marine sponge Dysidea dendyi. *Journal of Natural Products*. **2001,** 64, 151-153.
184. Haglund, P. On the identity and formation routes of environmentally abundant tri- and tetrabromodibenzo-*p*-dioxins. *Chemosphere*. **2010,** 78, 724-730.
185. Olsman, H., Engwall, M., Kammann, U., Klempt, M., Otte, J., van Bavel, B., and Hollert, H. Relative differences in aryl hydrocarbon receptor-mediated response for 18 polybrominated and mixed halogenated dibenzo-*p*-dioxins and -furans in cell lines from four different species. *Environmental Toxicology and Chemistry*. **2007,** 26, 2448-2454.
186. Rayne, S. and Forest, K. pKa values of the monohydroxylated polychlorinated biphenyls (OH-PCBs), polybrominated biphenyls (OH-PBBs), polychlorinated diphenyl ethers (OH-PCDEs), and polybrominated diphenyl ethers (OH-PBDEs). *Journal of Environmental Science and Health, Part A*. **2010,** 45, 1322-1346.
187. Bader, H. and Hoigne, J. Determination of ozone in water by the indigo method. *Water Research*. **1981,** 15, 449-456.
188. Kim, Y.M., Murugesan, K., Schmidt, S., Bokare, V., Jeon, J.R., Kim, E.J., and Chang, Y.S. Triclosan susceptibility and co-metabolism - A comparison for three aerobic pollutant-degrading bacteria. *Bioresource Technology*. **2011,** 102, 2206-2212.
189. Chen, X., Richard, J., Liu, Y., Dopp, E., Tuerk, J., and Bester, K. Ozonation products of triclosan in advanced wastewater treatment. *Water Research*. **2012,** 46, 2247-2256.

190. Tixier, C., Singer, H.P., Canonica, S., and Muller, S.R. Phototransformation of triclosan in surface waters: A relevant elimination process for this widely used biocide. *Environmental Science and Technology*. **2002,** 36, 3482-3489.
191. Environmental Protection Agency. Exposure and human health reassessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. Part I: Estimating exposure to dioxin-like compounds---Volume 2: Sources of dioxin like compounds in the United States. EPA/600/P-00/001Bb. Draft Final Edition. **2000,** Washington, DC.
192. Fiedler, H. National PCDD/PCDF release inventories under the Stockholm Convention on Persistent Organic Pollutants. *Chemosphere*. **2007,** 67, S96-S108.
193. Liu, H., Zhao, H., Quan, X., Zhang, Y., Chen, S., and Zhao, H. Formation of 2'-hydroxy-2,3',4,5'-tetrabromodipheyl ether (2'-HO-BDE68) from 2,4-dibromophenol in aqueous solution under simulated sunlight irradiation. *Chemosphere*. **2011,** 84, 512-8.
194. Suzuki, G., Takigami, H., Watanabe, M., Takahashi, S., Nose, K., Asari, M., and Sakai, S.I. Identification of brominated and chlorinated phenols as potential thyroid-disrupting compounds in indoor dusts. *Environmental Science and Technology*. **2008,** 42, 1794-1800.
195. Sim, W., Lee, S., Lee, I., Choi, S., and Oh, J. Distribution and formation of chlorophenols and bromophenols in marine and riverine environments. *Chemosphere*. **2009,** 77, 552-558.

Appendix

Table A.1.

LOQs for Each Wastewater Matrix

	LOQs in pM (ng/L)*				
Wastewater Samples	Triclosan	4-Cl-TCS	6-Cl-TCS	4,6-Cl-TCS	6-OH-BDE-47
MWP – 09/11	100.8 (29.1)	0.1 (.03)	0.3 (0.1)	0.3 (0.1)	3.6 (1.8)
PAWP – 07/11; MWP – 11/11	64.1 (18.5)	2.9 (0.9)	7.5 (2.4)	7.9 (2.6)	0.7 (0.35)
PAWP – 01/12; MWP – 10/11; SJWP – 01/12	7.9 (2.3)	0.24 (0.08)	0.25 (0.08)	0.09 (0.03)	0.43 (0.21)
SJWP – 02/12	30.4 (8.8)	0.73 (0.24)	0.53 (0.17)	0.94 (0.33)	0.90 (0.45)
MWP – 04/12	13.5 (3.9)	0.07 (0.02)	0.01 (0.003)	0.15 (0.05)	6.6 (3.3)
WLSSD – 06/11; WLSSD – 4/12;	43.8 (12.7)	0.54 (0.17)	0.49 (0.16)	1.21 (0.43)	0.59 (0.3)

*LODs may be calculated from LOQs by multiplying by 0.3

Note: The following paragraph is a note regarding the samples from the first two runs on at the U of M Cancer Research Center included in this thesis: MWP – 09/11, WLSSD – 06/11, PAWP – 07/11, and MWP 11/11. I did not dilute the spiked samples at all the first time I ran these samples and my calibration curve did not go above the concentration found in these samples. Due to these two errors in judgment, relative recoveries for triclosan turned out much higher than they should have been (>130%). The $^{13}C_{12}$-triclosan signal is suppressed by high amounts of triclosan. Diluting the spiked samples reduces this effect. In an attempt to correct this mistake, I diluted the spiked extracts 5 times and reran them on the LC-MS/MS. I then used the concentrations of the spiked samples in that run to calculate relative recoveries for the aforementioned samples. Relative recoveries using this new data turned out a bit better for triclosan (68.7 – 96.8%), so these relative recoveries were used except in the case of Metro – 09/11 Post Chlorination. (Relative recoveries for triclosan, when using $^{13}C_{12}$-triclosan as a surrogate, should be 100%, as they are the same compound and exhibit the same properties except

mass.) In the end the relative recoveries for triclosan were averaged to 100.9 ± 34.4%. This is what mainly contributed to high amounts of error in these samples, but the results are statistically valid.

Table A.2.

Recovery Corrections

	Relative Recoveries for Individual Analytes (%)				
Wastewater Samples	Triclosan	4-Cl-TCS	6-Cl-TCS	4,6-Cl-TCS	6-OH-BDE-47
MWP – 09/11	100.9 ± 34.4	90.8 ± 39.7	94.4 ± 56.9	67.4 ± 29.3	49.9 ± 27.6
PAWP – 07/11; MWP – 11/11	70.9 ± 2.6	86.8 ± 10.1	80.2 ± 17.4	62 ± 7.9	50.4 ± 9.5
PAWP – 01/12; MWP – 10/11; SJWP – 01/12	97.3 ± 3.8	77.2 ± 11.7	62.1 ± 16.1	54.1 ± 12.1	49.1 ± 12.9
SJWP – 02/12	105.2 ± 2.8	97.8 ± 9.5	102.9 ± 5.1	71.1 ± 8	67.5 ± 3.5
MWP – 04/11	105.4 ± 1	82.5 ± 24.3	101.6 ± 28.2	75.7 ± 18.7	65.1 ± 5.2
WLSSD – 06/11; WLSSD 04/12;	93.7 ± 2.8	80.8 ± 9.5	47.8 ± 5.1	50.6 ± 8	65 ± 3.5

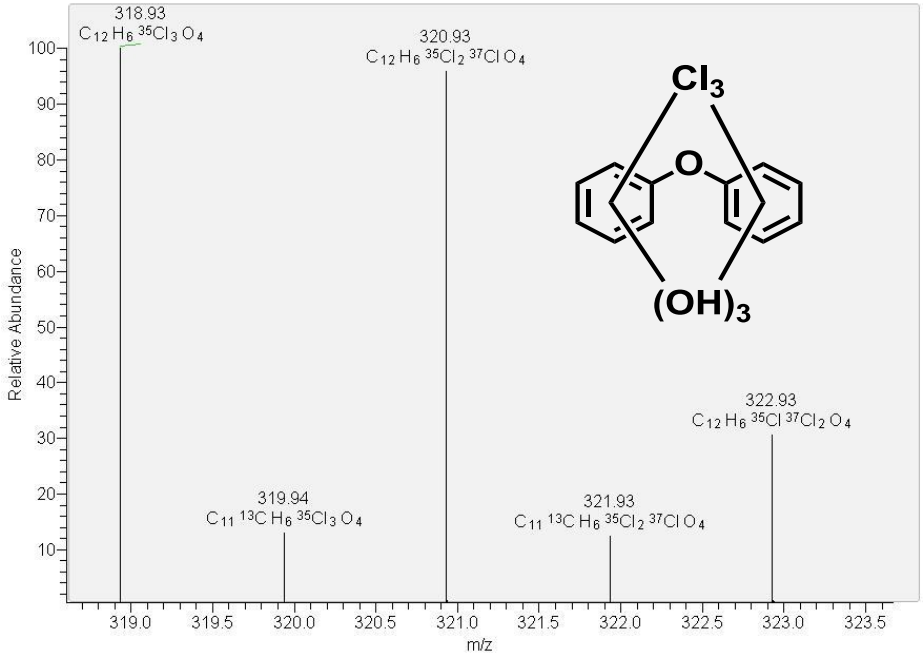


Figure A. Isotope Abundance of Dihydroxy-Triclosan.

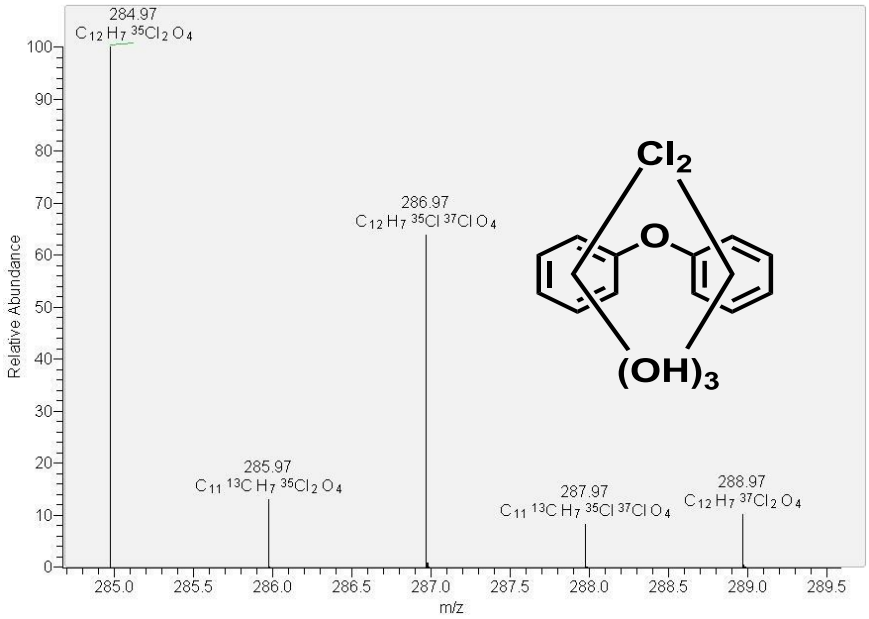


Figure A.2 Isotope Abundance Ratio for Trihydroxy-Dichloro-Diphenyl Ether.

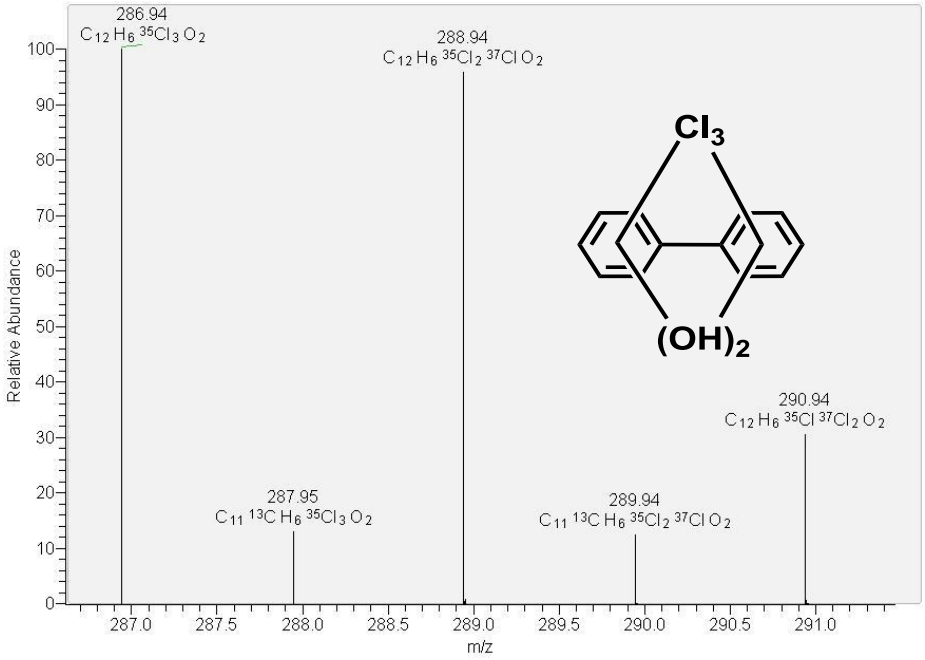


Figure A.3 Isotope Abundance for Deprotonated Dihydroxy-Trichloro-Biphenyl.

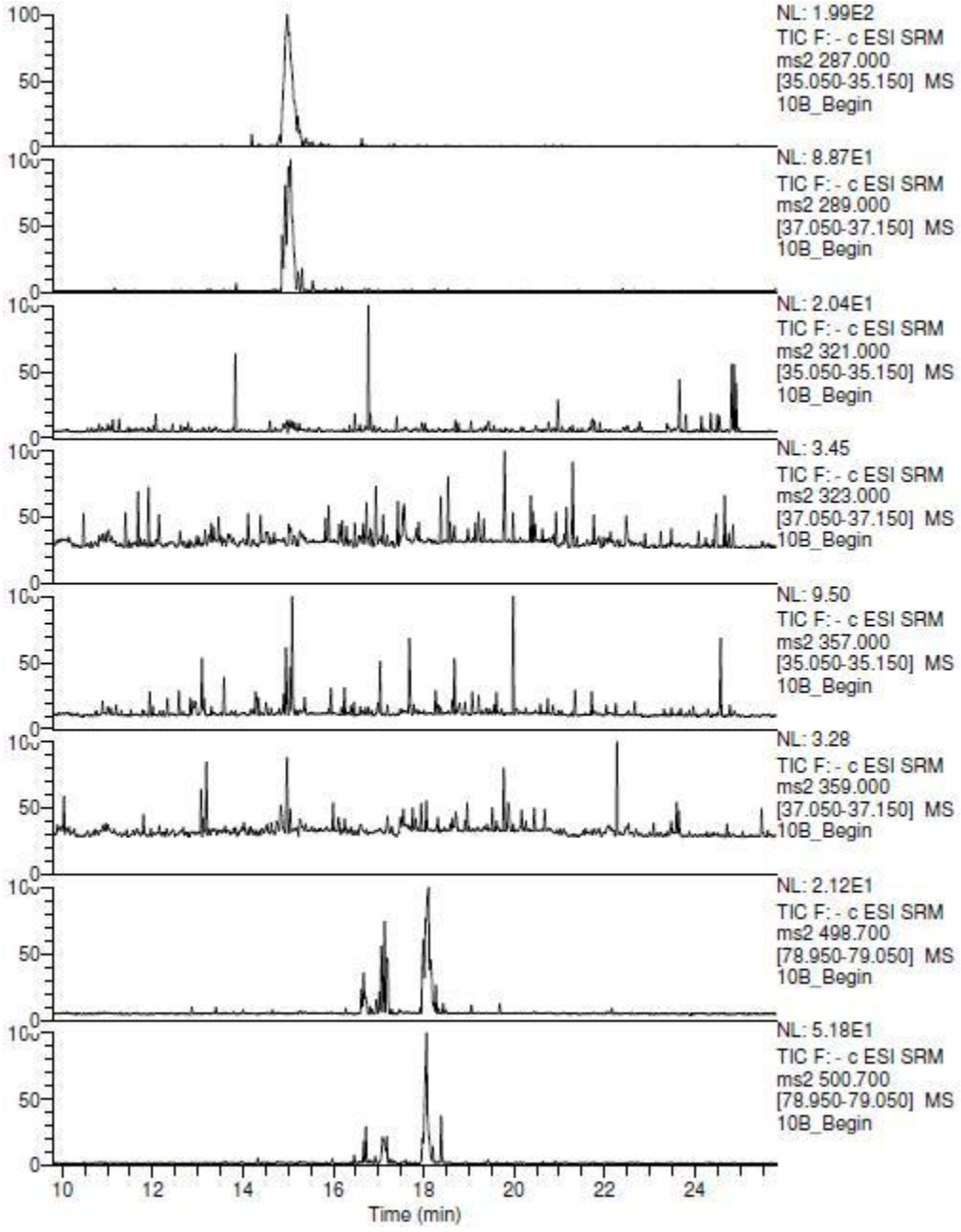


Figure A.4 Chromatogram of Representative Method Blank