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Influence of PCB-Contaminated Water Bodies on the PCB Content of Riparian Vegetation Through Foliar Deposition

Timothy Matthew Sattler
Clemson University

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INFLUENCE OF PCB-CONTAMINATED WATER BODIES
ON THE PCB CONTENT OF RIPARIAN VEGETATION
THROUGH FOLIAR DEPOSITION

A Master's Thesis
Presented to
The Graduate School of
Clemson University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science
Environmental Engineering and Science

by
Timothy Matthew Sattler
May 2013

Accepted by:
Dr. Cindy M. Lee, Committee Chair
Dr. John T. Coates
Dr. Thomas J. Overcamp

ABSTRACT

Polychlorinated biphenyls (PCBs) are a class of persistent organic pollutants that are generally considered semi-volatile. Volatilization and transport of PCBs from contaminated water bodies can result in PCB accumulation on terrestrial plant surfaces, acting as an entry point for contaminants into terrestrial food webs. Evergreen plants can serve as biomonitors of this PCB accumulation, as they often retain foliage for several years, allowing for greater concentrations to accumulate and subsequently be measured. *Rhododendron maximum* plants often grow in the riparian zone adjacent to water bodies in the Appalachian region, such as near Town Creek, Pickens, South Carolina. This research aimed to determine if PCBs in rhododendron leaves adjacent to PCB-contaminated Town Creek were due to gas phase deposition, primarily as a result of volatilization from the creek.

Town Creek and the riparian zone located off of Shady Grove Road, Pickens, SC, were chosen as the study site. Polyurethane foam (PUF) disks were selected as passive air samplers (PAS), which were deployed adjacent to rhododendron plants during August 2012 for one month, after which both samplers and rhododendron leaves were collected. Seven PAS were placed over an approximate 15 m gradient extending from the creek, including one directly over the surface of the water. Polyethylene (PE) passive aqueous samplers were used to confirm the presence of dissolved PCBs in Town Creek. PCBs were detected in all samplers and rhododendron leaves. Maximum PCB accumulations were detected nearest the creek for both PUF disks (2077.7 ng) and rhododendron leaf

samples (6670.8 ± 1400.9 ng/g lipid). Concentrations rapidly decreased in both media with increasing distance from the creek. PCB accumulations in PUF disks were dominated by tri-, tetra-, and penta-chlorinated congeners, while rhododendron leaf samples had primarily tetra- and penta-chlorinated congeners. PE samplers had primarily tetra-, penta-, and hexa-chlorinated PCBs, along with some tri-chlorinated congeners.

PCBs may be biodegraded in the environment via selective dechlorination (anaerobically) or ring cleavage (aerobically), yielding enantiomeric fractions (EFs) that differ from racemic (0.5). Chiral PCB congeners 91 (2,2',3,4',6-pentachlorinated biphenyl) and 95 (2,2',3,5',6-pentachlorinated biphenyl) were detected in PE, PUF disks, and rhododendron leaves. EFs were used to compare chiral signatures of these congeners among sampled media. PCB 95 was racemic or near-racemic in PE samples, and non-racemic (>0.5) in leaf samples. PCB 91 was non-racemic (>0.5) in both PE and rhododendron leaf samples. The difference in EF value between PE and leaf samples may be due to a secondary source of PCBs contributing to contamination in the leaves, such as volatilization from the soil.

These data show the importance of volatilization and atmospheric transport when considering the fate and transport of PCBs in aquatic and terrestrial environments. Collectively, Town Creek was shown to be the primary contributor to PCB contamination in adjacent rhododendron leaves due to gas phase deposition. This work supports the use of rhododendron leaves as biomonitors of atmospheric PCB pollution.

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TABLE OF CONTENTS

Section	Page
Title Page.....	i
Abstract.....	ii
Acknowledgments.....	iv
Table of Contents.....	v
List of Tables.....	vii
List of Figures.....	viii
Chapters	
1) Introduction	1
2) Materials and Methods.....	11
a. Experimental Layout.....	11
b. Materials and Methods.....	15
3) Results and Discussion.....	21
a. Achiral Results and Discussion.....	21
b. Chiral Results and Discussion.....	38
4) Conclusions.....	43
a. Conclusions.....	43
b. Future Work.....	45

Appendices.....	47
1) Appendix A.....	48
a. Materials and Methods.....	48
b. Polyethylene Passive Aqueous Samplers.....	51
c. Sample Extraction and Analyses.....	52
2) Appendix B	55
a. Achiral data for two- and three-month PAS.....	55
3) Appendix C.....	56
a. Total PCB accumulations and homolog distributions for two- and three-month deployments of PAS.....	56
Literature Cited.....	58

LIST OF TABLES

Table		Page
3.1	Mass (ng) of PCB 91 and 95 for each sample media. * Below detection limit on the achiral column for sample used to determine EF values.....	38
A.1	Relative positions of all rhododendron leaf samples and PAS in relation to Town Creek, SC.....	51
A.2	Total PCB concentrations comprising averages reported for all sampled media.....	55

LIST OF FIGURES

Figure	Page
1.1	Approximate location of Pickens, SC.....7
2.1	Location of the experimental site adjacent to Town Creek, Pickens, SC in relation to the former Sangamo Weston plant12
2.2	Relative positions of PAS (S1-S7) and preliminary rhododendron leaf samples (T-I - T-IV).....14
2.3	Relative positions of PAS (S1-S7) and final rhododendron leaf samples (T1-T5).....14
2.4	Inside of a PAS with PUF disk in wire carrier..... 16
2.5	Hydrograph of stream gage height for Twelvemile Creek near Liberty, SC (USGS Gage 02186000). The red star and line beginning at August 15 indicates the approximate height of PAS S1 two feet over the surface of Town Creek at the time of the one-month deployment.....17
3.1	Total PCB concentrations (ng/g lipid) for preliminary rhododendron leaf samples at Town Creek, SC. Concentrations are lipid normalized. Error bars represent standard deviation for n=3.....22
3.2	PCB homolog distributions for preliminary rhododendron leaf samples at Town Creek, SC. Error bars represent standard deviation for n=3.....23
3.3	Total PCBs (ng) for one-month deployment of PUF disks at Town Creek, SC. No error bars are shown because n=1.....25
3.4	PCB homolog distributions for one-month deployment of PUF disks at Town Creek, SC. No error bars are shown because n=1.....26
3.5	Total PCB concentrations (ng/g lipid) for final rhododendron leaf samples at Town Creek, SC. Concentrations are lipid normalized. Error bars represent standard deviation for n=3.....28

LIST OF FIGURES (Continued)

Figure	Page
3.6 PCB homolog distributions for final rhododendron leaf samples at Town Creek, SC. Error bars represent standard deviation for n=3.....	29
3.7 Total PCB concentrations (ng/g PE) for two sets of PE samplers at Town Creek, SC. Concentrations are an average of three replicates for each set, n=3. Error bars represent standard deviations.....	31
3.8 PCB homolog distributions for two sets of PE samplers in Town Creek, SC. Percentage values are an average of three replicates for each set, n=3. Error bars represent standard deviations.....	32
3.9 Enantiomeric fractions (EFs) for PCBs 91 and 95 in two sets of PE samplers deployed in Town Creek, SC. EF values are an average of three replicates. Horizontal lines at 0.5 indicate racemic EFs.....	41
3.10 Enantiomeric fractions (EFs) for PCBs 91 and 95 in final rhododendron leaves sampled from Town Creek, SC. EF values are an average of three replicates. Horizontal lines at 0.5 indicate racemic EFs.....	42
A.1 Fully assembled and installed PAS units (S2 and S3).....	49
A.2 PAS unit (S1) assembled and installed over Town Creek.....	50
C.1 Total PCBs (ng) for two- (S1) and three- (S6, S7) month deployments of PUF disks at Town Creek, SC. Error bars represent standard deviation for n=2.....	56
C.2 PCB homolog distributions for two- (S1) and three- (S6, S7) month deployments of PUF disks at Town Creek, SC. Error bars represent standard deviation for n=2.....	57

CHAPTER 1

Introduction

PCBs overview, history, and key physiochemical properties

Polychlorinated biphenyls (PCBs) are a subset of persistent organic pollutants (POPs) that were commercially produced until 1977 (USEPA, 2003). PCBs initially entered the environment during this manufacturing period and continue to be released through the ongoing use of their applications. A possible 209 congeners of PCBs exist, with one to nine chlorines attached in varying configurations to the biphenyl structure, which can be further categorized in subsets by the degree of chlorination, termed homologs (Henry and DeVito, 2003). Each homolog group consists of isomers with each one being defined by the location of chlorine atom(s) on the double ring structure. Furthermore, nineteen of 78 possible chiral PCB congeners are stable atropisomers at ambient temperatures due to chlorines attached *ortho* to both phenyl rings, restricting rotation (Haglund, 1996). Enantiomers of a chiral compound typically differ in their biological properties, such as rates of microbial degradation, resulting in PCB enantiomeric fractions that differ from racemic (0.5) in environmental systems (Garrison et al., 2000). Primarily atmospheric PCB emissions have been shown to be racemic or near-racemic (Desborough and Harrad, 2011), while chiral signatures in topsoil, water, aquatic life, and other environmental matrices are often non-racemic due to differences in enantioselective degradation through microbial transformation or metabolic activity (Pakdeesusuk et al., 2003).

Generally, PCBs are hydrophobic compounds, owing to their low aqueous solubility, large octanol-water partitioning coefficients, and relatively low vapor pressures (Shiu and Mackay, 1986). As the degree of chlorination increases, the log value of the octanol-water partitioning coefficient also increases, while the aqueous solubility tends to decrease. PCB congeners with fewer chlorine atoms attached (di, tri) tend to have higher rates of volatilization compared to moderately chlorinated (tetra, penta) and heavily chlorinated (hexa, hepta) congeners. Furthermore, lower chlorinated congeners more readily desorb from sediments, making them available for bio-uptake, volatilization, or other means of transformation (Wood et al., 1987). Henry's law constants for PCBs are low, making it challenging to predict and calculate the air-water partitioning constants for these compounds (Fang et al., 2006).

PCB volatilization is also a temperature dependent process, resulting in seasonal changes in atmospheric, biota, soil, and water PCB total concentrations, homolog distribution, and chiral signatures (Motelay-Massei et al., 2005). As a result, given constant emissions, sorption of PCBs to surfaces is likely at lower temperatures, while revolatilization from surfaces occurs at higher temperatures (Wania and Mackay, 1996). Such behavior results in sorption of PCBs to stream sediments and other surfaces, along with the potential for long-range atmospheric transport, contributing to the environmental persistence of PCBs (Shiu and Mackay, 1986).

Terrestrial plant surfaces can serve as an environmental compartment in the transport of semi-volatile organic compounds such as PCBs, acting as a major entry point for

contaminants into terrestrial food webs. Evergreens in particular can serve as biomonitors of this process, as they often retain foliage for several years, allowing for more significant accumulation of contaminants to occur and subsequently be measured (Bacci and Gaggi, 1987). Pine needle samples in the UK have been used to examine distribution of PCBs over urban areas (Tremolada et al., 1996). Rhododendrons can serve as a receptor of volatilized PCBs because they tend to grow in well-drained soils near streams and can keep their leaves for at least four to seven years depending on the subcanopy environment in which they grow (Nilsen, 1986).

PCB uptake via roots and small-scale volatilization from soil (vertical trends)

It has been shown previously that volatilization from soil may impact PCB chiral signatures in grass (Harrad et al., 2006), but it is believed this contribution is limited to only plant surfaces at the soil:air interface, as air sampling at 1.5 meters above the ground did not show a significant contribution from soil PCBs (Robson and Harrad, 2004). It may be possible to develop a height-dependent scale of respective contributions to PCB content from soil volatilization and atmospheric deposition if PCB concentrations are significantly high ($>1 \mu\text{g g}^{-1} \sum\text{PCB}$), although the contribution from soil volatilization may not be discernible at heights above a few centimeters (Harrad et al., 2006).

Desborough and Harrad (2011) measured concentrations and EFs of PCB 95 in air, soil, and grass samples in a two-year study. Samples from 2009 were taken during the warm summer months, while 2010 samples were collected in spring. PUF disk passive

air samplers were deployed at heights of 3, 10, 40, 90, and 130 cm above the soil surface, while grass and soil samples were taken from the same plot (Desborough and Harrad, 2011). A non-racemic EF for PCB 95 in soil samples was observed, while air samples from ≥ 10 cm were found to be racemic. In the 2009 sampling, EFs in air at 3 cm matched that of the soil closely, while grass samples had an intermediate EF between that of the soil and the racemic air samples for PCB 95. In 2010 sampling, EFs from air at 3 cm did not match those of the soil as closely, likely due to cooler temperatures compared to the 2009 sampling. Furthermore, grass samples from 2010 displayed EFs closer to racemic, although still intermediate between the EFs of air at 3 and 10 cm, which led to the conclusion that PCBs in grass arise substantially from deposition of PCBs volatilized from soil (Desborough and Harrad, 2011). Although the volatilization of PCBs from soil appears to contribute significantly to grass PCB content, I believe the intermediate EFs measured in grass reflect a mixed contribution from both atmospheric PCBs and those volatilized from soil, especially during cooler months in which volatilization of PCBs from soil is not as great.

Concentrations of semi-volatile organic contaminants (polychlorinated dibenzo-p-dioxins and dibenzofurans) in grass samples were shown to be primarily from atmospheric deposition, while contributions from root uptake were negligible (Welsch-Pausch et al., 1995). A review by Fries (1995) revealed that atmospheric deposition is the primary means by which plants are contaminated with halogenated hydrocarbons such as PCBs.

Observed PCB chiral signatures (seasonal and horizontal trends)

Harrad et al. (2006) studied PCBs# 95 and 149 chiral signatures in grass, which were compared with previously reported outdoor air and topsoil measurements from the same location in the U.K. West Midlands (Robson and Harrad, 2004). PCBs in outdoor air measured at 1.5 meters were racemic, i.e. from a primary source, while soil and grass samples were not. Their comparison of PCB chiral signatures in outdoor air and topsoil samples revealed that PCBs in topsoil undergo significant enantioselective degradation, while PCBs in the atmosphere were racemic or near-racemic (Robson and Harrad, 2004).

While results from a three month experiment were less clear, a 12 month seasonal experiment showed a clear trend of chiral signatures in grass changing from racemic or near-racemic (at the beginning of growth season) to increasingly non-racemic (during the growing season), and returning to near-racemic (end of winter) (Harrad et al., 2006). From their results, it can be expected that multiple PCB sources (air, soil, water) of differing chiral signatures may result in an intermediate chiral signature in plants, alluding to the relative contribution various PCB sources may have on the overall PCB content in plants.

Atmospheric PCBs have been measured along an urban to rural gradient using PUF disk samplers, showing a clear trend of decreasing PCB concentrations with distance from an urban area (Harner et al., 2004). The spring to summer sampling period had the greatest PCB concentrations when compared with summer-fall and fall-winter sampling periods, all conducted in an area ~75 km from urban Toronto to the surrounding rural

areas (Motelay-Massei et al., 2005). The increased concentration of atmospheric PCBs during spring and early summer months is consistent with higher rates of volatilization as air temperatures increase.

PCBs present in rhododendron leaves, soils, and dissolved phase at Town Creek, Pickens

Several PCB congeners have been found in both the dissolved phase in Town Creek, Pickens, SC, as well as the leaves of *Rhododendron maximum* plants directly adjacent to Town Creek next to Shady Grove Road. The approximate location of Pickens, SC, is shown in Figure 1.1. Preliminary results show higher total PCB concentrations in leaves collected during the fall and winter (4153.4 ± 601.6 and 3722.2 ± 93.3 ng/g lipid, respectively) than in spring (727.4 ± 199.5 ng/g lipid) (Dang, 2012). PCBs were also measured in topsoil samples near the rhododendrons. PCB homolog distributions in Town Creek and rhododendron leaf samples were found to be similar, while that of soil samples were different (Dang, 2012). Homolog distribution in the rhododendron was dominated by tetra and penta PCBs (>90%), with some contribution from di and tri PCBs, which was consistent between seasons (Dang, 2012).

Significant PCB concentrations were measured in two groundwater wells (1800 ng/L and 6535 ng/L average concentrations) near Town Creek using polyethylene (PE) passive samplers (Hahn, 2012). PCBs were also measured in the aqueous phase of the creek using PE samplers (Dang, 2012).



Figure 1.1: Approximate location of Pickens, SC

Use of passive air sampling in PCB gas phase detection and polyethylene passive samplers in PCB dissolved phase detection

It has been shown previously that use of polyurethane foam (PUF) in passive air samplers (PAS) is both a cost and labor effective method of evaluating PCB and other POP concentrations in the atmosphere (Du et al., 2009; Motelay-Massei et al., 2005). PUF PAS have previously been used to investigate both spatial and seasonal trends in atmospheric PCB concentrations along an urban-rural transect in Canada (Motelay-

Massei et al., 2005). Equilibrium with atmospheric concentrations is usually not achieved with passive air sampling, but background concentrations can be estimated by deploying samplers for varying lengths of time (Khairy and Lohmann, 2012). Additionally, PAS can be used for insight into seasonal patterns of POP atmospheric concentrations (Devi et al., 2011).

Polyethylene devices (PEs) have been shown to be effective passive samplers for measuring dissolved PCBs, with concentrations achieved on the PE being in proportion to the true aqueous concentrations (Adams et al., 2007). Increasing the exposure time of passive samplers with the dissolved phase can result in greater amounts of PCBs collected to a certain point, but longer periods of time can result in fouling of the samplers, potentially interfering with the transfer of PCBs to the sampler. PCB levels in a given water body can vary on a daily or even hourly basis due to changes in suspended particulate matter and DOC. Because of this, passive sampling usually gives more consistent results than whole-water samples (Allan et al., 2009).

Hypotheses

Hypothesis 1: The primary source for PCB contamination in rhododendron leaves is via gas-phase deposition. The PCB homolog distribution and chiral signatures of rhododendron leaves and passive air samplers from the same height off the ground and location were expected to correlate strongly. Since it is unlikely that equilibrium would be achieved at the PAS:air and rhododendron:air interfaces, total PCB concentrations in PAS and rhododendron leaves were not expected to match. Volatilization of PCBs from contaminated soils was expected to have an insignificant impact on even the lowest of air samplers and rhododendron leaves, given their height off the ground. However, if this impact was discernible, PCBs in lower rhododendron leaves would likely have intermediate EFs, somewhere between the EFs of upper leaves and that of the soil samples. In the absence of soil contribution, measured PCB content in leaves and air samplers would be indicative of another gas phase source, such as Town Creek.

Hypothesis 2: PCB-contaminated water bodies, such as Town Creek, have a strong contribution to the PCB content of leaves on adjacent rhododendron plants through foliar deposition. Without contribution from PCB-contaminated water bodies, PCB content measured in rhododendrons would be representative of another source, if any. The PCB contribution from Town Creek was expected to be non-racemic in nature based on previous sampling (Dang, 2012). Because of the rural location, a primary (and therefore racemic in nature) atmospheric PCB source was not expected to be significant, but could not be ruled out entirely. Rhododendron plants upstream of the Sangamo-Weston site

would indicate if an additional long-range atmospheric source of PCBs was present. Total PCB concentrations in rhododendron leaves (and therefore air samplers) were expected to decrease with increasing distance away from the surface water. PCBs measured in Town Creek and the closest rhododendron leaves sampled were expected to have similar homolog distributions and chiral signatures. PCBs measured in rhododendron leaves sampled furthest from the creek may have significantly different chiral signatures if another atmospheric PCB source was present, although this was not expected to be significant. PCB enantiomeric fractions measured in rhododendron leaves were expected to trend along this transect between the EFs measured at each end location, representative of the decreasing influence of PCB-contaminated water bodies with increasing distance.

CHAPTER 2

Experimental Layout

I chose the experimental site to be the riparian zone adjacent to Town Creek, located along Shady Grove Road (State Road S 39-32) in Pickens, SC. This site was chosen due to the history of PCB contamination in Town Creek (Dang et al., 2010; Pakdeesusuk et al., 2003) and the presence of rhododendron plants adjacent to the creek. The site location is approximately two kilometers downstream of the former Sangamo Weston Plant, as shown on the map in Figure 2.1, before the confluence of Town Creek with Twelvemile Creek. The GPS coordinates for this location are N34° 52.960', W82° 44.144' (degrees, minutes, and seconds). Initially, I collected rhododendron leaf samples at this site and analyzed them for total PCB concentrations. These data were used to establish the range and gradient for the passive air sampling experiment. Based on these preliminary rhododendron leaf samples, which showed little to no PCBs in leaves at 10 and 20 meters away from Town Creek, passive air sampling was conducted in the riparian zone extending ~10 meters from Town Creek. Preliminary rhododendron leaf sample T-I was from the west shore of Town Creek, while samples T-II, T-III, and T-IV were from the east shore. More rhododendron plants were located on the east shore of the creek, which rose in elevation with increasing distance from the creek (Figure 2.2, Table A.1). The approximate location of preliminary rhododendron leaf samples in relation to the PAS is shown in Figure 2.2.

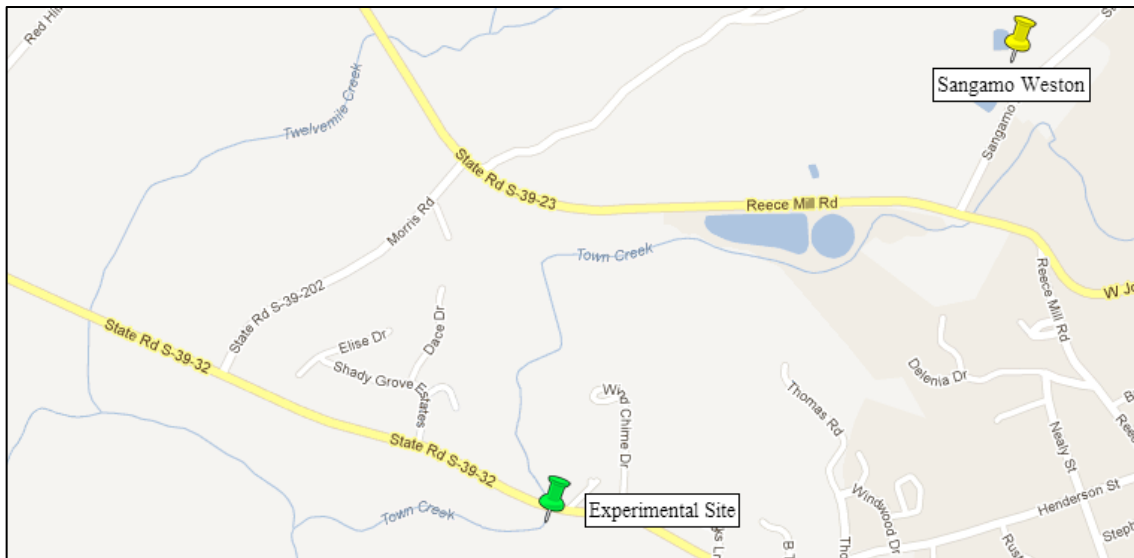


Figure 2.1: Location of the experimental site adjacent to Town Creek, Pickens, SC in relation to the former Sangamo Weston plant.

I deployed a total of seven passive air samplers (PAS) for a period of one month beginning on August 15, 2012, locating them directly adjacent to each rhododendron plant at heights corresponding with the leaves to be sampled (lowest foliage where available and 2 meters), with additional PAS placed directly above the creek and soil surfaces to help test the hypotheses. A PAS directly above the creek would help confirm Town Creek as a source of atmospheric PCBs, while PAS at soil surfaces may show differences in PCBs accumulations compared to PAS at 2 m height. The relative positions of PAS and final rhododendron leaf samples are shown in Figure 2.3. At the same time, I deployed polyethylene devices (PEs) in Town Creek near the rhododendron plants to measure dissolved PCBs. PE devices were deployed as two sets of three devices each. Rhododendron leaf and soil samples were collected in conjunction with the PUF

disks after one month, whereas PE samplers were collected after a two-month exposure period (October 15, 2012). Field blanks of PUF disks and PE were treated in the same manner as experimental samplers in cleaning and transport to and from the field, with the exception of actual deployment. A lab blank PUF disk was also prepared in the same manner. Rhododendron blanks were collected from the South Carolina Botanical Gardens (Clemson, SC) in winter (January, 28, 2013). All samples were wrapped in aluminum foil, transported on ice back to the laboratory, and stored at -4°C until analysis. Lab blanks were stored in the same manner as samples until their analysis. The relative positions of all rhododendron leaf samples and PAS in relation to Town Creek are provided in Table A.1 in Appendix A.

Immediately following the one-month exposures, I deployed a second round of PUF disks at the same locations for a planned exposure period of three months. Due to flooding and loss of the PUF disk, the PUF located directly above the stream (S1 in Figures 2.2 and 2.3) was replaced one month into the exposure, giving the new PUF a two-month exposure. At the time of collection, PUF disks 2, 3, 4, and 5 (S2-S5 in Figures 2.2 and 2.3) of the three month exposure were found to be damaged beyond use due to tree collapse that had damaged the PAS and changed the relative position of those samplers. The PUFs in these samplers were fouled with soil and water due to the damage incurred.

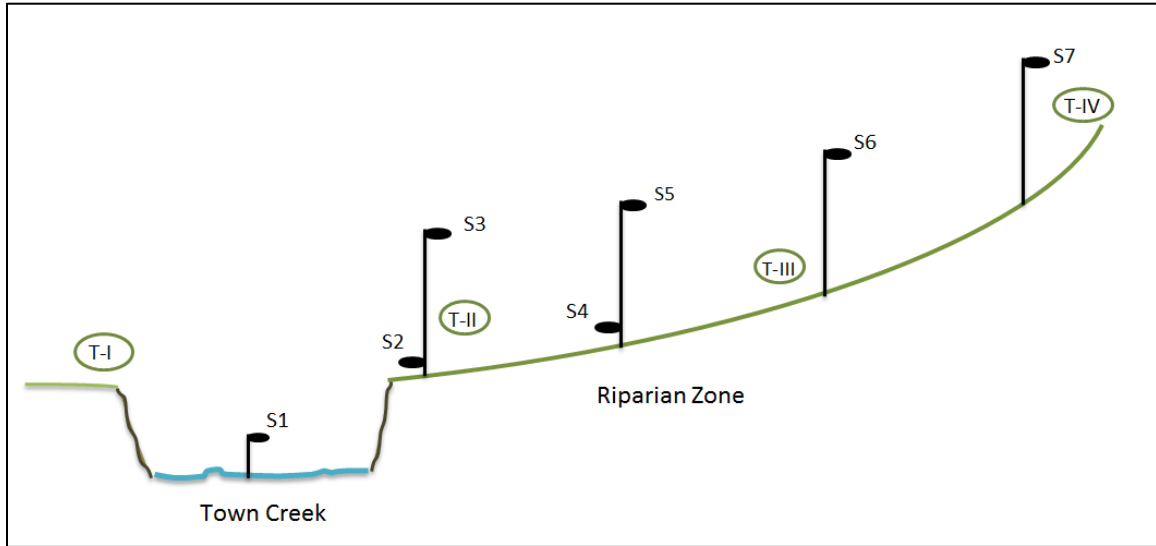


Figure 2.2: Relative positions of PAS (S1-S7) and preliminary rhododendron leaf samples (T-I - T-IV).

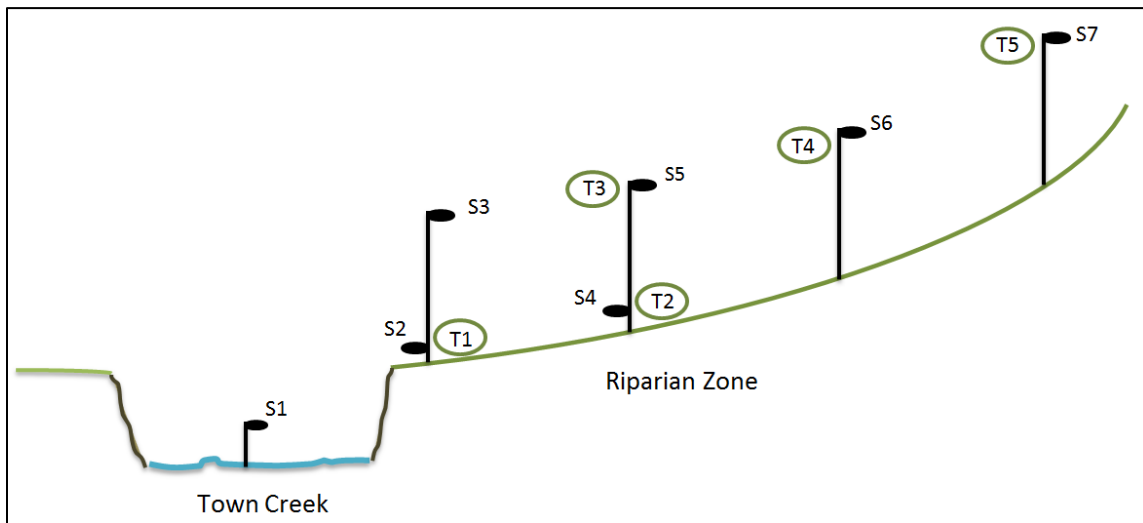


Figure 2.3: Relative positions of PAS (S1-S7) and final rhododendron leaf samples (T1-T5).

Materials and Methods

Rhododendron Leaves

I collected preliminary rhododendron leaf samples in March 2012 from the general vicinity of the study site (within 100 m). Total PCB data from these samples were used to set the scale of the main experimental setup. Leaves were collected compositely from each rhododendron plant, avoiding leaves in the outermost whorl or cohort on any branch. These leaves are the youngest on the plant, and thus have had the least exposure time for potential accumulation of PCBs (Nilsen, 1986). Deciduous leaves, with exposures times of less than 1 year, were found to have no detectable PCBs (Dang, 2012). For this reason, rhododendron leaves less than 1 year old were avoided in the collection of composite leaf samples to maximize the potential for detectable PCBs. Final rhododendron leaf samples were collected in a similar manner from heights corresponding with PAS (lowest foliage available and 2 m).

Passive Air Samplers

I chose passive air sampling using PUF disks as the means of air sampling because of not only their relatively low cost and ease of use, but also their previous use in investigating PCB spatial concentration trends (Motelay-Massei et al., 2005). I cleaned PUF disks with a Dionex 200 Accelerated Solvent Extractor using a 1:1 (v/v) hexane:acetone solvent mixture and then allowed them to air dry in a fume hood to remove excess solvent (USEPA, 2005; Reddy et al., 2011). Cleaned PUF disks were wrapped in hexane-rinsed aluminum foil and stored at -4°C until deployment. All PUF

disks were handled using solvent-rinsed tongs and gloved hands to avoid contact with skin. The inside of the PAS with a PUF disk is shown in Figure 2.4.



Figure 2.4: Inside of a PAS with PUF disk in wire carrier.

Polyethylene Passive Aqueous Samplers

Dang (2012) previously used low-density polyethylene membrane as a passive sampling medium to measure aqueous PCB concentrations in a stretch of Town Creek near the former Sangamo Weston plant site. PE sampling devices are described in full in Appendix A. PE samplers were deployed from the same metal post in the stream bed that was used to hold the first PAS (S1 in Figure 2.2). A deployment period of 60 days was

chosen based on previous research maximizing PE exposure while minimizing biofouling (Dang, 2012). Biofouling would physically cover the surface of the PE, which would likely alter the partitioning of PCBs from the water column into the sampler. Careful monitoring of current stream levels through the United States Geological Survey (USGS Gage 02186000) online national water information system was done to ensure the PE remained below the stream surface during the two-month exposure period, as shown in Figure 2.5. At the time of deployment, the PAS above Town Creek (S1) was positioned approximately two feet above the surface of the creek.

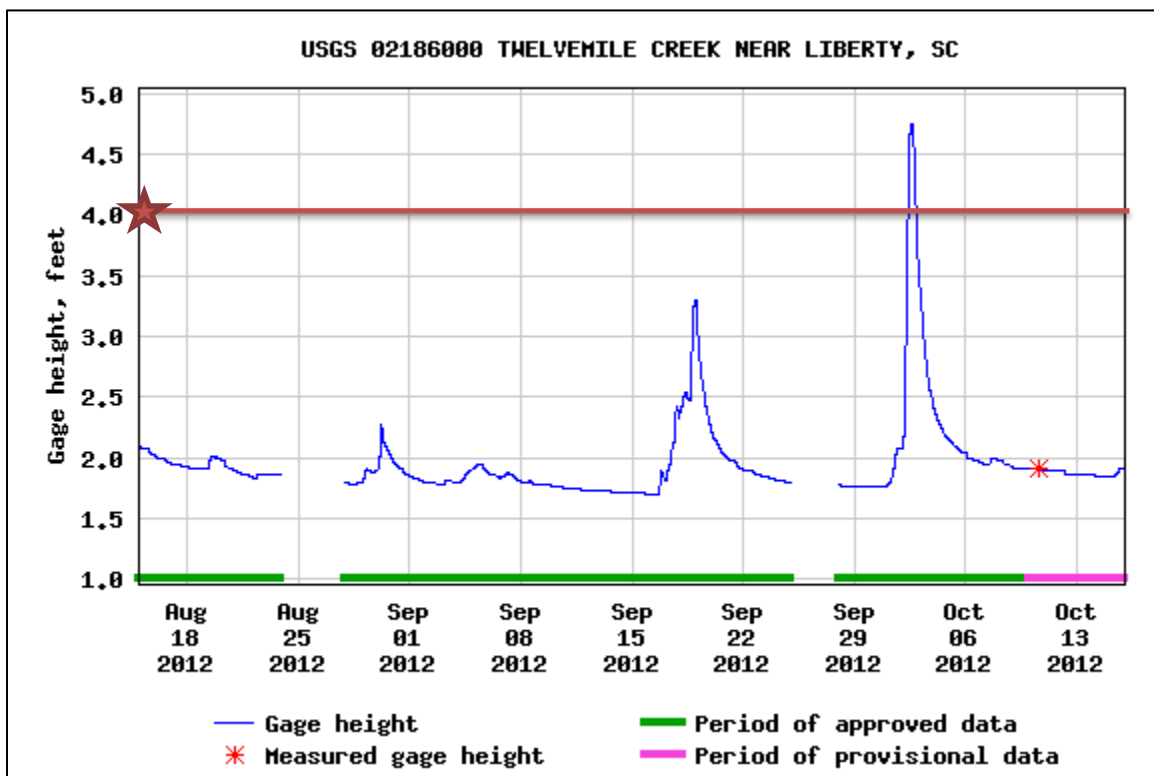


Figure 2.5: Hydrograph of stream gage height for Twelvemile Creek near Liberty, SC (USGS Gage 02186000). The red star and line beginning at August 15 indicates the approximate height of PAS S1 two feet over the surface of Town Creek at the time of the one-month deployment.

Sample Extraction & Analyses

Rhododendron leaf samples were air dried in the fume hood for 48 hours prior to extraction. Leaves were chopped into small pieces using a blender and divided into ~4 gram replicates. Samples were spiked with 200 ng surrogate standards (PCBs 14 and 169) prior to extraction to determine experimental method recovery, the efficiency of transfers and losses during extraction and cleanup. Extraction of PCBs from all solid samples, including rhododendron leaves and PUF disks, was accomplished with a Dionex 200 Accelerated Solvent Extractor (ASE) using 1:1(v/v) hexane: acetone solvent (USEPA, 2005). Generally, the blender and ASE sample vials were cleaned with soap and water, followed by a solvent rinse to reduce potential cross contamination.

PE samples used the same 200 ng surrogate standard spike, but were extracted by dichloromethane (DCM) dialysis. This was done by first soaking samples in ~20 mL DCM on a shaker table for several hours before covering and soaking them overnight at 4°C. The solvent layer was then removed and saved, rinsing the PE and vial with DCM at least three times to assist analyte transfer. The 24-hour dialysis process was then repeated for each sample, and extracts were combined at the end.

I processed samples through a baked sodium sulfate drying column to remove excess solvent and water. Gravimetric analysis was used to determine lipid content for leaf samples (USEPA, 2008). Samples were then cleaned on an alumina (Level III – 6% deactivated) column. PE sample extracts only required a drying column. Rhododendron leaf samples required an additional sulfuric acid cleanup step to remove baseline

interferences observed in gas chromatography analyses. PUF samples from longer exposures required an acid cleanup step as well. Following all necessary cleanup steps, I performed a solvent exchange to replace the extract solvent (hexane or DCM) with isooctane (2,2,4-trimethylpentane).

I analyzed samples with gas chromatography, using an achiral column to determine total PCB content and homolog distribution, and a chiral column for enantiomeric fractions of individual PCBs. Achiral PCB analysis was performed on a HP 6890-GC equipped with a RTX-5 column (Restek, Bellefonte, PA; 60 m length, 0.25 mm diameter, and 0.25 μm film thickness) and a ^{63}Ni electron capture detector (ECD). Achiral analysis methods are described in full in Appendix A and are adapted from previous work (Dang, 2012). Briefly, the GC conditions began with an initial oven temperature of 115°C for 2 min. The temperature then ramped to 185°C at a rate of 8°C/min, which was held for 8 min. The oven then ramped to a final temperature of 260°C at a rate of 2°C/min, which was held for 20 min, yielding a total run time of 78.75 min. This method accounts for 140 target PCB congeners, quantified as 92 domains as a result of co-elution of some congeners on the column. I constructed a calibration standard curve using six concentration levels of a 1:1:1 (w/w/w) mixture of Aroclors 1016, 1254, and 1260, with two injections of each level. Two internal standards (Aldrin and PCB 204) were used to quantify PCB concentrations.

Chiral PCB analysis was performed on an Agilent 6850 GC-ECD and a 30 m Chirasil-Dex column (0.25 mm diameter and 0.25 μm film thicknesses). Chiral analysis methods

are describe in full in Appendix A and are adapted from previous work (Dang, 2012). Briefly, the GC conditions began with an initial oven temperature of 105°C for 2 min. The temperature then ramped to 135°C at a rate of 10°C/min, which was held for 5 min. The temperature then ramped to 165 °C at a rate of 1.2 °C/min, which was held for 10 min. The oven then ramped to a final temperature of 185 °C at a rate of 5 °C/min and was held for 20 minutes, yielding a total run time of 69.0 min. Enantiomeric fractions (EFs) and column performance were checked by including a neat standard for each selected chiral congener in each batch, for which the EF was then calculated. The EFs of standards for all chiral congeners selected were racemic (0.5) within 5% relative standard deviation.

CHAPTER 3

Achiral Results and Discussion

Total PCB Concentrations & Homolog Distributions

Appendix B contains details of the total PCB concentrations measured in all sampled media. Preliminary rhododendron leaves in the general vicinity of the main study site (within 100 m) had total PCB concentrations ranging from 198.2 ± 53.3 to 426.8 ± 125.1 ng/g lipid, as shown in Figure 3.1. No clear trend of decreasing total PCBs with increasing distance was observed, although the two leaf samples closest to the creek, T-I (opposite shore) and T-II, had higher average concentrations than those further away, T-III and T-IV. Leaf samples showed a strong preference for the accumulation of tetra- and penta-chlorinated congeners (Figure 3.2). There was high variation in total PCBs and homolog distributions measured in replicates of leaf samples, most likely due extraction inefficiencies. With preliminary leaf samples, a blender was not used prior to extraction with ASE, so the sizes of the leaf pieces varied between replicates and overall extraction efficiencies were low. It is likely that because of the variation in leaf size, concentration and homolog data presented here have significant error associated with them and were only used in experimental design. The PAS were established on the same shore as T-II through T-IV due to the presence of multiple rhododendron plants. T-I on the opposite shore had higher PCB concentrations measured, but rhododendron plants on this shore were limited in number. These data show that, generally, rhododendron leaves in the riparian zone directly adjacent to Town Creek accumulate PCBs.

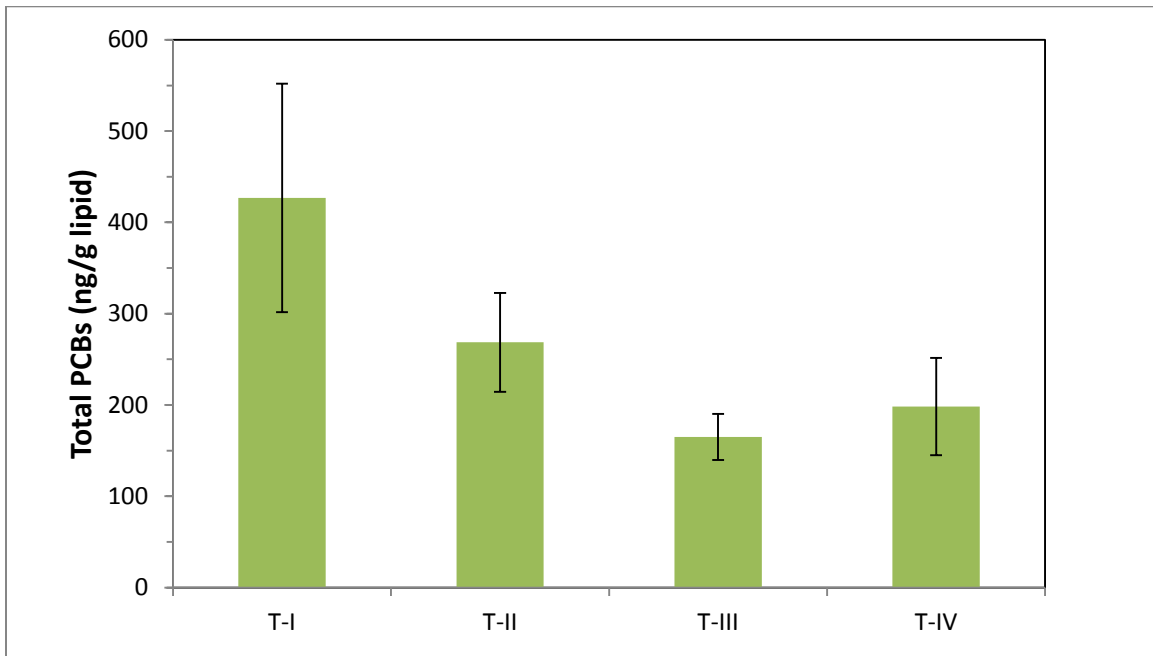


Figure 3.1: Total PCB concentrations (ng/g lipid) for preliminary rhododendron leaf samples at Town Creek, SC. Concentrations are lipid normalized. Error bars represent standard deviation for n=3.

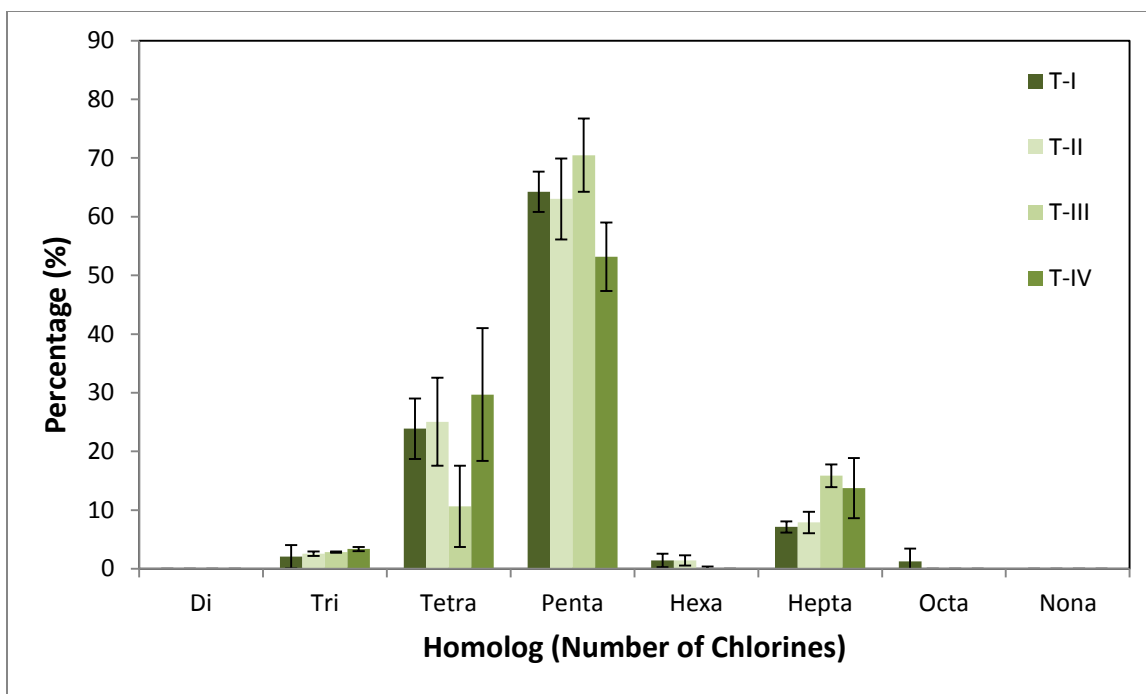


Figure 3.2: PCB homolog distributions for preliminary rhododendron leaf samples at Town Creek, SC. Error bars represent standard deviation for n=3 .

The one-month exposure of PUF disks yielded substantial accumulation of PCBs, with total PCB concentrations generally decreasing with increasing distance from the creek (Figure 3.3). PCB accumulation in PUFs was dominated by tri-, tetra-, and penta-chlorinated congeners (Figure 3.4). Little to no octa- and nona-chlorinated congeners were detected in passive air sampling, consistent with the idea that heavier chlorinated congeners are less likely to desorb from sediments (Wood et al., 1987). Maximum total PCB accumulations were measured in S1 (2077.7 ng), while minimum accumulations were measured in S5 (846.6 ng). The detection of more di-chlorinated congeners in S1 and S2 (19.1 and 16.8 % di-chlorinated congeners, respectively), the two PAS closest to Town Creek, is notable. The four PUFs farthest from the creek (S4-S7) had similar

accumulations of PCBs in both total mass and homolog distributions. Similar concentrations were measured for PUF disks located at different heights but on the same post (i.e. S2 and S3, S4 and S5). These data support the idea that any volatilization of PCBs from contaminated soil only contributes to PCB concentrations in plant surfaces at the soil:air interface. Previous work by Desborough and Harrad (2011) has shown that volatilization of PCBs from soil is not discernible in PAS at heights 10 cm and greater above contaminated soils.

Recoveries of surrogate standards for extraction from PUFs averaged 108.2% ($\pm 7.4\%$ SD) and 78.7% ($\pm 4.1\%$ SD) for PCBs 14 and 169, respectively. No corrections for recovery were made.

Recovery values were much lower for PUFs from the two- and three-month exposures due to the additional acid cleanup step required. Recovery of surrogate standards for S1 (two-month exposure) averaged 58.4% ($\pm 8.8\%$ SD) and 29.4% ($\pm 4.2\%$ SD) for PCBs 14 and 169, respectively. Recoveries for S6 and S7 (three month exposure) were even lower ($<25\%$). Total PCB concentrations of 19.9 and 0.4 ng/g PUF were measured for field and lab PUF blanks, respectively, although recoveries were too low ($<25\%$), thus these values have significant error associated with them. No corrections for field and lab blank concentrations in PUFs were made. Sulfuric acid cleanup was performed on these samples after a solvent exchange to isooctane had already occurred. DCM was used as the carrier solvent and solvent exchange back to isooctane was performed after acid cleanup. Recovery values for these PUF exposures are too low to merit inclusion of data

for total PCBs and homolog distributions. Total PCB accumulations and homolog distributions for two- and three-month exposures of PAS are provided in Appendix C, Figures C.1 and C.2.

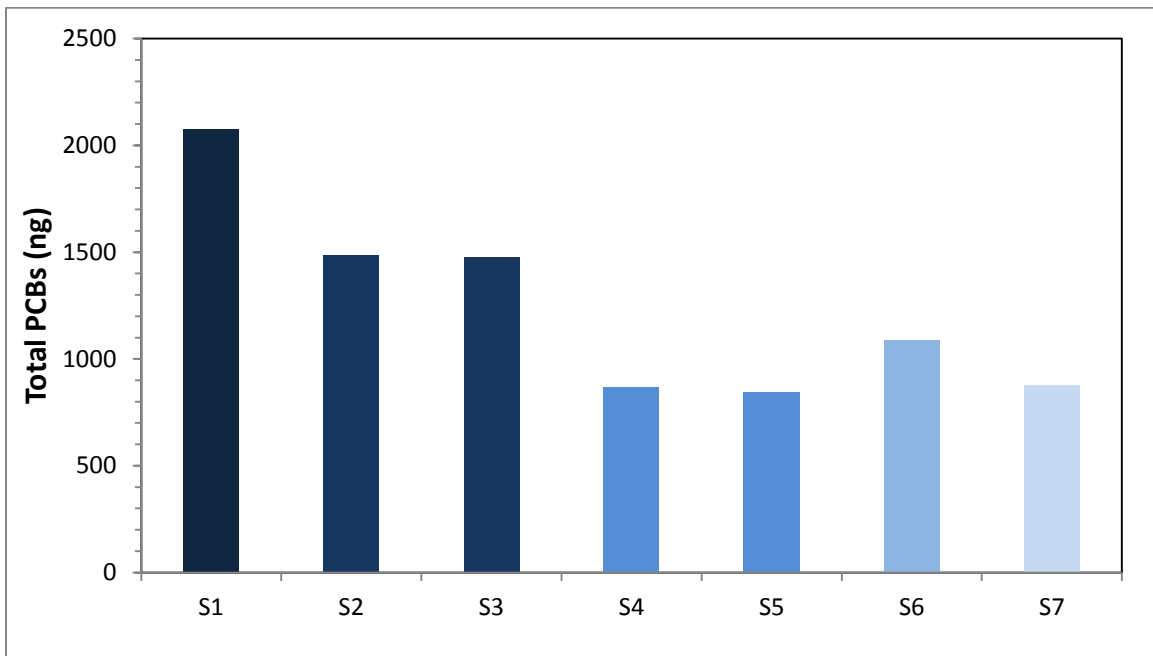


Figure 3.3: Total PCBs (ng) for one-month deployment of PUF disks at Town Creek, SC. No error bars are shown because n=1.

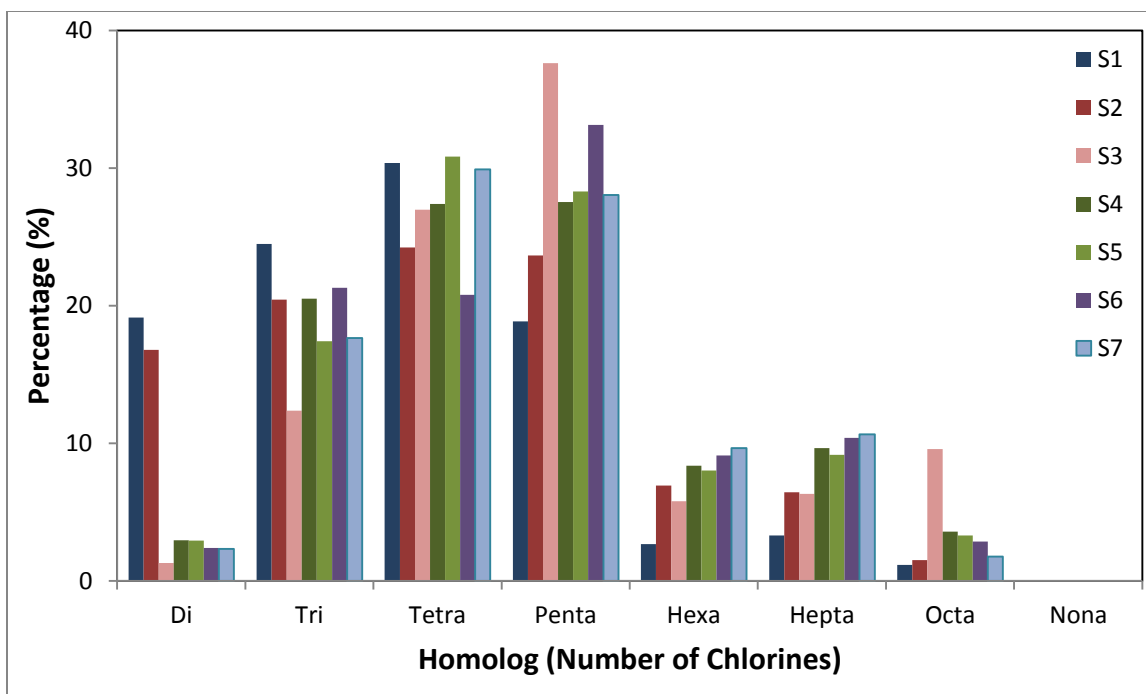


Figure 3.4: PCB homolog distributions for one-month deployment of PUF disks at Town Creek, SC. No error bars are shown because n=1.

Total PCB concentrations in the final rhododendron leaf samples were measured at a maximum in samples closest to the creek and decreased rapidly with increasing distance, as shown in Figure 3.5. Concentrations at T1 measured 6670.8 ± 1400.9 ng/g lipid, decreasing to 1713.3 ± 423.0 ng/g lipid at T2, just 3 m further away from the creek than T1 samples. Leaf samples at T5, a direct distance of about 15 m from the creek, contained 1052.2 ± 301.8 ng/g lipid. Total PCBs measured in the final leaf samples are much higher than in the preliminary leaf samples, due in part to the low extraction efficiency previously discussed for preliminary leaf samples. Rhododendron leaves in the riparian zone at Town Creek preferentially accumulate mid-weight PCB congeners, with tetra- and penta-chlorinated congeners comprising almost 90% of total PCBs (by

mass) in all samples (Figure 3.6). The accumulation of di-chlorinated congeners in T1, closest to the creek, is also notable. Final leaf samples T2-T5 had similar accumulations of PCBs in both total mass and homolog distributions. Total PCBs measured in rhododendron leaves from the Botanical Gardens, which were collected as references for background concentrations, were 18.5 ± 5.0 ng/g lipid, which is substantially lower than average concentrations in rhododendron leaves from the Town Creek study site. Recoveries of surrogate standards averaged 38.0% ($\pm 10.3\%$ SD) and 45.9% ($\pm 14.1\%$ SD) for PCBs 14 and 169, respectively, for rhododendron leaf samples using the extraction and cleanup methods described previously. Recovery values were reduced for select samples that required additional acid cleanup steps. No corrections were made for recovery values and blank concentrations.

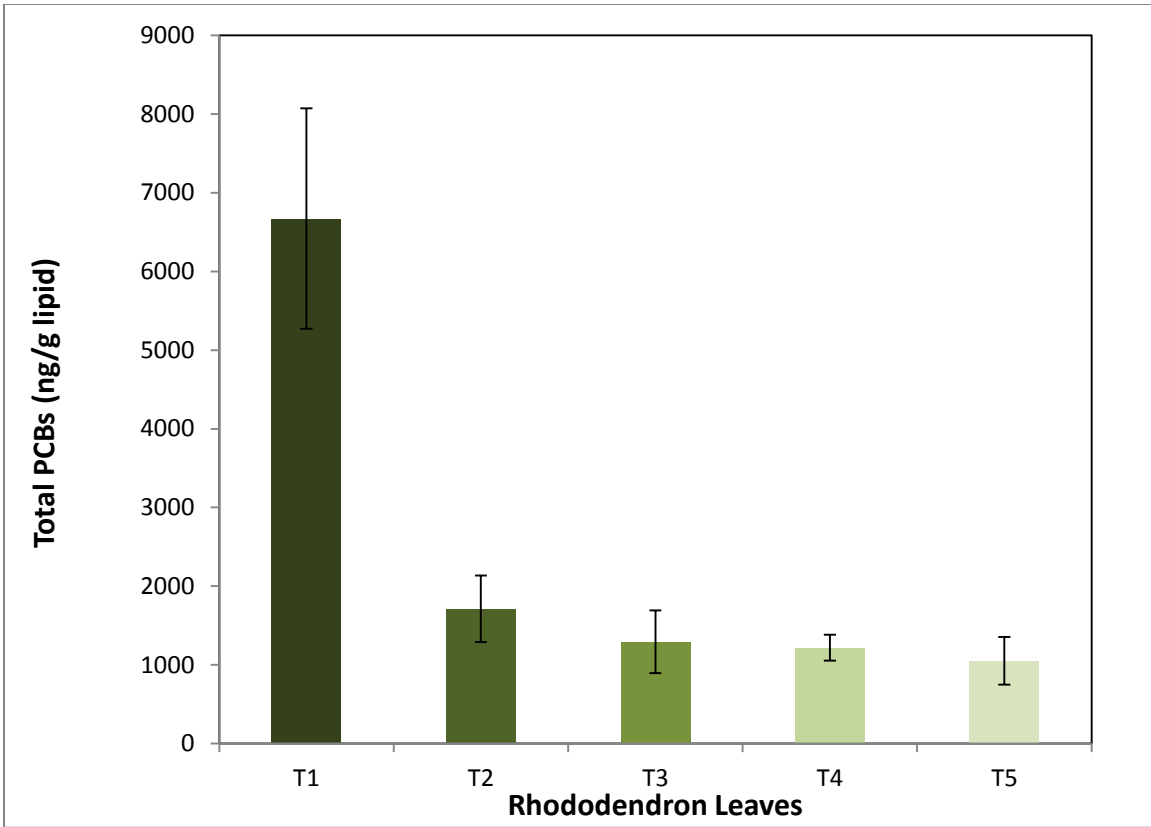


Figure 3.5: Total PCB concentrations (ng/g lipid) for final rhododendron leaf samples at Town Creek, SC. Concentrations are lipid normalized. Error bars represent standard deviation for n=3.

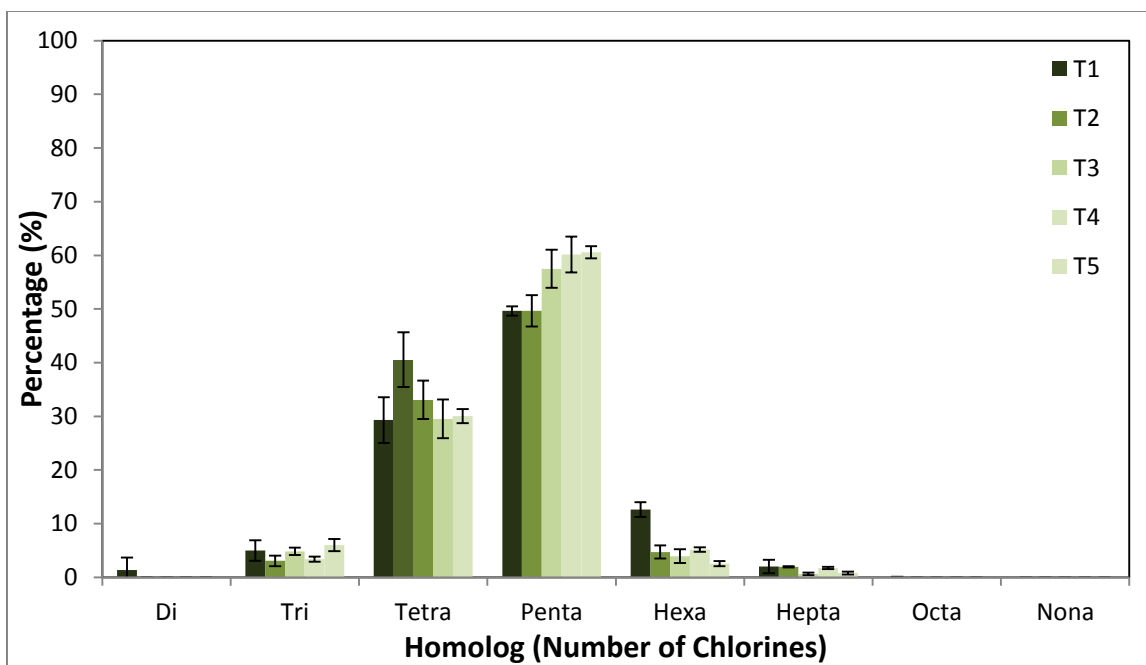


Figure 3.6: PCB homolog distributions for final rhododendron leaf samples at Town Creek, SC. Error bars represent standard deviation for n=3.

Generally, PE samplers from the study site had a strong preference for tetra-, penta-, and hexa-chlorinated congeners, with total PCB concentrations of 20289.5 ± 432.4 and 17975.7 ± 412.0 ng/g PE. Total PCB concentrations for PE samplers are shown in Figure 3.7, and homolog distributions are shown in Figure 3.8. Previous work by Dang (2012) measured maximum concentrations in PE just upstream (3568.6 ± 139.1 ng/g PE) and downstream (3547.6 ± 476.0 ng/g PE) of the study site during a similar 60 day sampling during December 2010 to February 2011. PE samplers from Dang (2012) show tetra-chlorinated congeners as the most dominant, followed by tri- and penta-chlorinated congeners. PE samplers from my study show hexa-chlorinated congeners as the most dominant, followed by tetra- and penta-chlorinated congeners. PE lab blanks averaged

total PCB concentrations of 115.7 ± 1.4 ng/g PE, for which no correction was made. Recoveries of surrogate standards averaged 50.04% ($\pm 4.33\%$ SD) and 91.37% ($\pm 0.01\%$ SD) for PCBs 14 and 169, respectively, for extraction from PE using the dialysis method described previously. When a working solution was prepared directly from the recovery standard used during the dialysis extraction (surrogate for 100% recovery), similar recovery values were obtained. This suggests loss of PCB 14 in the recovery standard solution used, as recoveries of $80.57 \pm 8.60\%$ and $87.02\% \pm 18.31\%$ for PCBs 14 and 169, respectively, were previously reported for the same PE dialysis extraction method used here (Dang, 2012). Average total PCBs reported were not corrected for recoveries.

Previous research by Dang (2012) covered an approximate 2.2 km range in Town Creek, SC, using PE sampling devices. The research by Dang (2012), which covered a range from just upstream of the original Sangamo-Weston effluent ditch and extended to just downstream of the bridge at Shady Grove Road, measured a fivefold increase in total PCBs. The high PCB concentrations measured in Town Creek and the food web at downstream locations suggest a possible ongoing source of PCBs to the creek (Walters et al., 2008).

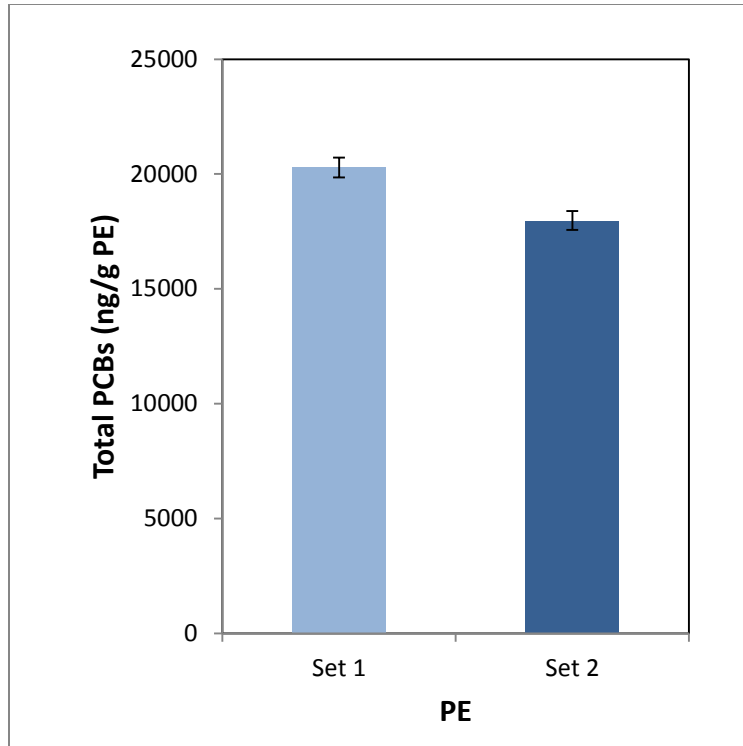


Figure 3.7: Total PCB concentrations (ng/g PE) for two sets of PE samplers at Town Creek, SC. Concentrations are an average of three replicates for each set, n=3. Error bars represent standard deviations.

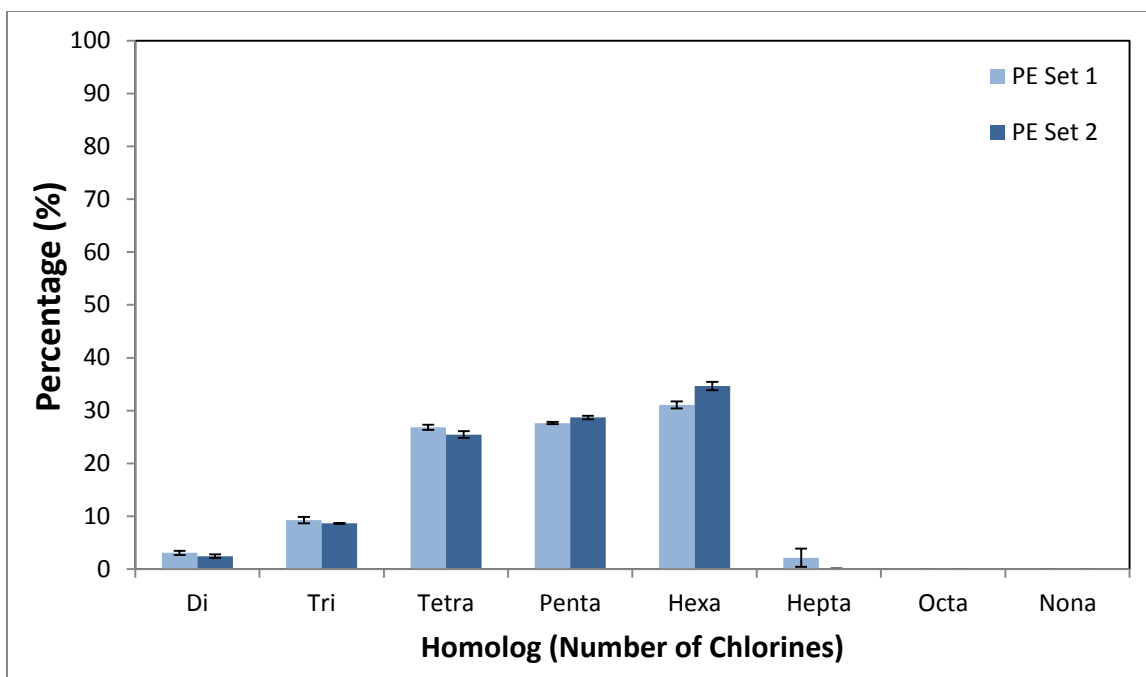


Figure 3.8: PCB homolog distributions for two sets of PE samplers in Town Creek, SC. Percentage values are an average of three replicates for each set, n=3. Error bars represent standard deviations.

PE samples from Town Creek, SC, had higher concentrations of total PCBs than either the rhododendron leaves or PAS. These results are logical, as higher concentrations in leaves or PUF disks when compared with PE samples would indicate rapid volatilization of PCBs from the creek or possibly another atmospheric source of PCBs contributing to accumulation in those samples. The results support the hypothesis that the creek is the main source of PCBs. However, total PCB concentrations among the three media are not directly comparable due to differences in normalization of concentration data.

Total PCBs measured in PE samplers confirm the presence of significant aqueous concentrations of PCBs in Town Creek, SC. Rhododendron leaves from the Botanical

Gardens in Clemson, SC had much lower PCBs, indicating that an additional long-range atmospheric source of PCBs is unlikely. Even if additional long-range sources of atmospheric PCBs do exist, it is likely that their impact on PCB accumulations in PAS and rhododendron leaves at Town Creek is indiscernible. The long-range atmospheric transport of PCBs from urban sources can result in detectable atmospheric concentrations of PCBs (1.66 to 6.50 pg m^{-3}) in more pristine regions such as Antarctica (Li et al., 2012). The PAS deployed directly over Town Creek accumulated the most total PCBs, showing that substantial PCBs are volatilizing from the Creek. PCB concentrations measured in PAS further away from the creek were lower by comparison, likely due to atmospheric mixing and dilution. PCBs were detected in all rhododendron leaves and passive air samplers at the same study site, indicating that PCB accumulation in rhododendron leaves can be attributed to gas-phase deposition, rather than uptake from the roots.

Volatilization of PCBs from contaminated soils primarily contributes to PCB contamination in plant surfaces only at the soil:air interface, with the exception of the most heavily contaminated soils over which an attenuation of concentrations with increased height may be observed (Lehmler et al., 2010). Moreover, Krauss et al. (2004) measured similar atmospheric PCB concentrations at three different heights using PUF samplers over soils contaminated with low levels of PCBs. However, PUF samplers over heavily contaminated soils (1.1-160 μg total PCBs g^{-1}) in the same study showed a measurable influence due to volatilization of PCBs from soils, observed as a decrease in concentrations as one moved upwards. Soil samples from Town Creek have less than 1 $\mu\text{g/g}$ (471.9 ± 87.6 ng/g, n=2) (personal communication, Dang, 2013).

Rhododendron leaves sampled in the riparian zone showed a similar trend in decreasing PCB concentrations with increasing distance from the creek. Similar to the PAS, rhododendron leaves sampled from closest to Town Creek had the highest total PCB concentrations. Rhododendron leaves further away from the creek had much lower concentrations of PCBs, although no clear trend of decreasing concentrations with increasing distance from the creek was observed in leaf samples T2 to T5. However, the highest total PCB concentrations in both PUF disks and rhododendron leaf samples were measured closest to Town Creek, indicative of the decreasing influence of PCB-contaminated water bodies with increasing distance.

Previous studies have investigated spatial trends in PCB concentrations using PUF disk passive air samplers. Harner et al. (2004) measured a gradient of decreasing atmospheric PCB concentrations (~5 –10 times) along a 75 km transect from downtown Toronto, Canada, to a nearby rural area, demonstrating urban areas as a source of atmospheric PCBs. Similar trends of decreasing contaminant concentrations were measured in surface films (windows, vegetation) along the same urban-rural transect (Gingrich and Diamond, 2001). Jamshidi et al. (2007) measured similar urban “pulses” in atmospheric PCB concentrations with PUF disks along an urban-rural transect in the West Midlands of the United Kingdom (UK). Concentrations of PCBs measured in films declined more rapidly for heavier congeners, indicating favored atmospheric deposition of these heavier congeners, whereas lighter congeners are more likely to (re-)volatilize and be transported further from the source. This observation is consistent with that of a passive air sampling study in the Philadelphia metropolitan area in which PUF canisters

were used to measure atmospheric PCB concentrations (Du et al., 2009). However, trends in these studies were seen over much larger distances (>5 km) when compared to my study (<20 m).

Li et al. (2012) used PUF passive air samplers to measure atmospheric PCB concentrations in King George Island, Antarctica. Here, homolog profiles for PCBs accumulated were dominated by di-, tri-, and tetra-chlorinated congeners. At Town Creek, homolog profiles for PUF disks were dominated by tri-, tetra-, and penta-chlorinated congeners. However, Antarctica represents a nearly pristine environment, and PCB concentrations and homolog profiles measured are the result of long-range atmospheric transport (Gambaro et al., 2005). The gradient of <20 m used at Town Creek is too small to see differences in homolog distribution due to the long-range atmospheric transport processes discussed here, with the exception of the di-chlorinated congeners that were only detected in T1, S1, and S2.

In the UK, pine needles have been sampled for PCBs, supporting the concept of evergreen foliage as possible biomonitors of atmospheric contamination (Tremolada et al., 1996). Similar to the studies using PUF samplers, PCB concentrations measured in UK pine needles correlated positively with population densities. However, total PCB concentrations measured in pine needles were considerably smaller than the PUF samplers and ranged only between 0.5 and 4.0 ng/g d.w. Tremolada et al. (1996) chose to use dry weight for the normalization to compare to other studies. Also, pine needle samples in the UK were taken from more rural areas, away from urban and industrial

centers. Regardless, total PCBs measured in rhododendron leaves in my study are orders of magnitude higher when normalized to dry weight (~27 –209 ng/g d.w.), which is a result of both different atmospheric PCB concentrations and the properties of each foliage type related to accumulation of PCBs. Bacci and Gaggi (1987) investigated accumulation and volatilization kinetics from azalea (of the genus *Rhododendron*) leaves in controlled greenhouse studies, with implications for using the plant as a biomonitor of atmospheric contamination. The authors suggested similar studies for other plant species such as pine needles, lichens, and mosses, noting that concentrations of accumulated contaminants vary between foliage types.

Of particular note are the different homolog distributions between PUF disks and rhododendron leaves. PUF disks had a greater accumulation of di- and tri-chlorinated congeners than did the leaves. A possibility for the difference may be due to differences in time of exposure at the PAS:air and rhododendron:air interfaces. PAS often do not achieve equilibrium with atmospheric concentrations, particularly over a relatively short exposure of one month (Khairy and Lohmann, 2012). In contrast, rhododendron leaves often live for four or more years (Nilsen, 1986), allowing substantial time to accumulate PCBs in the riparian zone. However, it should be noted that no specific measurements, such as depuration compounds or time-integrated studies, were used to determine rates of PCB accumulation or loss from any given media. Even though rhododendron leaves may remain on the plant for several years, factors such seasonal water and air temperature changes, fluctuations in atmospheric PCB concentrations, and other phenomena would prevent the leaves from reaching a true equilibrium state. Rather, partitioning

considerations here can be thought of as differences in time of exposure to the atmosphere for a given media. PCBs, particularly the lighter-chlorinated congeners, are more likely to revolatilize from surfaces at higher temperatures (Wania and Mackay, 1996), which likely contributes to the differences in homolog distributions seen between PAS and rhododendron leaves. The greater accumulation of di-chlorinated congeners in PAS compared to rhododendron leaves can be attributed to their shorter exposure time. It is possible that lighter congeners did not have enough time to revolatilize from the PAS, or that higher chlorinated congeners did not have time to accumulate in the PAS. The general lack of di-chlorinated congeners in rhododendron leaves is consistent with the idea that lighter congeners are more likely to revolatilize and be transported further from the source (Jamshidi et al., 2007; Du et al., 2009, Gambaro et al., 2005). Despite difference in exposure time at the sample:air interfaces, the greater accumulation of di-chlorinated PCBs in PAS S1 and S2 corresponds with the detection of di-chlorinated congeners in the final leaf sample closest to the creek, T1. This is consistent with the lack of detectable di-chlorinated PCBs in leaf samples T2-T5, and decreased accumulation of di-chlorinated PCBs in corresponding PAS S3-S7.

Chiral Results and Discussion

PCBs 91 & 95

PCBs 91 (2,2',3,4',6-pentachlorinated biphenyl) and 95 (2,2',3,5',6-pentachlorinated biphenyl) were the only two chiral congeners detected throughout all sampled media: PE, PUF disks, and rhododendron leaves. In aqueous PE samples, PCB 95 was racemic or near-racemic, while PCB 91 was non-racemic (>0.5) (Figure 3.9). These results are consistent with those of Dang (2012), indicating possible biodegradation of PCB 91 in Town Creek, but not PCB 95. Table 3.1 shows the mass of PCBs 91 and 95 for each sample media for which EF values were measured. PCB 91 was always found in lower concentrations than PCB 95, and was not detectable in all sample replicates.

Table 3.1: Mass (ng) of PCB 91 and 95 for each sample media. *Below detection limit on the achiral column for sample used to determine EF values.

<u>Sample Media</u>	<u>PCB 91 (ng)</u>	<u>PCB 95 (ng)</u>
PE B.	7.5±0.4	76.6±0.8
PE B.	5.9±0.3	62.2±1.3
R. T1	3.5±3.1	66.4±11.4
R. T2	0.7±1.2	19.8±4.4
R. T3	nd*	21.8±3.0
R. T4	1.0±1.8	16.8±2.0
R. T5	nd*	13.6±2.1

Biodegradation of PCBs involves either selective dechlorination (anaerobically) or ring cleavage (aerobically) of a congener. However, several different enzymatic processes may biodegrade PCBs in both aerobic and anaerobic environments (Lehmler et al., 2010). Aerobic oxidative processes degrade more lightly chlorinated congeners into the corresponding chlorobenzoic acids through the 2,3-dioxygenase pathway (Abramowicz, 1995). PCB 91 is more likely to undergo biodegradation due to available (no chlorine attached) adjacent *meta* and *ortho* positions on the phenyl rings, whereas PCB 95 has no available *meta* and *ortho* positions because of its chlorine substitution pattern (Dang, 2012). Furthermore, previous work by Dang et al. (2010) measured racemic or near-racemic EF values for PCB 95 for aqueous semi-permeable membrane devices in Twelvemile Creek, SC.

In rhododendron leaf samples from the riparian zone at Town Creek, both PCB 95 and PCB 91 were non-racemic (>0.5), as shown in Figure 3.10. Compared to PE samples, this shows a slight departure of PCB 95 from being racemic or near-racemic (<0.5) in aqueous samples to being non-racemic in leaf samples. A difference in EF values for PCB 95 between the creek and leaf samples may indicate preferential transport and/or accumulation of one enantiomer over the other, although this is not likely since PCB enantiomers for a given congener share identical physical and chemical properties associated with transport (mass, number and configuration of attached chlorines, air-water partitioning coefficients, etc.), with the exception of the rotation of polarized light (Lehmler et al., 2010).

A secondary source of PCBs (i.e. volatilization from the soil) containing PCB 95 of a higher EF value may result in an intermediate EF value in the leaves. If soil EF values for PCB 95 were non-racemic (>0.5), volatilization from the soil could contribute to the preferential accumulation of one enantiomer in the leaves. Desborough and Harrad (2011) has shown that volatilization of PCBs from soils may not be discernible at heights above a few centimeters (≥ 10 cm) when comparing EF values for soil and PUF samples. EF values for PUFs at heights ≥ 10 cm were racemic in nature, while EF values for soil samples and the PUF at a height of 3 cm were non-racemic. In my study, PUF disks located at different heights on the same post had similar overall accumulations of total PCBs, indicating that any volatilization from soil is indiscernible at even the lowest heights (>10 cm) sampled.

In contrast to PCB 95, EF values in both PE and rhododendron leaf samples for PCB 91 were similarly non-racemic (>0.5) (Figures 3.9 and 3.10). This does not suggest an impact on the PCB content of rhododendron leaves due to volatilization of PCBs from the soil, at least not that of a differing chiral signature. PUF disk samples were not quantifiable for EF values of PCB 91 and 95. Those further from the creek (S2-S7) did not contain sufficient quantities of either target congener, while PUF samples S1 (both one- and two-month exposures) had interference in elution of the target congeners on the chiral column.

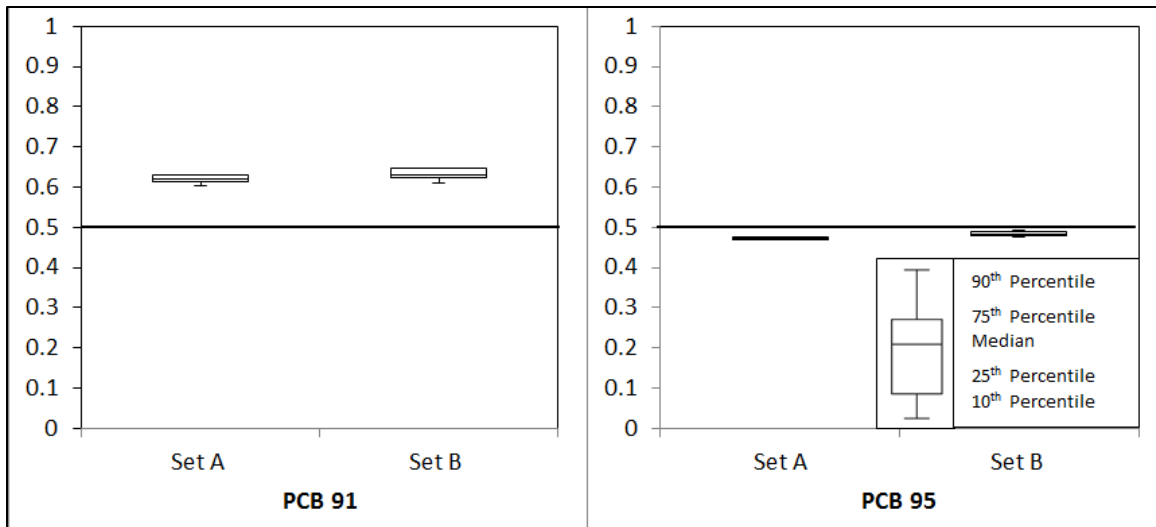


Figure 3.9: Enantiomeric fractions (EFs) for PCBs 91 and 95 in two sets of PE samplers deployed in Town Creek, SC. EF values are an average of three replicates. Horizontal lines at 0.5 indicate racemic EFs .

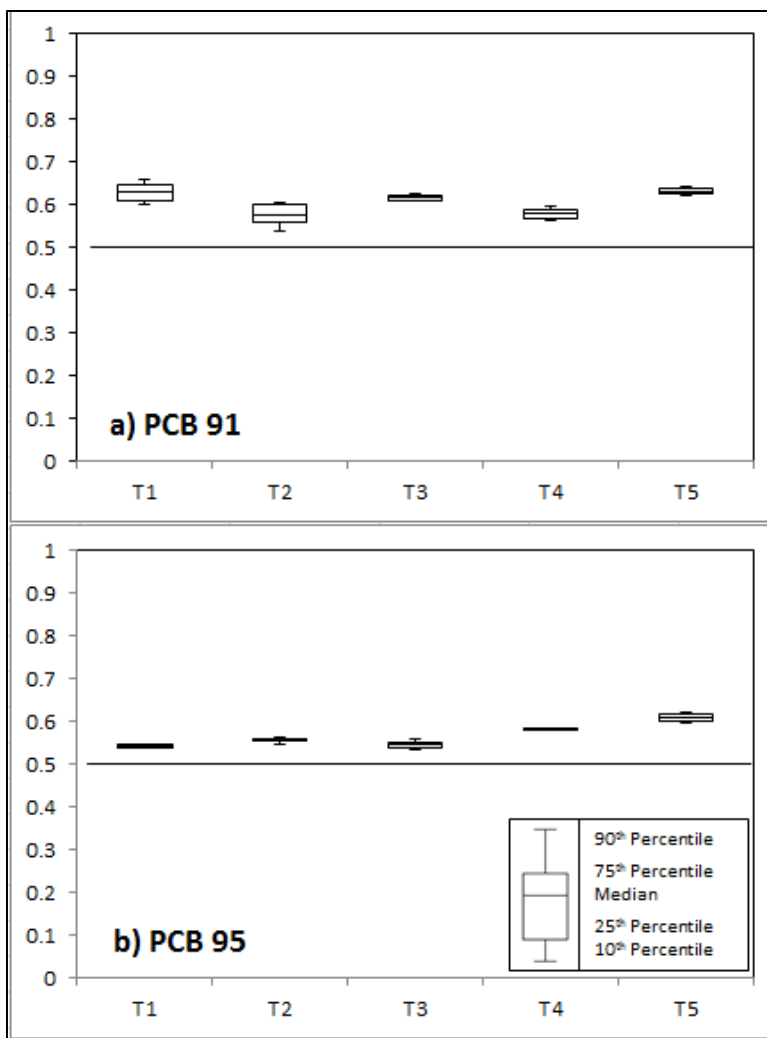


Figure 3.10: Enantiomeric fractions (EFs) for PCBs 91 and 95 in final rhododendron leaves sampled from Town Creek, SC. EF values are an average of three replicates. Horizontal lines at 0.5 indicate racemic EFs .

CHAPTER 4

Conclusions

These data provide evidence to support the hypotheses presented in Chapter 1. PCBs were detected in all rhododendron leaves and passive air samplers from the study site at Town Creek, SC, which supports that the primary source for PCB contamination in rhododendron leaves is via gas phase deposition. Furthermore, similar trends in decreasing total PCBs with increasing distance from the creek were observed in both rhododendron leaves and PAS. Although PAS and leaves from nearest the creek had the highest concentrations in comparison to leaves and PAS further away, there was no clear spatial trend of atmospheric PCB concentrations in the riparian zone adjacent to Town Creek. However, lower PCB concentrations measured at distances of up to 15 m from the creek are suggestive of a decreasing influence with increasing distance. These data are supportive of the hypothesis that PCB-contaminated water bodies, such as Town Creek, strongly contribute to the PCB content of leaves on adjacent rhododendron plants through foliar deposition.

When compared with rhododendron leaves from the Town Creek study site, rhododendron blanks collected from the Botanical Gardens have minimal detectable PCB contamination. Additionally, PCBs were detected in rhododendron leaves concurrent with PAS at Town Creek. These data provide support for the use of rhododendron leaves as biomonitors of atmospheric PCB pollution. Since rhododendron plants retain their broad, waxy leaves for four to seven years (Nilsen, 1986), low atmospheric PCB

concentrations may be detected due to the long exposure time of the leaves. Since collection of rhododendron leaf samples can be done with relative ease, they may prove to be a useful indicator of the presence of atmospheric PCBs in a given area. This approach would eliminate the cost and time required for passive atmospheric sampling, making rhododendron leaves useful in potential scoping projects.

This project has direct implications for the Twelvemile Creek/Lake Hartwell Superfund project. These data show that Town Creek is still contaminated with significant levels of dissolved PCBs, which is accumulating in the riparian vegetation immediately adjacent to the creek through atmospheric transport. The use of PAS in a site with contamination history can be used to evaluate the status of cleanup efforts. This approach could be strengthened by the inclusion of PE aqueous sampler. Although PCBs are a semi-volatile class of compounds, PCB fate and transport research often focuses on deposition of contaminated sediments and accumulation in the aquatic food web. PCBs detected in the PAS adjacent to Town Creek, a PCB-contaminated water body, show the importance of atmospheric transport as an additional consideration.

Future Work

Determination of EF values for PCBs 91 and 95 in soil samples (if present) would provide an additional line of evidence for the major source(s) of PCB accumulation in rhododendron leaves. Determination of EF values for PCB 95 in soil samples at Town Creek would provide additional explanation for the non-racemic EFs observed in rhododendron leaf samples. Determination of EF values for PUF samples would likely support the argument for Town Creek being the major contributor to chiral signatures of PCBs 91 and 95 observed in rhododendron leaf samples.

Seasonal trends in PCB concentrations and homolog distributions may be observed if sampling is repeated at the same locations. Changes in both water and air temperature impact the flux of PCBs between the water column and the atmosphere, as well as flux of PCBs between the rhododendron leaves and atmosphere. As a result, significant seasonal variations in total PCBs in all sampled media are likely. With longer deployments of PAS, it is possible that equilibrium at the PUF:air interface may be approached, resulting in more similar homolog distributions for PUF and rhododendron leaf samples. However, PUF samples from longer exposures will likely require more cleanup processes, while retaining satisfactory recovery values to quantify results.

Although direct atmospheric concentrations cannot be measured from PUF disks, an average daily flux of PCBs from a given media to the atmosphere can be estimated using air sampling volumes for PUF PAS, side-by-side active sampling measurements, or depuration compounds (Persoon, and Hornbuckle, 2009). Persoon and Hornbuckle

(2009) recommend the use of depuration compounds for back-calculating PCB air concentrations in outdoor passive sampling experiments using PUF disks. With this, seasonal or geographical differences in contaminated water bodies may be compared more easily.

APPENDICES

APPENDIX A

Materials and Methods

Passive Air Samplers

PAS (model TE-200) and PUFs (14 cm diameter, 1.35 cm thick, surface area 365 cm², density 0.0213 g cm⁻³) used were Tisch Environmental, Inc. (Cleves, OH) brand, purchased from Newterra (Beamsville ON, Canada). Generally, sampling devices were washed with soap and water prior to assembly. Immediately prior to PUF disk insertion in the field, I rinsed the interior of the sampling device with hexane and allowed it to air dry (Du et al., 2009). The PAS included a metal bracket for attaching the samplers to metal posts, along with a wire carrier insert to hold each PUF during deployment. I chose Unistrut[®] as the universal post material since shorter, pointed pieces could be driven into the ground before connecting a longer post (~2m) to attach the sampler(s). Sampler covers were kept closed with the included hook as well as a zip tie to reduce tampering. A fully assembled and installed PAS unit is shown in Figure A1. The PAS unit located over Town Creek (S1) is shown in Figure A2.



Figure A.1: Fully assembled and installed PAS units (S2 and S3).



Figure A.2: PAS unit (S1) assembled and installed over Town Creek.

Polyethylene Passive Aqueous Samplers

The same PE tubing from Brentwood Plastics, Inc. (St. Louis, MO) from Dang's research (2012) was used in this experiment. In a similar manner, small sheets of PE (2.5 X 5 cm) were prepared for each of two samplers. PE sheets were strung onto two coppers wires in triplicate for each device. The PE was cleaned by sequential soaks in dichloromethane, hexane, and methanol, each for 24 hours (Dang, 2012; Fernandez et al., 2009). Devices were deployed approximately 20 cm below the water's surface.

Table A.1: Relative positions of all rhododendron leaf samples and PAS in Relation to Town Creek, SC.

Sample I.D.	Distance from creek shore (m)	Approximate Height above water's surface (m)
T-I	3	N/A
T-II	10	N/A
T-III	20	N/A
T-IV	2.5 (opposite shore)	N/A
T1	2	2-2.5
T2	5	4-4.5
T3	5	6
T4	9	7
T5	12	9
S1	0 (over water)	0.5
S2	2	3
S3	2	5
S4	5	4
S5	5	6
S6	9	7
S7	12	9

Sample Extraction and Analyses

I baked sodium sulfate at 450 °C to ensure purity and dryness prior to use. Drying columns were plugged with fiberglass wool and 10 g sodium sulfate. I passed sample extracts in hexane through each drying column, followed by sample vial rinses with hexane at least three times to minimize analyte loss. The liquid sample was then evaporated to 5.0 mL using high purity nitrogen. I then removed 1.0 mL of sample and massed immediately in an aluminum weigh boat for lipid analysis. The weigh boat was allowed to dry overnight in the fume hood before re-weighing the aliquot to determine the mass of lipid in 1.0 mL of the sample extract. The remaining 4.0 mL of sample was cleaned on a column packed with 3.5 g alumina (Level III-6% deactivated) and 2 g baked sodium sulfate, which was prepped by rinsing with hexane just prior to passing the sample through. This was followed with several vial rinses with solvent to assist sample transfer through the column. Following the column cleanup, samples were again evaporated to 4.0 mL. I carried out an additional cleanup step for all leaf samples by combining the extract with ~ 5 mL of concentrated sulfuric acid in glass centrifuge tubes. These were then mixed by centrifuging at 2000 rpm for 20 minutes. I allowed the samples to settle and then carefully removed the upper solvent layer, mixing with 6 mL of isooctane for solvent exchange. I evaporated all samples to a final volume of 2.0 mL, transferred to glass GC vials, and stored at 4 °C until further analysis. Some leaf samples required repeated acid cleanups to fully clean samples to clearness, which reduced the recovery values for these samples.

I prepared individual samples for analysis using 250 μL of sample and 5 μL each of PCB 204 and Aldrin internal standards to quantify PCB concentrations. Individual calibration standards were prepared in a similar manner. The GC analytical method described in Chapter 2 on the 60 m achiral capillary column can quantify up to 140 target PCB congeners, with some congeners being co-eluted (Dang, 2012). Hydrogen (99.999% ultra high purity) and nitrogen (99.999% ultra high purity) were employed as carrier and make-up gases, respectively. The injection inlet was set to a temperature of 225°C, and the ECD detector was set to a temperature of 325°C. The column, anode, and make-up gas flow rates were set at 1.4, 6.0, and 90.0 mL/min, respectively. Recalibration was required if a change of >10% relative peak area count (PAC) was observed. Relative PAC was calculated using the ratio of PAC for a standard over PAC of an internal standard ($R_{\text{PAC}} = \text{PAC}_{\text{S}}/\text{PAC}_{\text{IS}}$). A procedural blank (isooctane solvent) and check standard (of 1:1:1 Aroclors 1016, 1254, 1260 at a given level) were included in every GC batch run. A splitless injection volume of 1.0 μL was used, with three syringe rinses in solvent before and after each column injection.

I prepared individual samples for chiral analysis using 250 μL of sample. EFs were determined by taking the ratio of peak area count for the first eluting isomer over the total peak area counts for a given chiral congener. Chosen chiral congeners were compared against separately prepared standards of 1:1:1 (v/v/v) Aroclors 1016, 1254, and 1260 in isooctane to check for coelution interferences. Hydrogen (99.999% ultra high purity) and nitrogen (99.999% ultra high purity) were employed as carrier and make-up gases, respectively. The injection inlet was set to a temperature of 225°C, and the ECD was set

to a temperature of 325 °C. The column and makeup gas flow rates were set at 2.0 and 48.0 mL/min, respectively. A splitless injection volume of 1.0 μ L was used, with three syringe rinses in sample before each injection, and six rinses in solvent after each column injection.

APPENDIX B

Table B.1: Total PCB concentrations comprising averages reported for all sampled media.

<u>Sample Media</u>	<u>Total PCBs</u>
PE Set 1	21155.3, 20236.6, 20959.7 (ng/g PE)
PE Set 2	18767.9, 18409.1, 18093.7
S1	2077.7 (ng)
S2	1485.5
S3	1478.4
S4	868.6
S5	846.6
S6	1089.5
S7	876.1
T-I	416.1, 456.6, 265.9, 568.5 (ng/g lipid)
T-II	335.3, 277.0, 203.8, 258.2
T-III	227.7, 204.2, 177.6
T-IV	235.8, 160.5
T1	7522.1, 7436.2, 5053.9
T2	2196.6, 1410.3, 1532.9
T3	1185.4, 962.1, 1736.4
T4	1194.6, 1065.0, 1393.2
T5	1400.7, 874.5, 881.5

APPENDIX C

Total PCB accumulations and homolog distributions are given for two- and three-month deployments of PAS S1 and S7, respectively. As discussed previously, recovery values for these PUF exposures are too low to merit any confidence in the data.

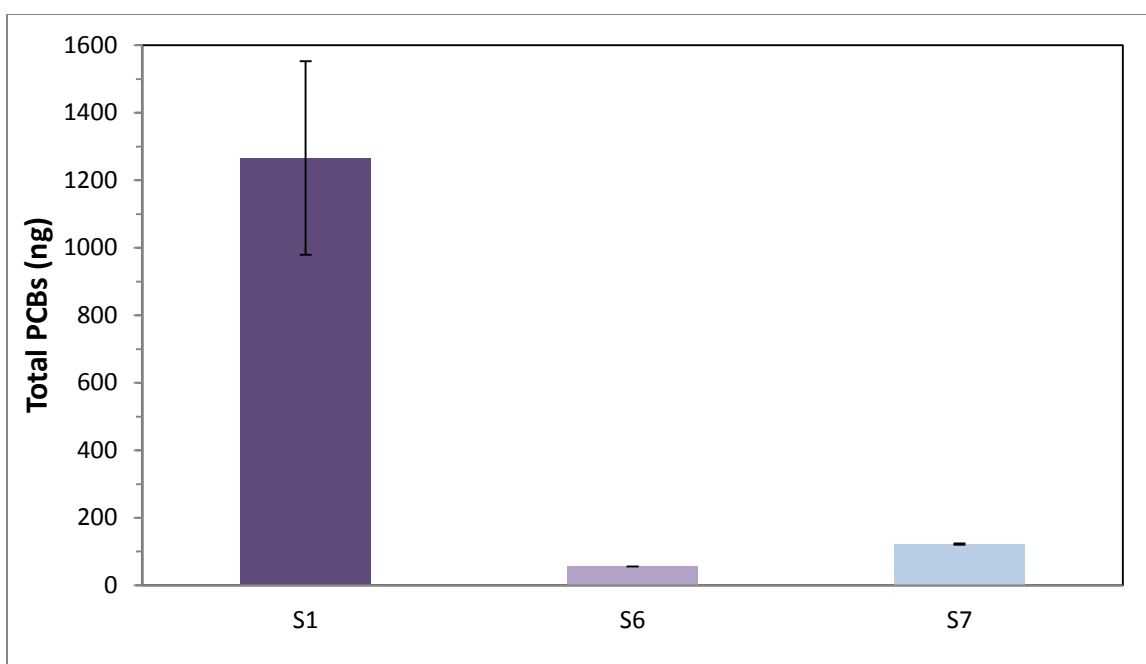


Figure C.1: Total PCBs (ng) for two- (S1) and three- (S6, S7) month deployments of PUF disks at Town Creek, SC. Error bars represent standard deviation for n=2.

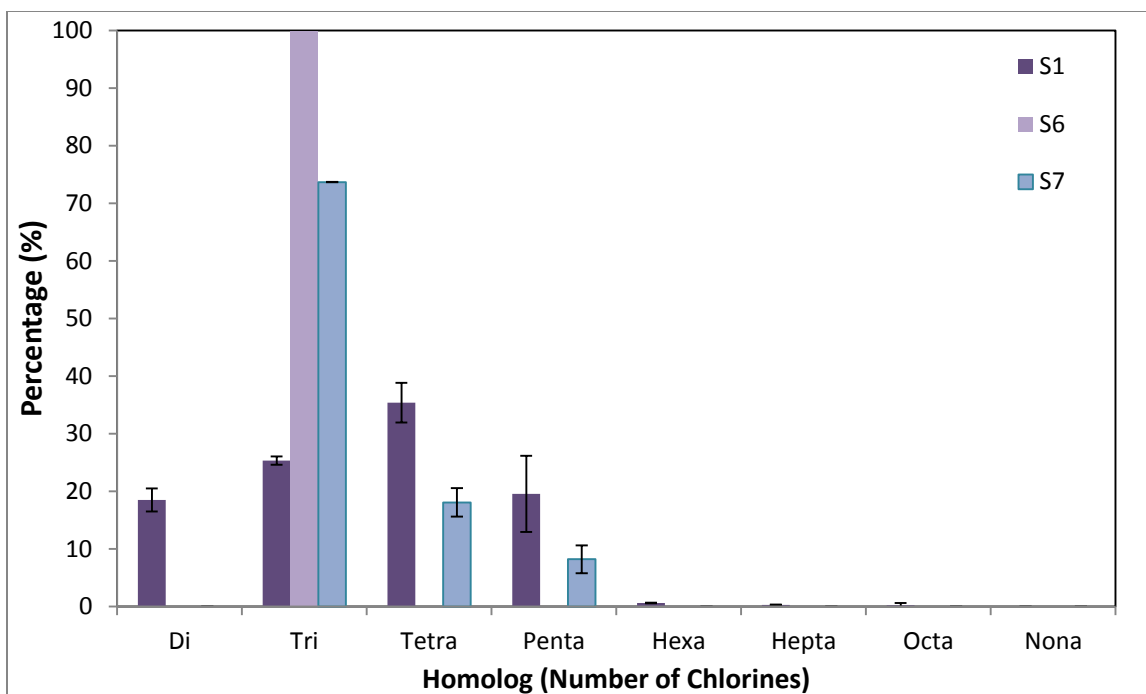


Figure C.2: PCB homolog distributions for two- (S1) and three- (S6, S7) month deployments of PUF disks at Town Creek, SC. Error bars represent standard deviation for n=2.

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