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Achiral and chiral analysis of polychlorinated biphenyls (PCBs) in the aquatic and riparian food webs in Twelve Mile Creek, South Carolina.

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ACHIRAL AND CHIRAL ANALYSIS OF POLYCHLORINATED BIPHENYLS
(PCBs) IN THE AQUATIC AND RIPARIAN FOOD WEBS
IN TWELVE MILE CREEK, SOUTH CAROLINA

A 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
Viet D. Dang
August 2007

Accepted by:
Dr. Cindy Lee, Committee Chair
Dr. Stephen Klaine
Dr. John Coates

ABSTRACT

Polychlorinated biphenyls (PCBs) contamination in the Twelve Mile Creek arm of Lake Hartwell, South Carolina, has been an issue for decades. Lake Hartwell, which is a reservoir located on the border between the states of South Carolina and Georgia, was contaminated with an estimated 440,000 pounds of PCBs. PCBs were released from the Sangamo-Weston capacitor plant located on Town Creek, which feeds into Twelve Mile Creek and ultimately Lake Hartwell. The Twelve Mile Creek tributary connecting the Sangamo-Weston plant to Lake Hartwell has had little attention. Understanding PCBs contamination in the aquatic and riparian food webs of Twelve Mile Creek has not been comprehensively investigated to date. With the dominant properties of this class of organochlorine compounds such as low solubility, and high lipophilicity, PCBs are considered resistant to degradation in the environment. Therefore, the goal of this thesis was to evaluate the pattern of the PCBs contribution to the aquatic and riparian food webs and biological processes governing transfer of PCBs through food webs in Twelve Mile Creek.

PCB analyses were conducted on sediments, aquatic and riparian organisms from Twelve Mile Creek, which were collected as part of a larger project by the US Environmental Protection Agency. Achiral PCBs analysis provided insight into total PCBs content of various trophic levels, and also on biomagnification of PCBs through the food webs. Chiral analysis of atropisomeric PCBs provided insight into biological discrimination between PCB congener enantiomers during uptake and biodegradation

processes. Atropisomeric PCBs analysis is a useful tool to track the potential biotransformation activity of chiral PCB atropisomers in the environment.

The aquatic food web including sediments, clams, mayflies, and yellowfin shiner (fish), was sampled in Fall 2005 from four sites along Twelve Mile Creek. Riparian species including spiders (*tetragnathidae*, *dolomedes*, and garden spiders) and amphibians (frogs, salamanders, and lizards) were collected in Spring, Summer, and Fall 2005 along Twelve Mile Creek from four sites. Achiral PCBs analysis demonstrated that total PCB concentrations were characterized by species-dependent patterns in the aquatic food web. No site or time-dependent patterns were consistently observed for total PCB levels in the riparian species. This observation demonstrated that PCBs biotransformation in the riparian was more variable than in the aquatic species. The observation is preliminary since the number of riparian species investigated was limited. Tetra-, penta-, and hexa-chloro biphenyl groups were almost always found as the most predominant contribution in the aquatic and riparian species accounting for 70% to 80% of the total PCB concentrations. PCB congeners 118 (245-34), and 153 (245-245) were almost always found to be abundant, confirming their highly persistent nature. PCBs 118 and 153 are present in Aroclor 1254 by a weight percent of 10.49 and 3.77, respectively. Aroclor 1254 was one of Aroclors mixture, which was released into the Lake Hartwell from the Sagamo-Weston plant during its manufacturing processes.

Enantiomers of atropisomeric PCBs were quantified to investigate the hypothesis that selective PCB biotransformation occurs in the food webs of the Twelve Mile Creek. Eight selected PCB atropisomers, 84 (236-23), 91 (236-24), 95 (236-25), 132 (234-236),

136 (236-236), 149 (236-245), 174 (2345-236), and 176 (2346-236) were measured in terms of enantiomeric fractions (EFs). Enantioselectivity was observed for PCBs 84, 91, 95, 136, and 149, but not at all sites studied. Meanwhile, enantioselectivity for PCB 174 was not observed. Evidence of metabolism was found for atropisomeric PCBs 84, 91, 95, and 149. However, only PCB congeners 95 and 149 were observed consistently between the aquatic and riparian species, which are also similar to previous observations for aquatic and riparian biota in Lake Hartwell, SC. A change in EF for PCB 149 occurred consistently in almost all sampling sites and seasons in the riparian species, but the EF of PCB 95 did not change consistently in most species investigated. The EF values for PCB 149 were found to be less than 0.5 in all species. The EF values for 95 were found to be less than 0.5 in clams, mayflies, and amphibians, while the values were found to be nearly racemic in fish and spiders. This observation lends support to the hypothesis that metabolism of PCB congeners occurs in the higher trophic levels.

DEDICATION

This research is dedicated to my family who encouraged me both physically and mentally during the years that I have studied at Clemson University. Their support helped me to overcome obstacles and complete my master's degree. On my behalf, this thesis is mostly dedicated to my father who has inspired in me heartfelt admiration, which was helpful motivation to enforce me going forward.

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LIST OF ABBREVIATIONS

Agency for Toxic Substances and Disease Registry	ATDSR
Enantiomeric Fraction	EF
Enantiomeric Ratio	ER
Gas Chromatography	GC
Gas Chromatography-Electron Captor Detector	GC-ECD
Internal Standards	ISs
Laboratory Control Sample	LCS
Laboratory Control Sample Duplicate	LCSD
Matrix Spike	MS
Matrix Spike Duplicate	MSD
National Fire Protection Association	NFPA
Organochlorine	OC
Polychlorinated Dibenzodioxins	PCDDs
Polychlorinated Dibenzofurans	PCDFs
Polychlorinated Biphenyls	PCBs
Quality Assurance and Quantity Control	QA/QC
Recovery Standards	RSs
Standard of Procedures	SOPs
South Carolina Department of Health and Environmental Control	SCDHEC
Standard Reference Material	SRM

List of abbreviations (continued)

United Nation Environmental Programme

UNEP

United State Environmental Protection Agency

USEPA

CHAPTER 1

INTRODUCTION

Polychlorinated biphenyls (PCBs) are anthropogenic organic compounds formulated as biphenyl rings with various numbers of chlorine atoms substituted onto its carbon positions. Theoretically, there are 209 possible PCB congeners (ranging from mono to decachlorinated isomers) in mixtures of technical product, which were produced commercially by catalytic chlorination of biphenyls (UNEP, 1999). The capability for long-range transportation and large-scale distribution makes PCBs truly global environmental pollutants. As one of structurally stable groups, PCB congeners have low aqueous solubility, high boiling points, and excellent dielectric properties. These characteristics made them very useful in diverse industrial applications, such as liquid components of transformers, heat-exchangers, and vacuum pumps. The commercial production of PCBs started in the late 1920s and was scaled back dramatically during the 1970s due to scientific and public concern. The total amount of PCBs produced during approximately 50 years of production has been estimated at greater than 1.5×10^5 metric tons (de Voogt and Brinkman, 1989). It was found that these chemicals were and are still present at low concentrations in most environmental matrices (i.e. fish, sediment, and human blood), and their great lipophilicity prompts their bioaccumulation and biomagnification throughout the food chain. This evidence has raised concerns about potential adverse human health effects, which has led to risk assessment guidelines by the United State Environmental Protection Agency (U.S. EPA). The guidelines help to limit

the PCBs level in various matrices and to urge better method development for chemical analysis of PCB congeners

Recently, research has addressed chiral PCBs analysis since it provides an opportunity to closely investigate PCB biodegradation mechanisms in the environment. Of the 209 different PCB congeners, there are 78 PCB congeners that are inherently chiral in which an asymmetric substitution of both phenyl rings lead to non-planar conformations, namely enantiomers (or atropisomers). However, only 19 of the 78 chiral PCB congeners with three or four ortho chlorine atoms are predicted to form stable atropisomers under normal environmental conditions (Kaiser, 1974). Of which, at least 12 atropisomers, PCBs 45 (236-2), 84 (236-23), 88 (2346-2), 91 (236-24), 95 (236-25), 132 (234-236), 136 (236-236), 144 (2346-25), 149 (236-245), 171 (2346-234), 174 (2345-236) and 183 (2346-245), have been detected in commercial PCB mixtures at levels greater than 1% (w/w) (Frame et al., 1996). It follows that exposure to certain enantiomers of chiral compounds have resulted in adverse biological and toxicological effects even though the two enantiomers have identical physical and chemical properties. A typical example is the (+)- and (-)-enantiomers of PCB 84, which have the same physical and chemical properties, but display a significantly different effect on protein kinase C translocation as determined by [³H]-phorbol ester binding in cerebellar granule cells (Rodman et al., 1991). More evidence has shown the differences that enantiomers of different PCBs atropisomers have in inducing cytochrome P450 activity, which metabolizes many xenobiotic compounds, and in accumulating uroporphyrin (Rodman et al., 1991). Wong et al. (2000; 2001b) reported that chiral PCBs, when released into the

environment, are racemic mixtures, which consist of equal amounts of each enantiomer and are defined by an enantiomeric fraction (EF) of 0.5. However, under microbial degradation, one enantiomer will be preferentially enriched and consequently produce non-racemic mixtures ($EF \neq 0.5$). This suggests that biotransformation may be controlled by enantioselective processes in certain circumstances.

Up to now, no individual chiral GC column has been produced that can enantiomerically separate all 19 PCB atropisomers. Some research has concluded that at least four columns are needed in order to resolve all 19 atropisomers (Blanch et al., 1996; Puttmann et al., 1986). Recently, it has been noted that the permethylated β -cyclodextrin (Chirasil-Dex) column is the most commonly used due to its bonded stationary phase, cross-linking and acceptable stability. On such a column, nine pairs of atropisomeric PCBs can be partially resolved including PCBs 91, 84, 135, 136, 174, and 176 or completely resolved including PCBs 95, 132, and 149 (Haglund and Wiberg, 1996). The partial or complete separation of atropisomers is based on the peak resolution (R) values on Chirasil-Dex column (Table 1). Therefore, this column was chosen for use in this research.

Table 1.1 Direction of the optical rotation [(+) and (-)], and peak resolution R values on Chirasil-Dex column (Haglund and Wiberg, 1996)

PCB#	Substitution	t _{R1}	t _{R2}	R
84	2,2,3,3,6-PentaCB (PeCB)	61.13(-)	61.32(+)	0.7
91	2,2,3,4,6-PeCB	59.05	59.32	0.9
95	2,2,3,5,6-PeCB	57.03	57.41	1.25
132	2,2,3,3,4,6-HexaCB (HxCB)	77.29(-)	78.02(+)	1.50
135	2,2,3,3,5,6-HxCB	68.22(+)	68.47(-)	0.8
136	2,2,3,3,6,6-HxCB	65.41(-)	65.66(+)	0.8
149	2,2,3,4,5,6-HxCB	69.03(+)	69.48(-)	1.25
174	2,2,3,3,4,5,6-HeptaCB (HpCB)	91.63(+)	92.13(-)	0.8
176	2,2,3,3,4,6,6-HpCB	76.80(-)	77.13(+)	0.8

PCBs contamination continues to be a topic of controversy in Lake Hartwell, South Carolina. Including the Twelve Mile Creek arm, the lower portion of Keowee River arm, and the upper portion of the Seneca River arm, Lake Hartwell is a large reservoir between the border of South Carolina and Georgia. The PCBs contamination source, which discharged into Lake Hartwell, has been attributed to the Sangamo Weston capacitor manufacturing plant which operated from 1955 to 1987 (EPA, 1993). Previous research has shown high concentrations of PCBs in the Lake Hartwell aquatic system consisting of fish and sediment (Germann, 1988; EPA, 1997). Nonetheless, much less scientific attention has been directed to Twelve Mile Creek, a small stream arm connecting Town Creek where the Sangamo Weston plant and Lake Hartwell. Therefore, this is the first project in the Environmental Engineering and Science Department focusing on the aquatic and riparian food webs in Twelve Mile Creek in order to provide a comprehensive picture of PCB biotransformation and bioaccumulation. By means of chiral analysis techniques, the results from this thesis will help future research, specifically in the interest area of food webs.

1.1 Literature review

1.1.1. PCB Properties.

PCBs are a class of man-made and structurally related chemicals manufactured in the US from 1929 to 1977. As a summary of Agency for Toxic Substances and Disease Registry (ATSDR) in 1993 stated approximately 99% of PCBs used by the US industry were produced by the Monsanto Chemical Company in Sauget, Illinois, until manufacture was discontinued in 1977 (ATSDR, 1993). The trade names used prevalently for PCB mixtures, like the ones in this research, are Aroclors, which are different concentration fractions of PCBs with different chemical and physical properties (Bench, 2003). Several common names are Aroclors 1016, 1254, 1221, 1248, 1242, 1262 and 1260. Prior to 1974, PCBs were mostly used for nominally-closed applications (e.g., capacitor and transformers) and in open-end applications (e.g., flame retardants, adhesive, and pesticide extenders, surface coatings, wire insulators) (Durfree, 1976; EPA, 1976). Their physical and chemical properties are factors that made PCBs commercially valuable and potentially detrimental to the environment.

1.1.2. Chemical and physical identity.

PCBs comprise a group of 209 structurally different compounds (called congeners) with the empirical formula $C_{12}H_{10-n}Cl_n$ ($n=1-10$). A general chemical structure of polychlorinated biphenyls is shown in Figure 1:

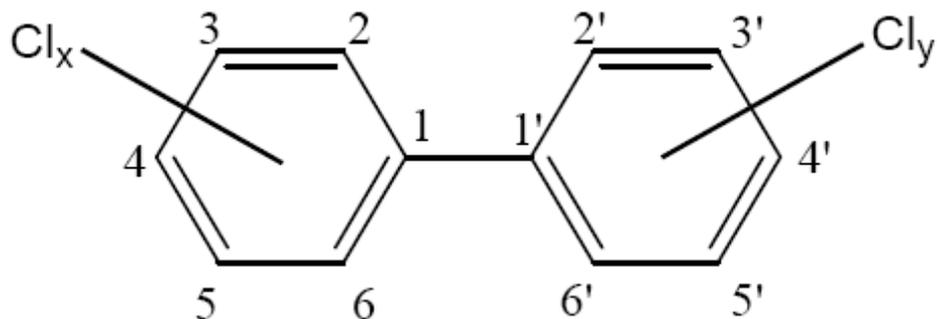


Figure 1.1 Generic polychlorinated biphenyl (PCB) structure.

Positions labeled by numbers can be substituted by chlorine atoms resulting in different congeners. In addition, the term “homolog” refers to all PCBs with the same number of chlorines while different substitution patterns are referred to as isomers. On a PCB, positions 2, 2', 6, and 6'; 3, 3', 5, and 5', and 4 and 4' are called its *ortho*, *meta*, and *para* positions, respectively. In principle, the biphenyl ring can rotate about the central C-C bond. Rotation, however, is hindered particularly by the presence of substituents in the *ortho* positions (2, 2', 6 and 6'). This resulted from a high free energy of activation, which is greater than 175 kJ/mol for tri-*ortho* atropisomers and approximately 250 kJ/mol for tetra-*ortho* atropisomers (Schurig et al., 1995). These values are well above the energy barrier required for rotational stability (~ 90 kJ/mol) at environmental temperatures and result in a half-life of thousand years for the labile atropisomers (Harju and Haglund, 2001). Meanwhile the free energy of activation is small (~ 4-19 kJ/mol) if the *ortho* positions are free (McKinney and Singh, 1988). Two extreme *ortho*-substituted configurations are found possible: 1) when the biphenyl ring is in the same plane (planar)

and 2) when the rings are at a 90^0 angle to each other (non-planar). The degree of planarity is largely dependent on the number of substitutions at the *ortho* positions. Inversely, co-planar or non-*ortho* substituted PCBs, particularly that have two *para*-chlorine atoms and at least one *meta*-chlorine atom (e.g. PCB 77 (34-34), 81 (345-4), 126 (345-34), and 169 (345-345), are of increasing concern because they exhibit toxicity similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin (EPA, 1998).

Products of PCBs were marketed in the US under trade name Aroclors. The Aroclors were identified by a four-digit numbering code, in which the first two digits represent the type of mixture and the last two are the approximate chlorine content (weight percent) of the congener. For example, Aroclor 1254 contains tri- through heptachlorinated homologs with an average chlorine content of 54%. An exception is Aroclor 1016 containing an average chlorine content of 41% (Hutzinger et al., 1974). An important physical property of PCBs is their general inertness. This means they resist both alkali and acid and have thermal stability as well. Because of these unique properties, PCBs were found to be useful in a wide variety of industrial applications including dielectric fluids and lubricants (Afghan and Chau, 1989). In general, PCBs are only slightly soluble in water and this solubility decreases with increasing chlorination (EPA, 1980). However, PCBs are soluble in nonpolar organic solvents and biological lipids making them persistent in fatty tissues (EPA, 1980). Hutzinger et al. (1985) found that certain PCBs are combustible liquids, and the products of combustion including polychlorinated dibenzodioxins (PCDDs), hydrogen chloride, and polychlorinated dibenzofurans (PCDFs) are even more hazardous than the initial materials.

1.1.3. PCB environmental fate and transport

PCBs are globally circulated and present in all environmental media. Due to the widespread commercial use and persistence in the environment, PCBs have been distributed worldwide. The presence of PCBs is even found in regions where they have never been used, such as in remote areas including open oceans, deserts, high mountain, the Arctic and the Antarctic (UNEP, 1992). PCBs are also noted for semi-volatility permitting these compounds to be either in the vapor phase or absorbed on atmospheric particles (UNEP, 1992). Thus, for example, the atmospheric fate of PCBs is highly dependent on the OH radical concentration, temperature, and the latter's influence on the gas/particle partitioning equilibrium (Wania and Daly, 2002). Additionally, PCBs in the atmosphere are transported by sorption to particles and will be removed by wet and dry deposition processes (Wania and Daly, 2002). In water, PCBs are transferred by diffusion and currents. The removal of PCBs from the water column is attributed to sorption by suspended solids and sediments as well as by volatilization from the water surface (Achman et al., 1992; Pearson et al., 1996).

PCBs degradation is primarily governed by the degree of chlorination on a congener. Normally, the higher chlorination, the less degradation occurs. The pattern of PCBs in the environment varies depending on the sources of PCBs and physical, chemical, and biological transformation processes. For instance, some authors suggested that anaerobic dechlorination has caused changes in PCBs pattern in sediments from the Hudson River and Twelve Mile Creek arm of Lake Hartwell (Bush et al., 1987; Pakdeesusuk et al., 2003).

1.1.4. Human health potential

Because PCBs are no longer manufactured or widely used today, there are relatively few ways which people can be exposed to concentrated PCBs. The common exposure routes can be counted as including food, surface soils, drinking surface water and groundwater, indoor air, and working place (ATSDR, 2000a). Among the exposures, the most crucial ways populations are exposed to PCBs may be from digesting contaminated food, especially fish from PCB-contaminated waters, and from inhaling contaminated air. PCB exposure has also been attributed to inhalation of contaminated indoor air where the use of PCBs production is on-going. This occupational exposure can be orders of magnitude higher than the exposure of the general population. Upon entering into the body, PCBs have potential toxic effects on human health. The toxic effects of PCBs were brought to public awareness by the Yusho incident in Japan in 1968, where more than 1,800 people were injured due to consumption of PCB-contaminated rice oil (Kuratsune, 1996). According to the US EPA, PCBs are also probable human carcinogens and also can cause non-cancer human and animal effects, such as reduced ability to fight infections, and problems with immune and reproductive systems. Some *ortho*-substituted congeners, especially chiral PCBs such as PCB 95 and 149, cause neurotoxicity and developmental toxicity (Schantz et al., 2003)

1.2. Twelve Mile Creek.

The Twelve Mile Creek is a tributary of Lake Hartwell, a reservoir located at the head of the Savannah River between South Carolina and Georgia. A sketch of Twelve Mile Creek is given in Figure 1.2. It is also the source of drinking water for the public water supply systems owned and operated by the Town of Pickens and the City of Easley. The total population using the Creek as a source of drinking water is approximately 43,022 persons (EPA, 1994). As proposed by EPA, the Sangamo Weston Superfund site consists of Sangamo Weston, Inc., which is a former capacitor plant located on Town Creek, and portions of the Twelve Mile Creek and of the Twelve Mile Creek arm of Lake Hartwell. The EPA issued a Record of Decision (ROD) for the Sangamo Weston/Twelve Mile Creek/Lake Hartwell PCB-contaminated Superfund Site in Pickens County in June 1994 (EPA, 1994). The site was listed as Operational Unit 1 (OU-1) and Operational Unit 2 (OU-2). OU-1 addressed mostly the land-based source area which included the Sangamo Weston plant and six satellite disposal areas (EPA, 1994). OU-2 addressed the contamination of fish, surface water and sediment at the location, and biological migration pathways downstream from the source areas (EPA, 1994). Research conducted by South Carolina Department of Health and Environmental Control (SCDHEC) beginning from 1977 to 1985 showed that fish in Twelve Mile Creek had the highest PCBs concentration indicating the capacity of PCBs to bioaccumulate in tissue samples and also in food webs (EPA, 1994). Because of PCBs contamination from the Sangamo site to fish and sediment in Twelve Mile Creek and Lake Hartwell, the SCDHEC and EPA issued an advisory against eating fish from the lake in 1976. The advisory has been

modified periodically and reevaluated annually on the basis of fish and sediment sampling conducted by SCDHEC. The advisory limit of PCBs in the lake has been thus set at 2 ppm at present (EPA, 1997).



Figure 1.2 Map of Twelve Mile Creek with sampling locations noted by numbers (Walters et al., 2005).

1.3. Chiral PCBs in food webs.

Chiral PCBs in food chains have recently received attention with a number of papers released. Enrichment of several PCB enantiomers from biological environmental samples of seabirds (Warner et al., 2005), dolphins (Reich et al., 1999), and fish species (Wong et al., 2004) has been reported. Data obtained are generally supporting previous conclusions in which atropisomeric PCBs are enantioselective through food webs. For example, Wong et al. (2004) measured enantiomeric composition of chiral PCB congeners in the aquatic food web of Lake Superior sampled in 1998. Biomagnification was observed for all chiral PCB congeners studied (PCBs 91, 95, 136, 149, 174, 176, and 183). PCB atropisomers were racemic in zooplankton and phytoplankton while macrozooplankton (such as diporeia and mysids) had significantly nonracemic residues for most chiral congeners. This suggested that higher aquatic species generally have greater capability to biotransform persistent contaminants. Lower aquatic species are considered to have poor biotransformation capability toward persistent xenobiotic compounds due to lower levels and activities of cytochrome P-450 1A and 2B isozymes (Stegeman and Kloeppersams, 1987; Kevin et al., 1987). Another chiral PCBs study has been done in an arctic marine food web from the Northwater Polynya in the Canadian Arctic by Warner et al. (2005). Highly nonracemic EFs were measured in predators (several seabirds and ringed seals), but racemic EFs were found in prey (zooplankton and Arctic cod). Similarly, nonracemic EFs were also found for PCBs 91, 95, 136, and 149 for aquatic and riparian biota from Lake Hartwell, SC (Wong et al., 2001b). Research showed that PCB EFs had species-dependent patterns, which suggest differences in the

ability of different species to bioprocess PCBs enantioselectively. In addition, according to Chu et al. (2003), the enantiomeric composition of PCB atropisomers measured in livers of harbor porpoises from the southern North Sea was related to age. Racemic values were found in almost juvenile porpoises, while non-racemic values were found in adult porpoises indicating different biotransformation behavior of PCB enantiomers with age. Thus, chiral analysis can play an important role in understanding contaminant dynamics in food webs.

1.4. Chiral analytical methods

In recent years, interest in the development of analytical methods for separation of enantiomers of organic compounds has been considerable because of their importance in the biochemistry and pharmaceutical industry. Numerous organochlorine (OC) compounds exist as a pair of enantiomers in which bioactivity of one enantiomer is different from the other in the environment. In order to understand the real environmental characteristics of chiral compounds, enantioselective methodologies will be useful to assess the separation of enantiomers and to discriminate biological processes controlling enantiomers. Frequently, enantioselective analysis of OC contaminants is preferably performed with high-performance liquid chromatography (Ellington et al., 2001) and capillary electrophoresis (Lewis et al., 1999). Alternatively, there are fewer publications done on the use of gas chromatography (GC) in the enantiomeric separation of these contaminants. Separation of enantiomers by GC is conventionally based on an empirical selection of a commercial chiral column, in which modified cyclodextrins are bound to

the stationary phase. As shown in Figure 2, cyclodextrins are cyclic α -(1- \rightarrow 4) glucose oligomers with 6, 7 and 8 glucose units corresponding to α -, β -, and γ -CD, respectively.

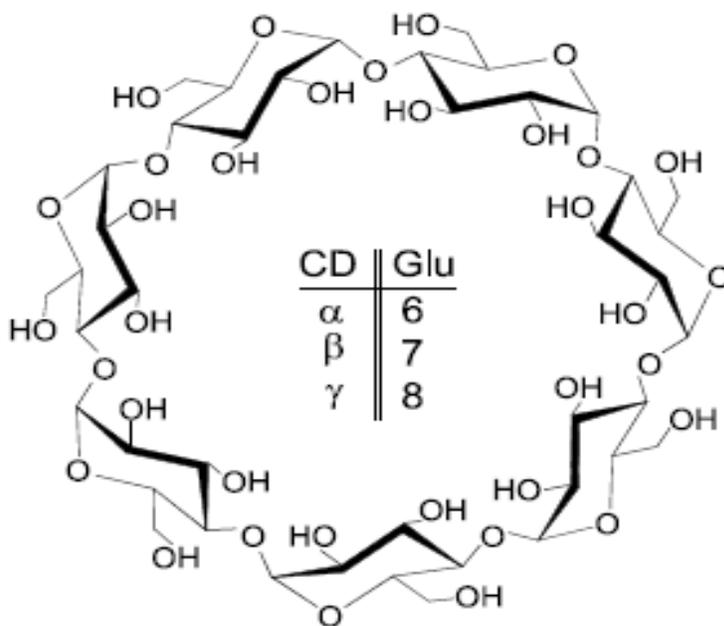


Figure 1.3 Chemical structure and geometry of cyclodextrins (Szejtli, 1998)

Different chiral columns separate different congeners, and to differing degrees. Among commercially available chiral GC columns, Chirasil-Dex column has been reported as efficient for the enantioseparation of chiral PCBs (Schurig and Glausch, 1993). In Chirasil-Dex, permethylated β -cyclodextrin is linked via a single octamethylene spacer to polydimethylsiloxane. This column is able to resolve enantiomers presenting a 2,3,4,6-substitution pattern such as PCBs 91(236-24), 95(236-25), 84(236-23), 132(234-236), 132(236-236), 149(236-245), 174(2345-236), and 176(2346-236) (Vetter et al., 1997). Wong and Garrison (2000) noted that enantiomers of PCBs 132 and 176 are co-

eluted on this column. Because it is one of the most commonly used and efficient, the Chirasil-Dex column was chosen for chiral PCBs analysis in this research.

1.5. Data presentation

There are many ways to express chiral composition. One of these, the enantiomeric ratio (ER) was the most frequently used descriptor for the relative abundance of environmental pollutants in the past. ER is expressed by:

$$ER = A_+/A_-$$

where A_+ and A_- correspond to the peak area of the (+) and (-) enantiomers, respectively. Thus, a racemic mixture has an ER of 1. However, there are several limitations in using ER, one of which is misleading data presentation because ER can range from zero to infinity. Therefore, the enantiomeric fraction (EF) was proposed to provide a better understanding of the chiral signatures, which is defined by:

$$EF = A_+/(A_+ + A_-) \text{ or } EF = A_1/(A_1+A_2)$$

where A_1 and A_2 represent the first and second eluting enantiomers on chiral columns when the identity of the (+) and (-) forms is not known. So, the EF can only range from 0 to 1. Racemic enantiomer distributions have an EF of 0.5, whereas EFs of 0 or 1 indicate pure single enantiomers (-) or (+), respectively.

CHAPTER 2

RESEARCH OBJECTIVES

The main goal of this work is to explore the usefulness of chiral PCBs in understanding PCBs movement through food webs in Twelve Mile Creek. Three hypotheses were evaluated to support this goal including:

2.1. Chiral analysis is a useful tool for investigating the mechanism of PCB bitransformation and degradation in food webs. The enantioselective degradation of PCB atropisomers may be an important indicator for assessing the environmental characteristics of natural contaminants.

2.2. The change in EFs for PCBs in the aquatic and riparian species compared to EFs for the sediment compartments suggests greater metabolic degradation of PCBs at higher level of food web.

2.3. The distribution of PCBs through the food webs depends on biological and environmental factors such as species, time, and contamination sources.

CHAPTER 3

EXPERIMENTAL METHODS

3.1. Chemicals

Solvent chemicals purchased from Fisher Scientific were all pesticide grade: hexane, methylene chloride, acetone, isooctane, and methanol. In addition, concentrated sulfuric acid (95-98%) purchased from Merck was trace metal grade. The concentrated acid was used during the centrifuge processes for lipid removal. Anhydrous sodium sulfate (Na_2SO_4) in powder form and alumina (chromatographic grade, 80-200 mesh) were purchased from EMD Chemicals Inc (Gibbstown, NJ). Sodium sulfate was prepared by drying for 24 hours at 105°C and then stored in desiccators for further use. Alumina was prepared by drying at 400°C for 4 hours and then activated by adding 10% water before use. Aroclor mixtures 1016, 1254, and 1260 were purchased from AccuStandard (Hew Haven, CT) as 1000 $\mu\text{g}/\text{ml}$ in isooctane. Working standards mixture was made in a concentration range of 3, 1.5, 0.3, 0.15, and 0.03 ppm each in isooctane. Recovery standards (RSs) including 3,5-dichlorobiphenyl (PCB 14) and 3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169) in 100 $\mu\text{g}/\text{ml}$ isooctane stock, internal standards (ISs) including Aldrin and 2,2',3,3',4,4',5,5',6,6'-hexachlorobiphenyl (PCB 209) in 100 $\mu\text{g}/\text{ml}$ isooctane stock, and neat standard PCB congeners 84, 95, 91, 132, 149, 136, 174, and 176 in 35 $\mu\text{g}/\text{ml}$ isooctane) were purchased from AccuStandard (New Haven, CT). A chiral PCB working solution was prepared by diluting stock standards including PCBs 84, 95, 91, 132, 136, 149, 174, and 176 in isooctane at a concentration level of 0.2 $\mu\text{g}/\text{ml}$

so that it would be within the range of the GC detection limit. Working solution of RSs and ISs was prepared in isooctane at a concentration of 2 µg/ml for each congener.

3.2. Samples collection.

The aquatic samples including sediments, *Corbicula* (clam), *Stenonema* (mayfly), and yellowfin shiner (fish) were collected by the US EPA in Fall 2005 from sites 2, 3, 4, and 6 spanning 25 stream-km (Figure 2). Clams were collected at a reference site marked as site 0 (Figure 2). The clam samples were then deployed at all four sites for up to 60 days. By the end of the deployment, they were sampled for further PCBs analysis. Site 2 was located on Town Creek, which is a tributary of Twelve Mile Creek. The Sangamo Weston plant, which discharged the PCBs, is located along Town Creek. Sites 3, 4, and 6 were on Twelve Mile Creek. Site 0 was the upper Twelve Mile Creek and upstream from the Sangamo site.

Riparian samples, including spider species (*Dolomedes*, *Tetragnathidae*, and Garden spider) and amphibian predators (Northern Cricket Frog, Fowler's Toad, Spring Peeper, Red Spotted Newt, and Pickerel Frog), were sampled by the US EPA in the Spring, Summer, and Fall 2005 from sites 1, 2, 3, and 6 (Figure 2). It should be noted that riparian samples collected are only a small portion of the food web and sample numbers were limited at sites and seasons.

3.3. Analytical methods.

In general, the extraction and analysis procedures used were modified from other methods, which followed EPA Method 1668 (EPA, 1999) and met the required Quality Assurance and Quantity Control (QA/QC) standards. Sediment and tissue samples were extracted for PCBs.

3.3.1. Sediment sample preparation.

In order to minimize the volume of solvent, extraction of the sediment samples was conducted as described by Dunnivant and Elzerman (1987) and modified by Pakdeesusuk et al. (2005). Briefly, PCBs were extracted from sediment samples into acetone via a sonication method. In addition, duplicate or triplicate extractions were completed for several random samples to monitor the method precision. Prior to sonication, samples were spiked with 100 μ L RSs.

3.3.2. Macro-invertebrate and tissue sample preparation.

For macro-invertebrate and fish tissue, a Soxhlet extraction was done following EPA Method 1668 (Revision A) (EPA, 1999). In summary, the Soxhlet apparatus was pre-extracted for 3 hours by approximately 300 ml of methylene chloride with a pre-weighed (empty) cellulose thimble included. For the sample preparation, 8-10 g of sample was mixed with 15-20g of dried anhydrous sodium sulfate. The sample and sodium sulfate mix was quantitatively transferred to the pre-extracted thimble. One hundred microliters of the RSs solution was spiked with the mix and then extracted for 18 to 24 hours. After the allotted time, the extracts were evaporated to approximately 5 mL.

The 5-mL extracts were eluted through a drying column comprised of glass wool and 10 g anhydrous sodium sulfate. The residues were further cleaned by a second step using 4 g of activated alumina and 0.5 g Na₂SO₄ for water removal. The extracts were centrifuged with 2 mL concentrated sulfuric acid in order to remove the residue of lipids. Hexane was added into the extracts up to 10 mL and was then reduced to 2 mL under the high purity nitrogen gas. Eight mL of isooctane was eventually added to 2-mL of hexane extracts and were concentrated to 2-mL final extracts. This method was employed for tissue samples including, but not limited to, fish, spiders, and other macro-invertebrates.

3.3.3. QA/QC sample preparation

Quality control samples included a procedural blank, laboratory control sample (LCS), laboratory control sample duplicate (LCSD), matrix spike (MS), matrix spike duplicate (MSD), and standard reference material (SRT). Quality assurance was accomplished by a check standard and an isooctane blank in every sequence of GC analysis. A check standard, which is similar to one of working standard mixture, was prepared with a concentration of 0.3 ppm for each Aroclor. The check standard was included with every sequence of samples. If a response factor, which is the ratio of peak area to concentration of any individual congener in the check standard, was more than 5% different from previous ones in the calibration curve, the calibration standard curve would be reconstructed.

3.3.4. PCBs achiral analysis

Congener-specific PCBs analysis was conducted on an Agilent 6890-GC equipped with a 60-m fused silica column (Rtx-5, Resteck, Bellefonte, PA; 60m length,

0.25mm diameter, 0.25µm film thickness) and a ⁶³Ni electron capture detector (ECD). GC parameters were modified from those described by Germann (1988) and Pakdeesusuk (2002). Generally, an initial oven temperature of 100⁰C was held for 2.5 min, followed by heating at 10⁰C/min to 150⁰C (0.5 min hold time), 1.1⁰C/min to 225⁰C (5 min hold time), and 10⁰C/min to 260⁰C (45 min hold time). The injector and detector temperatures were set at 250⁰C and 325⁰C, respectively. High purity helium was chosen as the carrier gas with a flow rate set at 2.0 ml/min. Anode and makeup gas nitrogen flow rates were set at 6.0 ml/min and 60.0 ml/min. Split vent flow was 60 ml/min initiated at 0.75 min. One µl of extract was injected on the GC.

Blank solvents (isooctane) were run after approximately every five GC samples to reduce analyte carry-over between consecutive injections. A check standard (0.3 µg/ml mixture of Aroclor 1260, 1254, and 1016) was run before every batch of samples. A calibration curve was constructed using five levels of a 1:1:1 mixture of the three Aroclors with two injections at each level. The quantification of PCBs in all samples was performed within the linear range of multiple calibration standards and based on the internal standards, Aldrin and PCB 209.

3.3.4.1. PCB peak assignment and congener-specific quantitative analysis.

To assign unknown peaks and absolute retention times (RTs), a mixture of Aroclors 1016, 1254, and 1260 (1:1:1 w/w/w) was used. Table 3.1 shows the IUPAC numbers and RT of PCB congeners in the standard mixture of three Aroclors on the 60-m fused silica GC column. A chromatogram of individual PCB congeners assigned in the mixture of three Aroclors is given in Figure A.1 in the Appendix.

Table 3.1 Assigned PCB congeners and RTs on 60-m GC column in 3-Aroclor mixture.

IUPAC Congeners	RTs	IUPAC Congeners	RTs	IUPAC Congeners	RTs
1	25.15	74	65.50	163,164,138	87.44
3	29.64	70	66.12	158, 160	87.72
4,10	32.27	100	63.91	178,126,129	88.37
9,7	35.44	63	64.88	175	88.98
6	36.89	76	66.47	182 ,187	89.30
5,8	37.71	95 ,66	66.85	183	89.87
14 (RS)	39.60	91	67.91	167,128	90.58
19	40.61	92	68.57	185	91.11
12,13	43.34	56,60	69.67	174	92.03
18	43.82	84	70.37	202,177	92.69
17,15	44.13	101,89, 90	70.62	156,171	93.29
27,24	45.41	99	71.48	201,157,173	94.01
31,16	46.71	119	72.53	172	94.53
29	48.63	83	73.35	197	94.91
26	49.40	97	74.21	180	95.34
25	49.80	81 ,87,115	75.07	193	95.67
31	50.70	85	75.78	191	96.24
28	50.91	136	76.24	200	96.98
21 ,33,20,53	52.44	77,110	76.72	169 (RS)	98.05
51	53.34	82,151	78.80	190,170	99.29
22	53.66	135,144,124	79.60	198	99.96
45	54.48	107,109,147	80.42	199	100.43
46	55.75	123,139, 149	81.00	203,196	101.21
52	56.37	118	81.32	189	103.39
43,49	57.07	134	82.69	195,208	105.92
47	57.53	114	82.87	207	106.96
48	57.71	131,122	83.25	194	109.25
Aldrin (IS)	58.70	146	83.65	205	110.24
44	59.73	153, 184	84.33	206	116.60
59	60.08	132	84.68	209 (IS)	123.66
37,42	60.29	105	84.71		
71	61.52	141	85.74		
41,64	61.75	179	85.96		
96	62.60	137	86.53		
40	63.08	130, 176	86.81		

Bold: congeners added to the mixture

Sivey (2005) suggested that the further the PCB congeners eluted from an internal standard, the less accurate the quantification. Therefore, two internal standards, Aldrin and PCB 209, were used to enhance the quantitative precision in this research. Congeners eluted from PCB 1 to PCB 81, 87, 115 were quantified based on Aldrin, while the rest of the eluted congeners were based on PCB 209. Data of recovery standards that were spiked into samples prior to extraction, showed that a recovery factor of 70-75% for PCB 14 and 50-60% for PCB 169 was obtained in the riparian samples (some samples showing PCB 169 below 40% were not included in the results and discussion section), and increased to 80-90% for PCB 14 and 70-80% for PCB 169 in the aquatic samples. A summary of recovery percent for PCBs and 169 is shown in Table A.4 and A.5 in the Appendix. It has been noted that recovery percent of PCB 14 was always higher than that of PCB 169 in both food webs. A low percent result for recovery standard PCB 169 could be explained by the higher amounts of fatty tissue in riparian samples favorably accumulating higher chlorine congener PCB 169, which was then removed partially during the lipid quantification process. However, the fat tissue was not saponified, this statement was thus not verified.

3.3.5. Chiral PCB analysis

All extracts were analyzed for eight chiral congeners (PCBs 95, 91, 84, 136, 149, 132, 174, and 176) according to the methods described by Wong et al. (2001a) and Hall (2004). The target chiral analytes were separated using a Hewlett-Packard 6850 capillary GC accompanied with a ^{63}Ni ECD and a Chirasil-Dex column (Rtx-5, Restek, Bellefonte, U.S.A; 30m length \times 0.25mm diameter Rtx-5 capillary column, and a 0.25 μm film

thickness). Helium and nitrogen were employed as the carrier and make-up gas. The GC conditions began with the initial oven temperature at 60⁰C for 2 min. The temperature was then increased to 150⁰C at a rate of 10⁰C/min and in turn to 200⁰C at a rate of 1⁰C/min. It was held at 200⁰C for a total running time of 61 minutes. The injector and detector temperatures were set at 210⁰C and 350⁰C, respectively. The flow rate of the carrier gas, anode, and make-up gas was conditioned sequentially by 2.0, 6.0, and 60.0 ml/min. Spilt vent flow was initiated at 0.75 min with a flow of 60 ml/min. Autosampler injection volumes were 1 µl.

3.3.5.1. Elution orders and retention time (RTs) of atropisomeric PCBs.

A working standard of nine standards was injected into the GC to identify RTs. Table 3.2 present RTs of enantiomers of eight PCB standard atropisomers assigned on 30-m Chirasil-Dex column. Elution order of enantiomers was obtained from Wong and Garrison (2000). Since PCB 132 and 176 coelute on Chirasil-Dex column, there were only six atropisomers (PCB 95, 91, 84, 136, 149, and 174) evaluated in this research (Figure 3.1). To evaluate PCB enantiomers, the EF value was used as defined in Chapter I (above). It is noted that the optical rotation of PCBs 84, 136, 149, and 174 on Chirasil-Dex GC column has been experimentally assigned using pure enantiomers (Haglund and Wiberg, 1996; Harju and Haglund, 2001). Therefore, the (-)-enantiomer elutes before (+)-enantiomers for all atropisomers, except PCB 174 for which elution order is reversed and PCB 95 and 91 in which the elution order is unknown. Table 3.3 gives the EF values of five injections of a working standard solution on the Chirasil-Dex GC column. A check standard was included in every sequence of running samples. A confident interval of 95%

(± 0.026) was used as a conservative measure of EF precision for the racemic standards (EF=0.5).

Table 3.2 Retention times (RTs) of PCB atropisomers on Chirasil-Dex.

PCB congeners	RT(1)	RT(2)
PCB 95 (236-25)	35.80	36.14
PCB 91 (236-24)	37.36	37.63
PCB 84 (236-23)	39.04	39.26
PCB 136 (236-236)	42.78	43.06
PCB 149 (236-245)	46.03	46.43
PCB 132 (234-236)	51.39	51.65
PCB 176 (2346-236)	51.64	52.42
PCB 174 (2345-236)	59.46	59.70

Table 3.3 Enantiomeric fractions (EFs) of atropisomeric PCBs in standard.

PCB congeners	EF values
PCB 95 (236-25)	0.50 ± 0.002
PCB 91 (236-24)	0.50 ± 0.007
PCB 84 (236-23)	0.49 ± 0.014
PCB 136 (236-236)	0.49 ± 0.012
PCB 149 (236-245)	0.50 ± 0.009
PCB 174 (2345-236)	0.50 ± 0.004

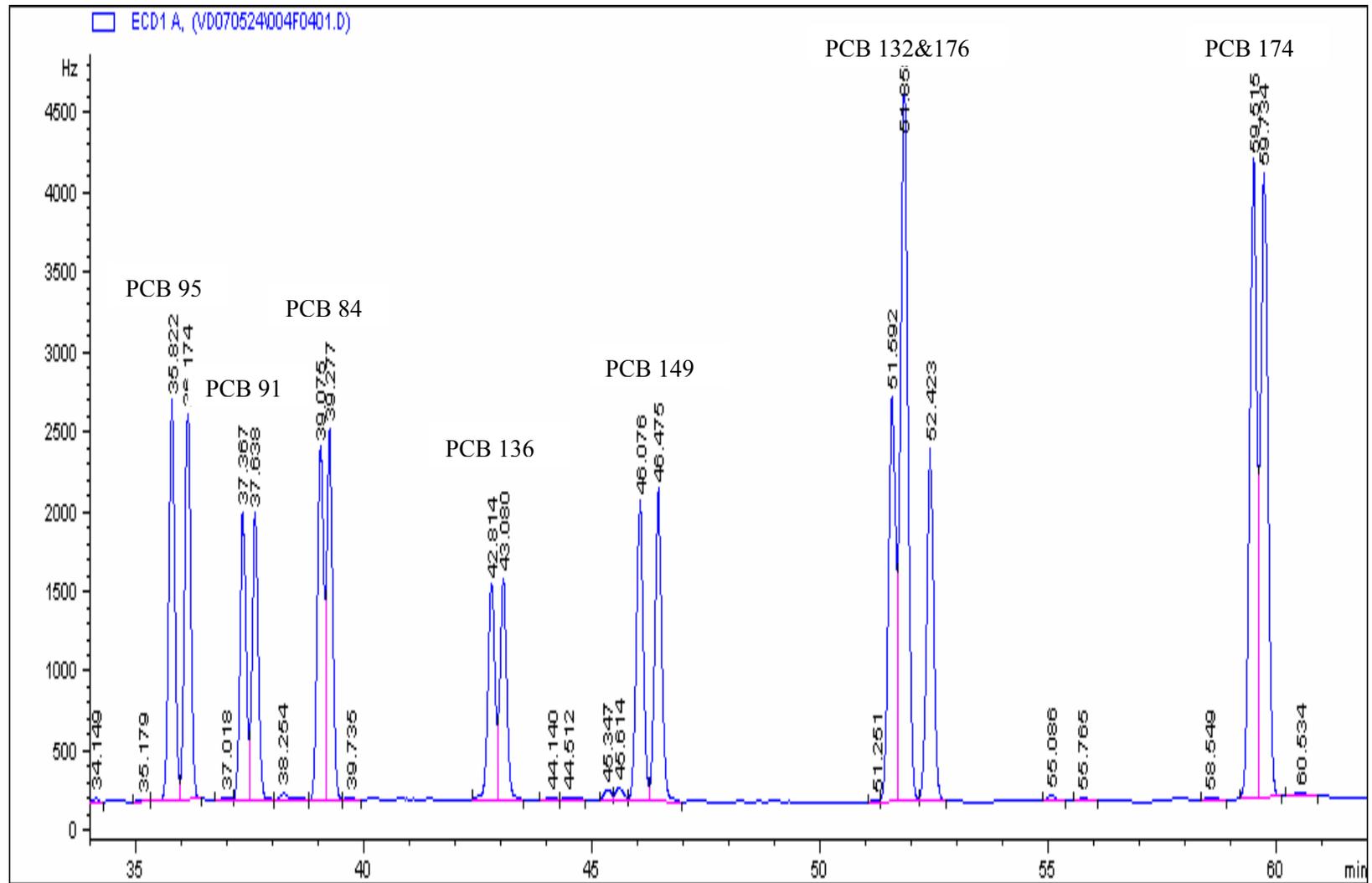


Figure 3.1 Chromatogram of atropisomeric PCBs standard on GC Chirasil-Dex column.

CHAPTER 4

RESULTS AND DISCUSSION

The results of the analysis of achiral PCBs will be presented in this section and will be followed by results of the chiral PCB analysis. Results obtained from the chiral analysis will be used to provide a better understanding of the enantioselectivity of PCB atropisomers in food webs, an understanding which can not be confirmed from congener-specific PCBs analysis alone. Comparisons with previous research at Lake Hartwell and other locations will also be discussed.

4.1. Achiral PCBs analysis

4.1.1. Achiral PCBs analysis in the aquatic food web.

Total PCB concentrations were found to be lowest in sediments (15.00 ± 0.14 ng/g wet wt) and increased through clams, mayflies, and fish (2680.66 ± 187.06 ng/g wet wt); both extremes were measured in samples from site 3 (Table 4.1 and Figure 4.1). Sample size for each type and location are shown in Table A.2 in the Appendix. Generally, fish had much higher total PCB concentrations at all sampling sites compared to the other samples, supporting the common observation that PCB concentrations are significantly related to lipid content (Table 4.2). Mayflies had greater levels of PCBs than clams, implying that time could also play a role in bioaccumulation of PCBs, because larval mayflies generally live in surface sediments for one year or more taking up PCBs from particulate organic matter. Clams were deployed at all four sites for up to 60 days, which

may not be adequate time to reach equilibrium or steady state for the bioaccumulation of PCBs.

The lowest levels of PCB concentrations were determined in sediment samples and ranged from 15.00 ± 0.14 ng/g at site 3 to 25.00 ± 3.25 ng/g at site 4. No general trend showing decreasing concentration with distance from the source was observed in the sediment samples, which differs from previous observation by Farley et al. (1994) and. However, these values are well below $19,910 \pm 502$ ng/g and $2,970 \pm 200$ ng/g, which were measured in surface sediments by Germann (1988) and Sivey (2004), respectively, at one site (G30) in the Twelve Mile Creek arm of Lake Hartwell. This significant difference is probably due to transportation and then depositional characteristics of PCBs as they move from a more turbulent environment (stream) to a more quiescent environment (lake). Another possible reason for a low concentration of PCBs in sediment in Twelve Mile Creek is that the sediment samples contained a significant sand layer, which potentially has a lower total organic carbon (TOC) content, leading to less PCB-adsorbent capacity.

Due to their low solubility, PCBs were likely sorbed to detritus or algae that were filtered by the clams during the 60-day deployment. PCB bioaccumulation in the clams decreased from 195.74 ± 30.41 ng/g wet wt at site 2 to 39.61 ± 9.24 ng/g wet wt at site 6 with increasing distance from the source of contamination (Table 4.1). Mayflies and fish did not follow the same trend of decreasing total PCBs concentration with distance from the source. Highest PCB concentrations were measured for mayflies at site 2, but total PCB concentrations did not decrease consistently from site 2 through site 5. However, the

upstream sites (sites 2 and 3) had higher total PCBs levels than the downstream sites (sites 4 and 6) in clam and fish samples, which support the general observation of decreasing concentration with distance. But this observation was not found for the mayflies in the aquatic food webs. A species-dependent PCB pattern was observed in the aquatic food web. There was also a difference for total PCB concentrations between the more mobile species (fish and mayflies) and the stationary species (clams).

The contribution of each PCB homolog group to total PCBs in the aquatic food web was also examined. In sediment, tetra-chloro biphenyls were found to be dominant at all sites and hexa and hepta were important at two sites (Figure 4.2). These contributions were similar to the observation by Sivey (2004) for near-surface sediments in the Twelve Mile Creek arm of Lake Hartwell, in which higher chlorinated congeners (tetra- and hepta-chloro biphenyls) dominated.

A Gaussian or normal distribution with penta-chlorobiphenyls dominating was observed for fish, but was not observed for sediment, clams and mayflies (Figures 4.2, 4.3, 4.4, and 4.5). A bimodal distribution was observed for sediment at sites 2, 4, and 6, in which a overlapping Gaussian or normal pattern was represented from tri- to penta-chlorobiphenyls for all three sites, while each site displayed a different trend from penta- to octa-chloro biphenyls. This observation could be more likely related to transport of particles than to distribution in the Aroclors, because if the observation was caused by the distribution in the Aroclors, a bimodal distribution would have been observed in all samples. Clams show a pattern that is statistically different from a normal or Gaussian distribution at site 2 (upstream), in which the tri-, tetra-, and penta-chloro biphenyl

groups occurred more abundantly. Tri and tetra groups are lower chlorinated congeners, account for most of the weight percent in Aroclor 1016 (Appendix-Table A1) and also have higher aqueous solubility, which may lead to higher concentrations in the water columns and thus more sorption to suspended particles. The clams from the other sites have tetra- and penta-chlorobiphenyls at higher levels and the distributions were also statistically different from normal. Time could be a factor for this difference, suggesting that 60-day deployment was not enough time for clams to take up PCBs and reach a steady state or equilibrium. In general, the homolog distribution of tetra-, penta-, and hexa-chlorobiphenyls was dominant for species except tri-chlorobiphenyls for clams at site 2, while tetra-, hexa-, and hepta-chlorobiphenyls dominated in sediments. The tetra-, penta-, and hexa-chlorobiphenyl PCB homologs are highly persistent components in the environment. The achiral analysis indicates that the homologs were apparently not metabolized in the sampled components of the aquatic food web. Of these groups, only PCBs 118 (2,3',4,4',5-pentachlorobiphenyl) and 153 (2,2',4,4',5,5'-hexachlorobiphenyl) were always found as the most abundant congeners. Concentration of PCBs 118 and 153 in the aquatic and riparian samples is given in Tables 6 and 7 in the Appendix. This finding is similar to previous observations of aquatic biota in the Twelve Mile Creek and in the Twelve Mile Creek arm of Lake Hartwell (Walters, 2006, personal communication) supporting observations obtained from the aquatic food web.

Table 4.1 Total PCB concentrations \pm standard deviations (ng/g wet wt) in the aquatic samples (Fall-05). Samples size is shown in Table A.2 in the Appendix.

	Sediments	Clams	Mayflies	Fish
Site 2	19.01 \pm 3.56	195.74 \pm 30.41	750.14 \pm 69.07	2436.84 \pm 168.04
Site 3	15.00 \pm 0.14	70.77 \pm 13.47	187.78 \pm 20.67	2680.66 \pm 187.06
Site 4	25.96 \pm 9.70	71.55 \pm 6.61	342.58 \pm 47.29	1827.94 \pm 162.84
Site 6	25.11 \pm 3.54	39.61 \pm 9.24	273.14 \pm 37.03	2121.18 \pm 162.84

Table 4.2 Lipid percent (%) \pm standard deviations in the aquatic species (Fall-05)

	Clams	Mayflies	Fish
Site 2	0.172 \pm 0.078	0.207 \pm 0.019	0.740 \pm 0.063
Site 3	0.185 \pm 0.026	0.172 \pm 0.061	1.049 \pm 0.230
Site 4	0.191 \pm 0.077	0.182 \pm 0.039	1.701 \pm 0.464
Site 6	0.239 \pm 0.026	0.142 \pm 0.009	0.717 \pm 0.147

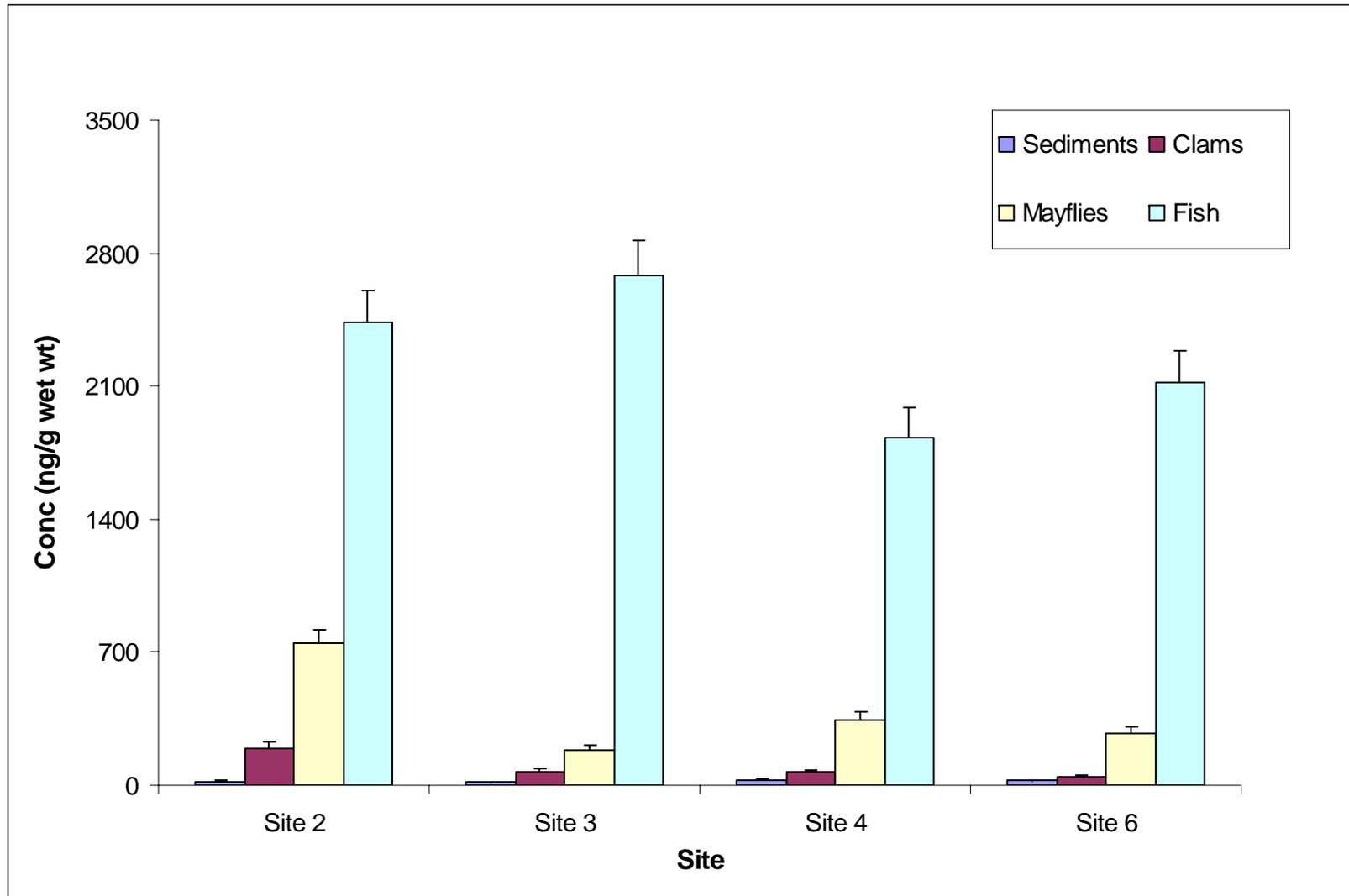


Figure 4.1 Total PCB concentrations in the aquatic food web (Fall-05); error bars represent standard deviations.

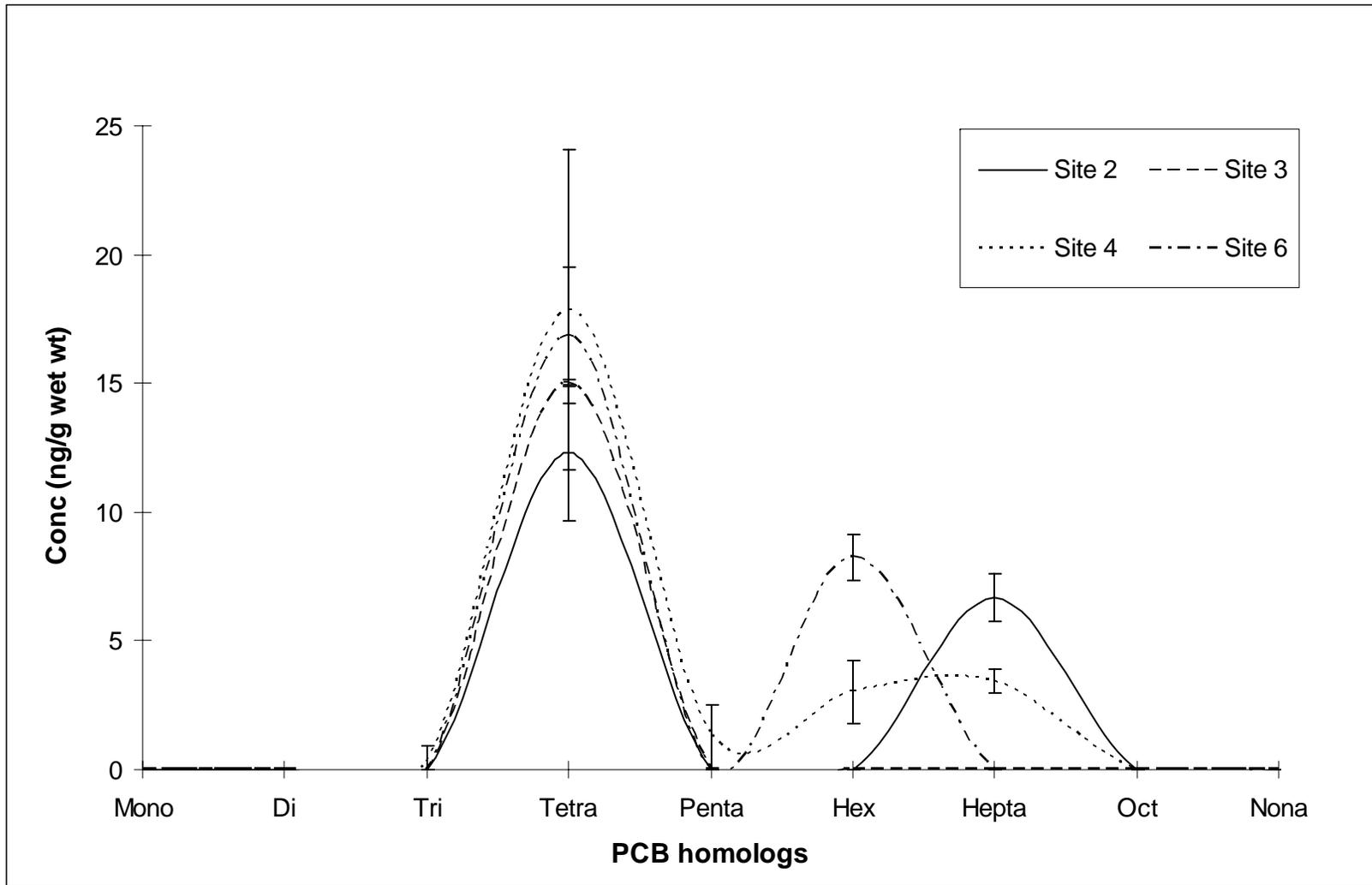


Figure 4.2 Distribution of PCB homologs concentration in sediments (Fall-05); error bars represent standard deviations of PCB groups measured

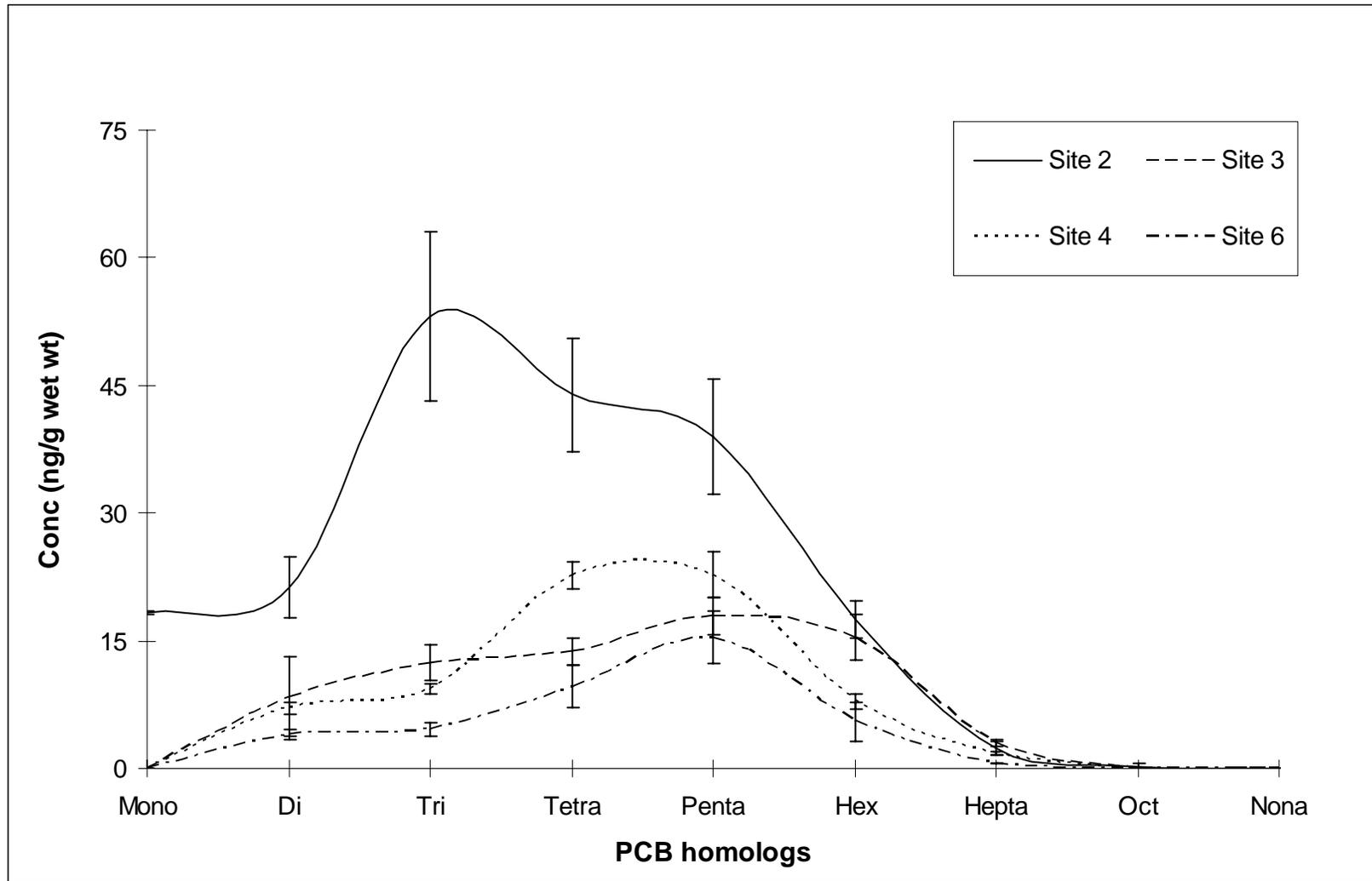


Figure 4.3 Distribution of PCB homologs concentration in clams (Fall-05); error bars represent standard deviations of PCB groups measured.

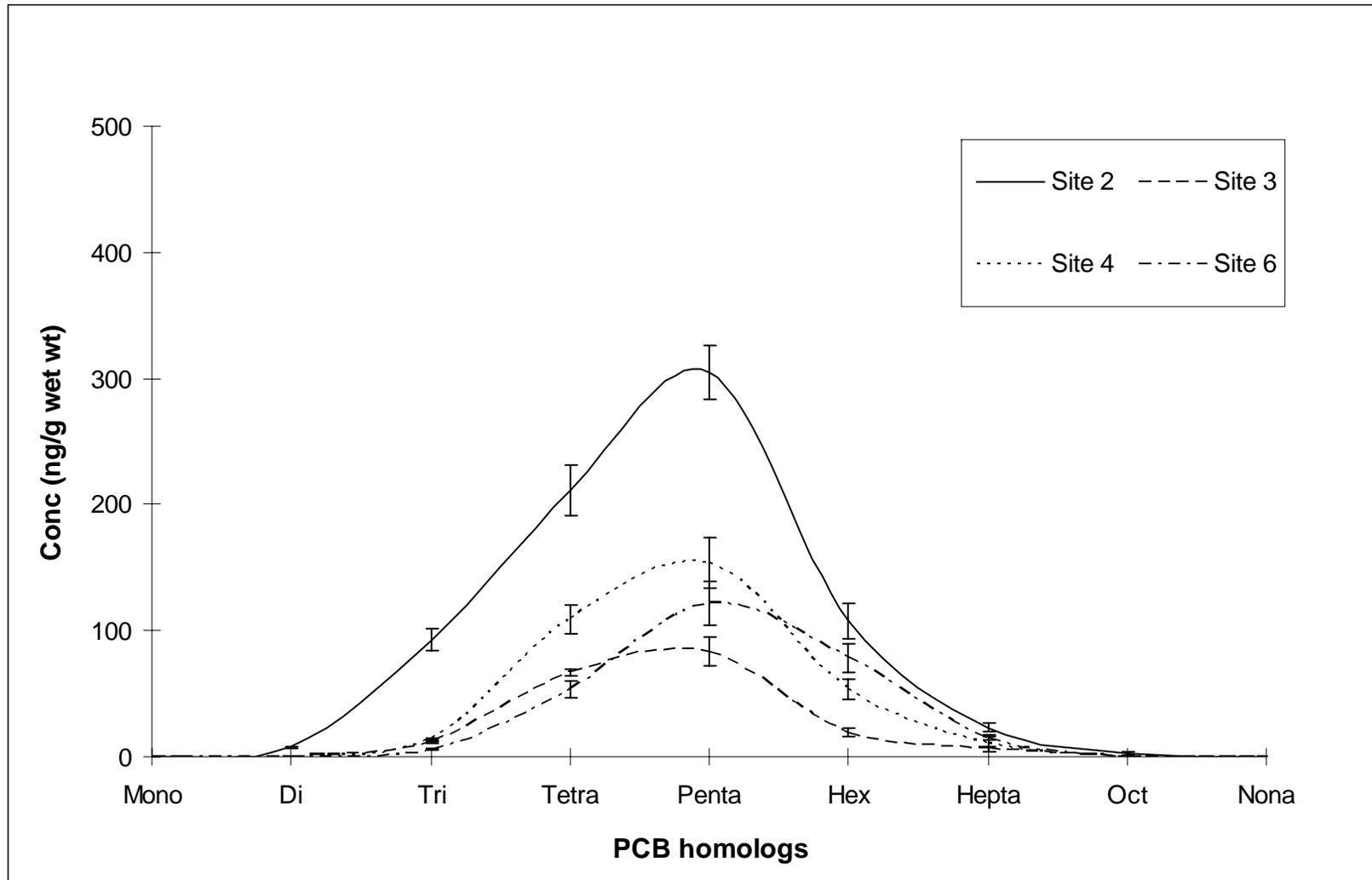


Figure 4.4 Distribution of PCB homologs concentration in mayflies (Fall-05); error bars represent standard deviations of PCB groups measured.

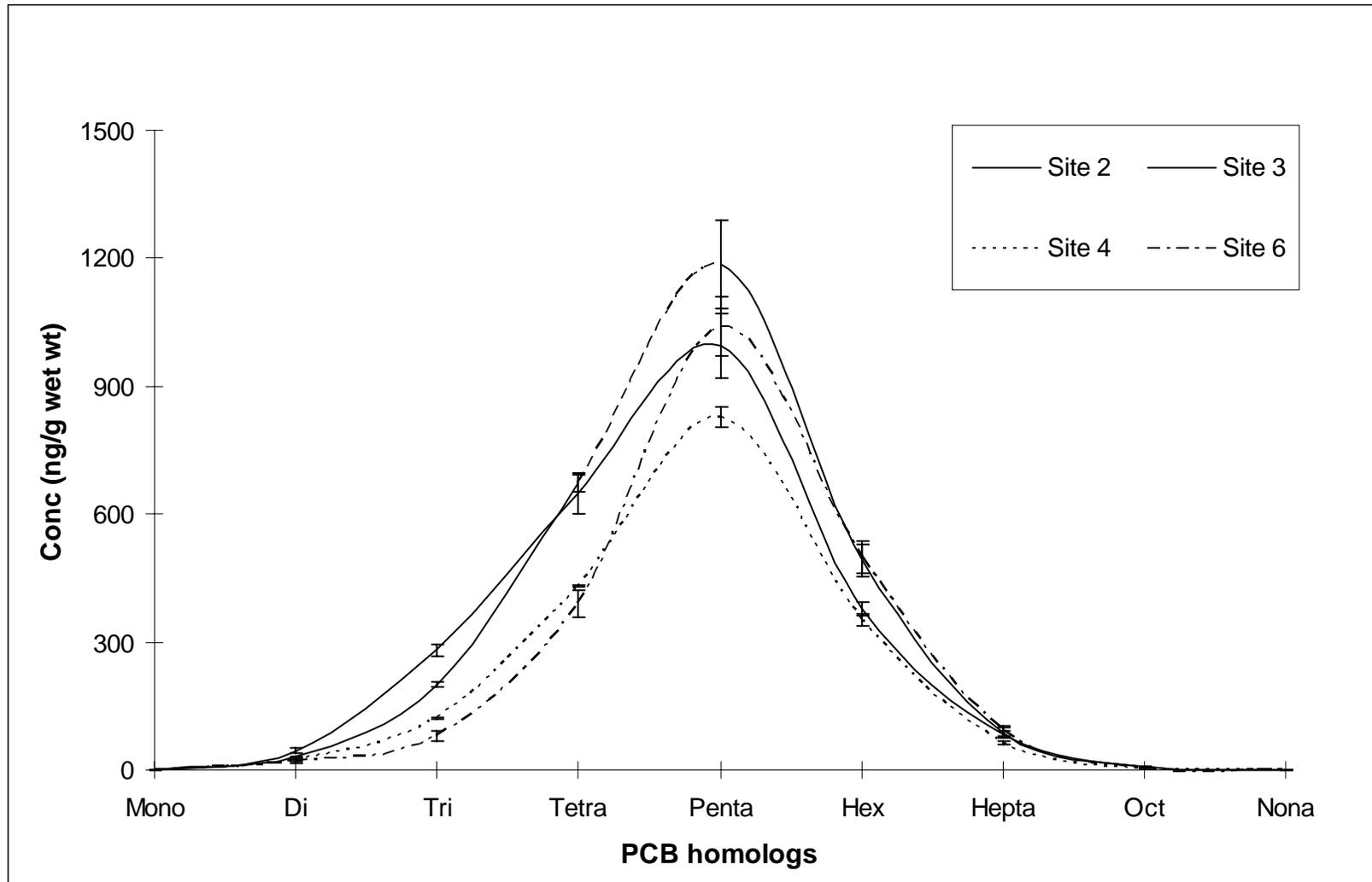


Figure 4.5 Distribution of PCB homologs concentration in fish (Fall-05); error bars represent standard deviations of PCB groups measured.

4.1.2. Achiral PCBs analysis in riparian samples.

Total PCB concentrations varied among sites and seasons (Figure 4.6). In spiders, the total PCBs concentration at site 3 was found to be highest in Spring 2005 (2430.50 ± 306.59 ng/g wet wt) and also found to be lowest in Summer 2005 (387.91 ± 175.55 ng/g wet wt) (Table 4.3). Note the samples size can be found in the Table A.3 in the Appendix. This finding suggests a relative variability of PCBs with time at site 3, which may be caused either by an abundance of contaminated prey sources in the spring or a change in prey diet in the summer with more prey from uncontaminated sources. A comparison of total PCB concentrations in Fall 2005 between mayfly and spider samples at sites 2, 3, and 6 showed that concentrations were all magnified in spiders, suggesting that adult mayflies could be a source of prey for the spiders. Since most spiders live for only one or two years, PCBs biotransformation is not likely to occur in the spiders. Similar total PCB levels in both Summer and Fall samples for the upstream (site 1) and downstream (site 6) sites suggest a similar mechanism of PCBs biotransformation in spider species or similar patterns of contamination in prey at both sites. Additionally, a similar trend with time was observed between sites 1 and 6, in which concentrations decreased from Spring to Summer and then significantly increased from Summer to Fall (Figure 4.6). This could be due to an increase in concentration in the summer and then diluting concentration in PCBs-contaminated or less contaminated prey in the spring and fall. No change occurred between the Spring and Summer at site 2 (Table 4.3), implying similar prey sources for the two seasons. Thus, no site-dependent PCB patterns were

observed for species of spider. However, total PCBs concentrations showed changes by season for each site, except for site 2 in Spring and Summer.

The patterns of PCB homologs distribution in spiders with season are presented in Figure 4.7, 4.8, and 4.9. The curves consistently followed a Gaussian distribution in Summer and Fall at all four sites, but not in Spring 2005. This difference might be explained because the spiders were immature in Spring leading to less PCBs bioaccumulation compared to Summer and Fall. The finding is similar to what was found in fish in the aquatic food web, inferring that PCBs contamination in these species is potentially derived from the same source or that the fish and spiders are higher in the food web than clams and mayflies.

Table 4.3 Total PCB concentrations \pm standard deviations (ng/g wet wt) in spiders with seasons and sites. Values are the average of any detection in spider species. The number of samples is shown in Table A.3 in the Appendix.

	Spring-05	Summer-05	Fall-05
Site 1	755.70 \pm 269.21	536.53 \pm 157.16	1505.91 \pm 332.25
Site 2	1814.24 \pm 231.02	1830.24 \pm 449.23	1346.88 \pm 409.61
Site 3	2430.50 \pm 306.59	387.91 \pm 175.55	558.28 \pm 169.85
Site 6	866.98 \pm 177.65	534.55 \pm 267.37	1680.27 \pm 405.09

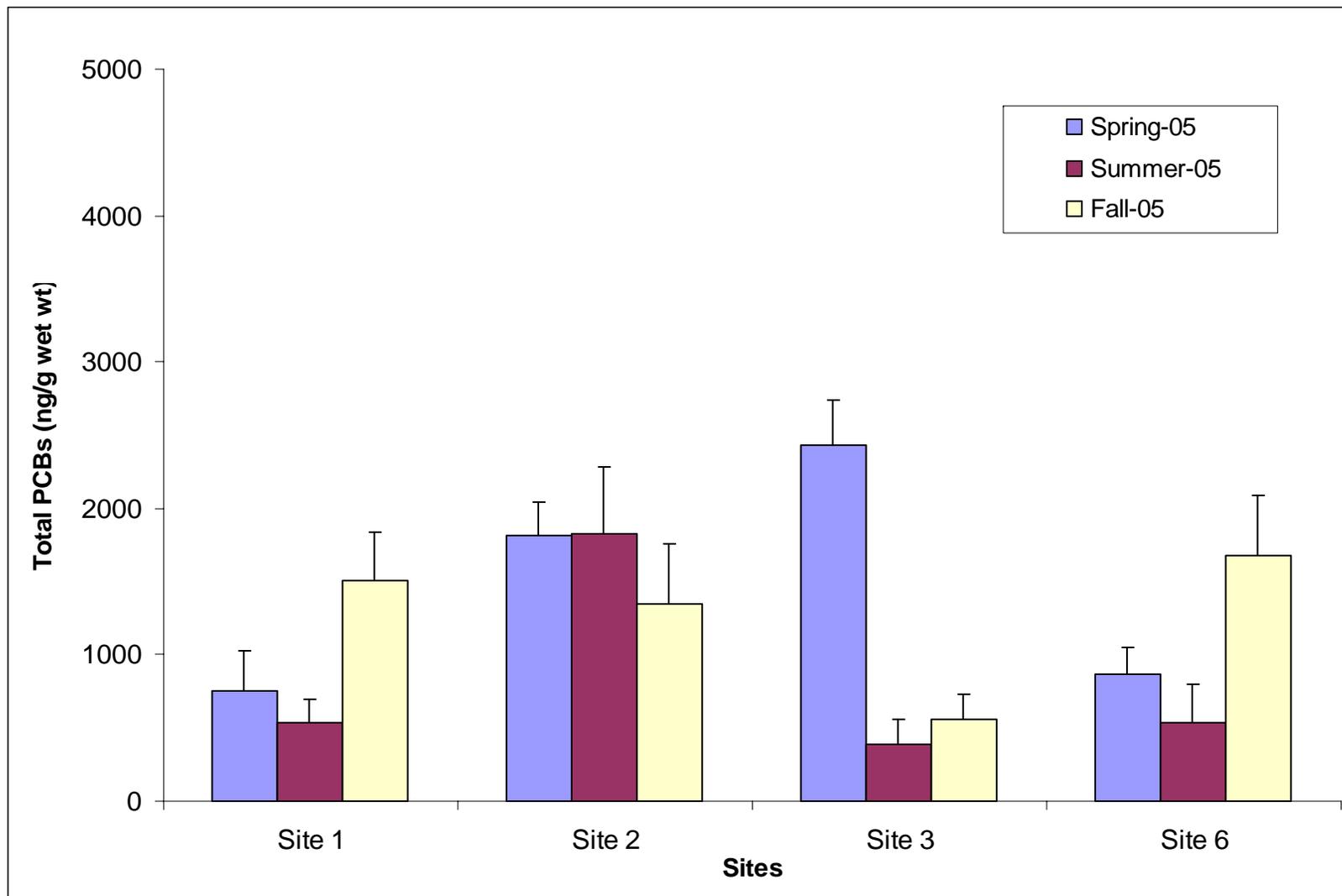


Figure 4.6 Total PCB concentrations in spiders with seasons and sites; error bars represent standard deviations among species.

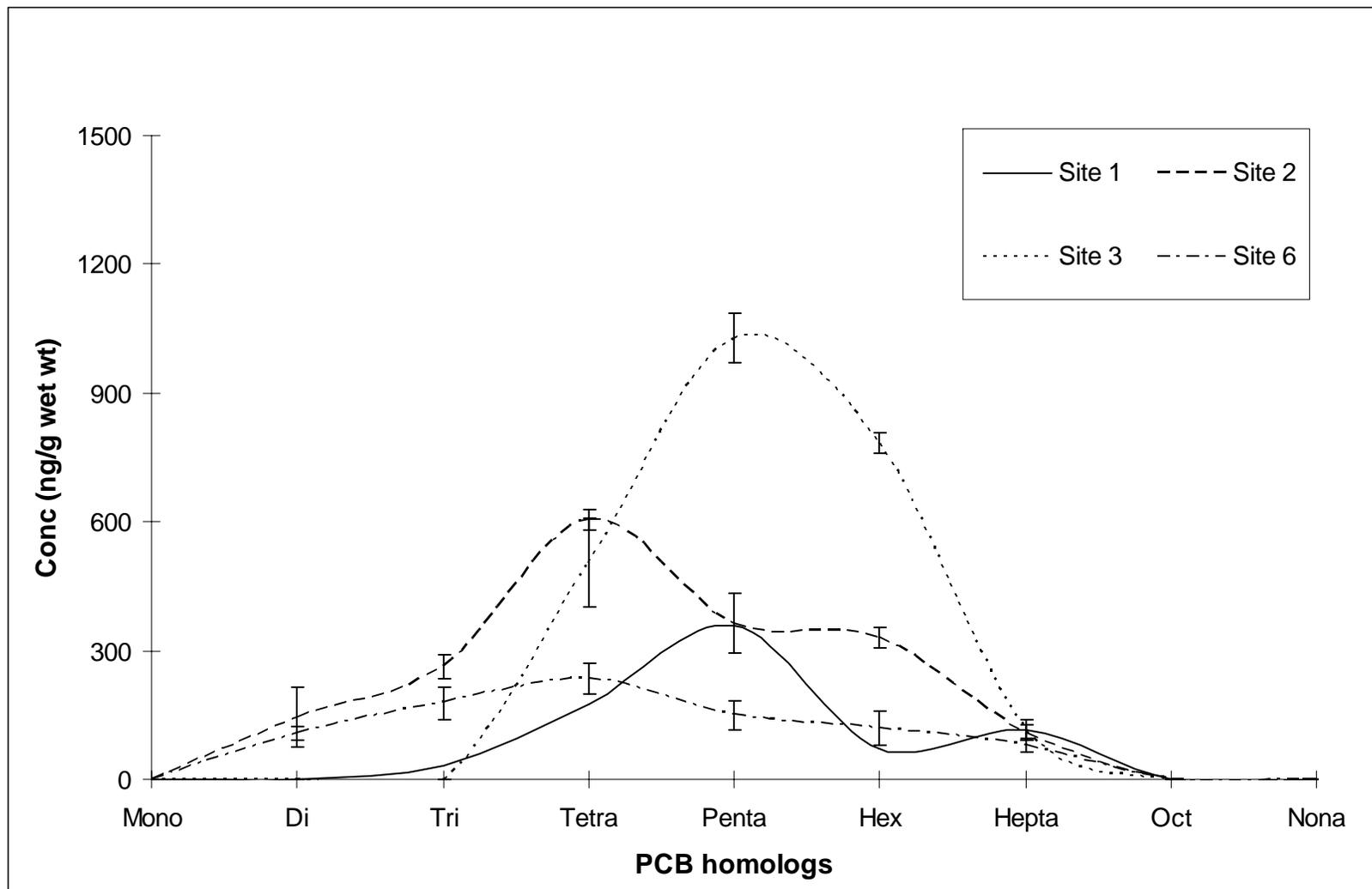


Figure 4.7 Distribution of PCB homologs concentration in spiders (Spring-05); error bars represent standard deviations of PCB groups measured

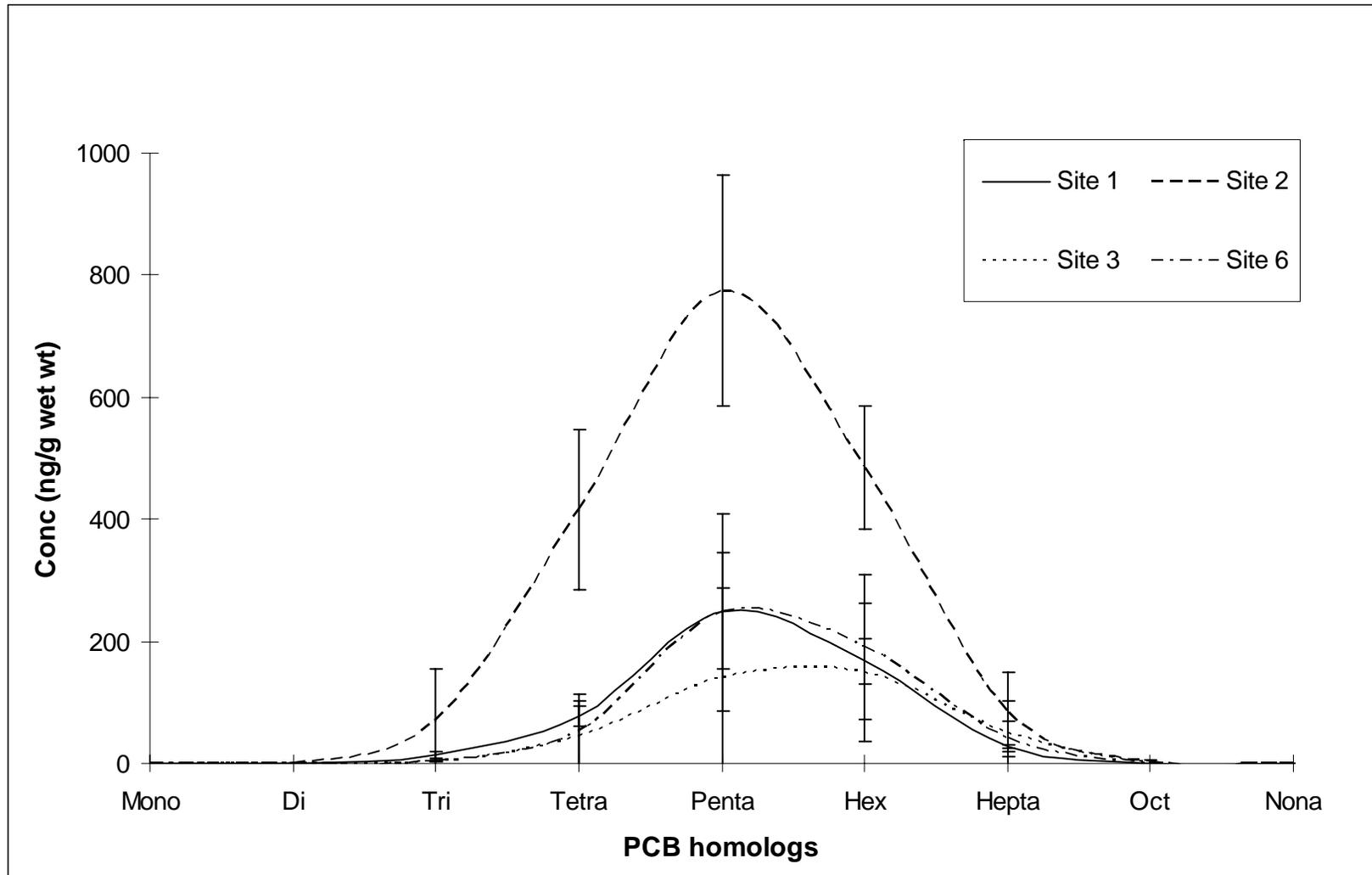


Figure 4.8 Distribution of PCBs homologs concentration in spiders (Summer-05); error bars represent standard deviations of PCB groups measured.

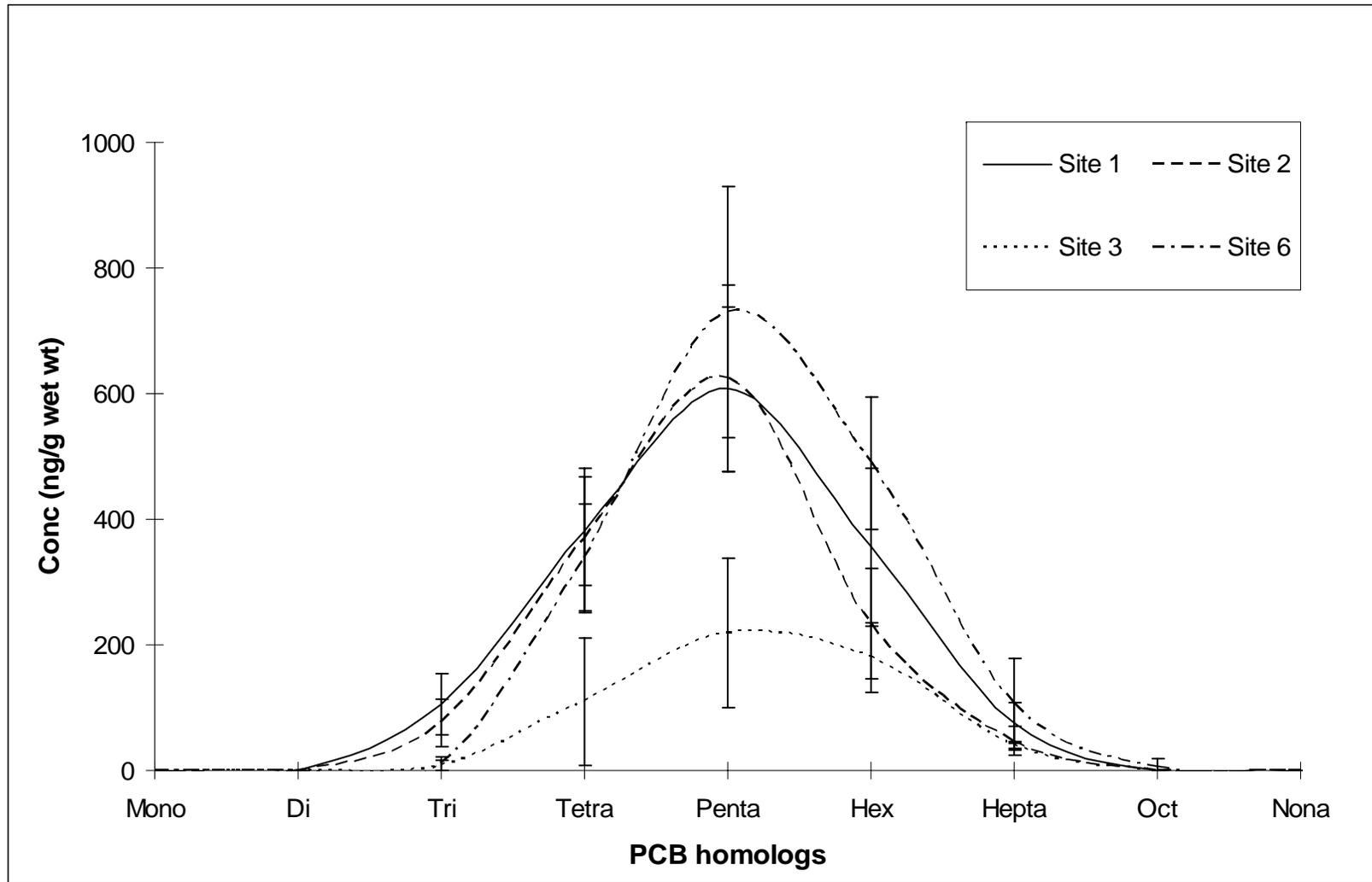


Figure 4.9 Distribution of PCB homologs concentration in spiders (Fall-05); error bars represent standard deviations of PCB groups measured.

PCBs in amphibians were only measured in Summer and Fall 2005 at sites 2, 3, and 6 due to limited samples. The total PCBs concentration was highest at site 6 in Fall 2005 (3207.25 ± 357.64 ng/g wet wt) and lowest at site 2 in Summer 2005 (30.19 ± 5.65 ng/g wet wt) (Table 4.4). Number of samples can be found in Table A.3 in the Appendix. The relatively low concentrations at site 2 and 6 in Summer could be evidence that a biodegradation process was more significant than the bioaccumulation process. PCB concentrations increased significantly from the Summer to Fall at sites 2 and 6 but increased only slightly at site 3 (Figure 4.10). The significant increases at sites 2 and 6 could have resulted from more contaminated prey than at site 3. Different total PCB concentrations between spiders and amphibians were also found. The spider samples have higher total PCBs at sites 2 and 6 in the summer while the amphibians have higher concentrations at site 3 in both Summer and Fall. A Gaussian distribution was also observed for amphibians at site 2 in Summer and all three sites in Fall (Figure 4.11 and 4.12). The distribution reflected the changes in total level of PCBs. Most of the total PCBs concentration resulted from the tetra-, penta-, and hexa-chlorobiphenyl groups, which represent the more bioaccumulative PCB congeners.

Table 4.4 Total PCB concentrations \pm standard deviations (ng/g wet wt) in amphibians in Summer and Fall 2005. Values are the average of any detection in amphibian species. Samples size is shown in Table A.3 in the Appendix.

	Summer-05	Fall-05
Site 2	30.19 ± 5.65	438.51 ± 74.90
Site 3	787.46 ± 166.19	844.27 ± 145.18
Site 6	31.02 ± 9.62	3207.55 ± 357.64

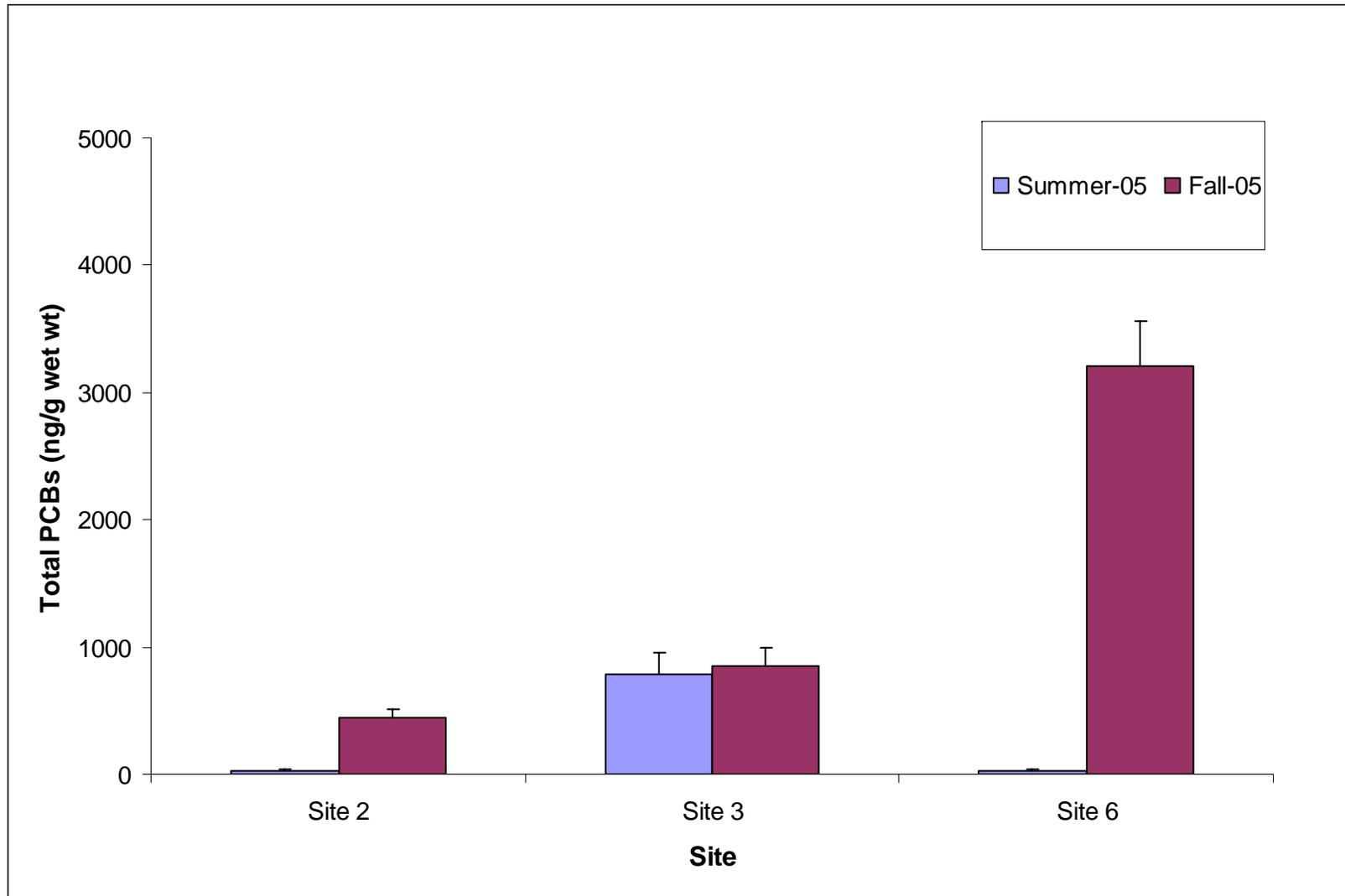


Figure 4.10 Total PCB concentrations in amphibians in Summer and Fall 2005; error bars represent standard deviations.

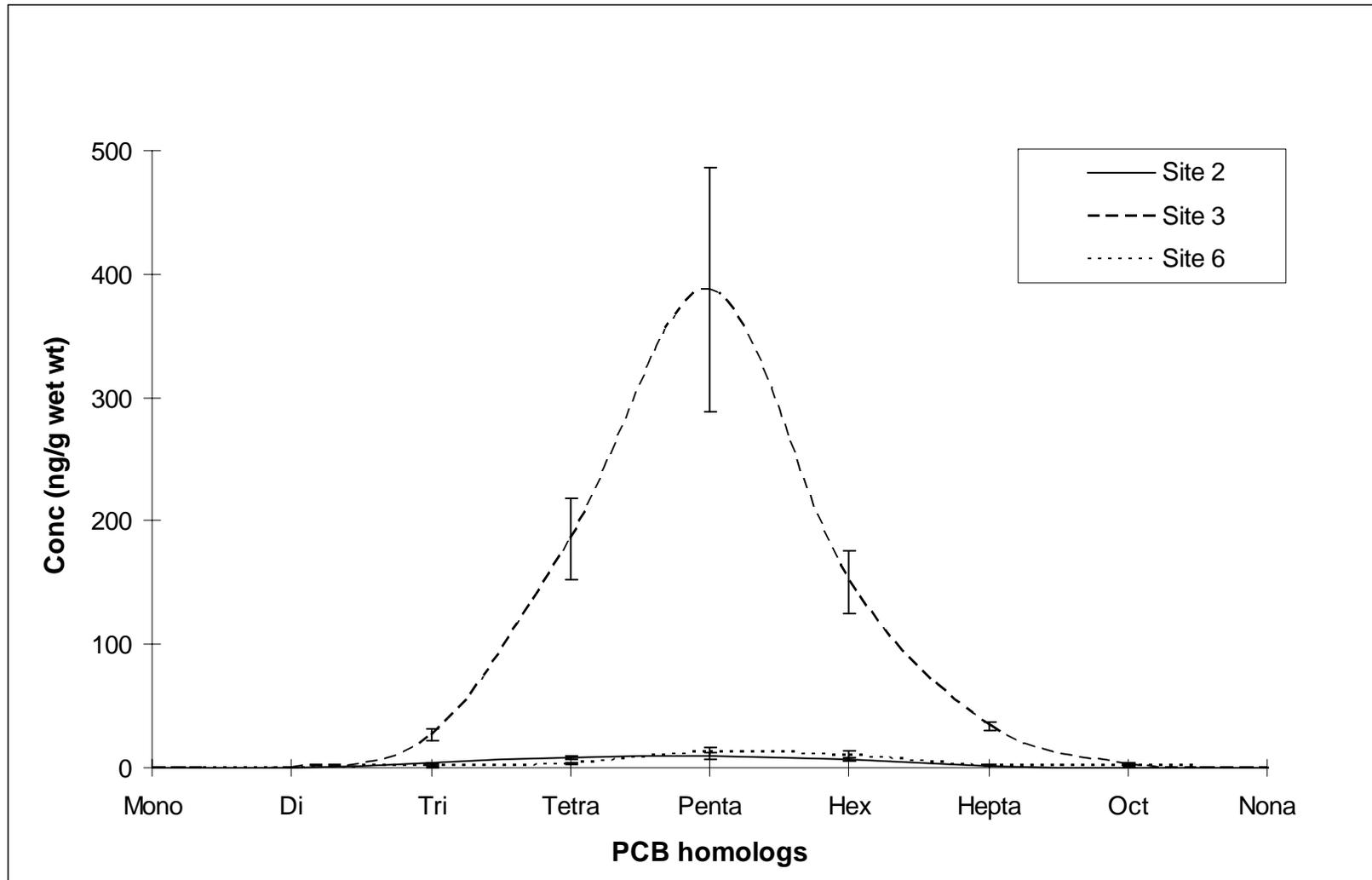


Figure 4.11 Distribution of PCB homologs concentration in amphibians (Summer-05); error bars represent standard deviations of PCB groups measured.

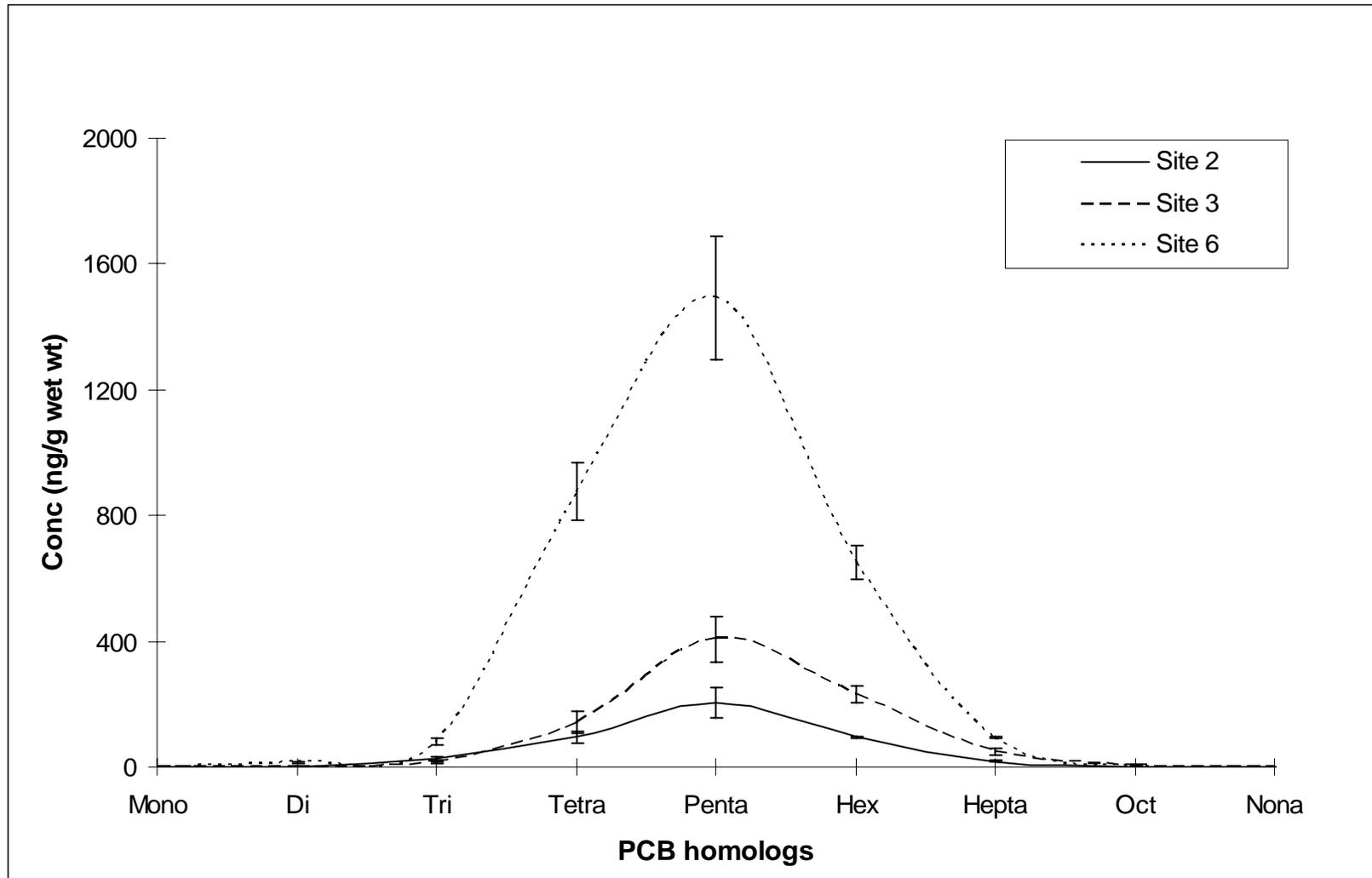


Figure 4.12 Distribution of PCB homologs concentration in amphibian (Fall-05); error bars present standard deviations of PCB groups measured.

Achiral PCBs analysis has provided a general picture of PCB variations in the aquatic and riparian food webs in Twelve Mile Creek. There were no consistent site- or time-dependent variations in PCBs for riparian samples because of differences in concentrations between the amphibians and spiders, which are likely to be prey for the amphibians. The lack of a pattern may be due to changes in the abundance of contaminated prey. However, a species-dependent PCBs pattern was observed for samples in the aquatic food web. Fish samples had consistently higher concentrations of PCBs compared to the other samples, which could be due to higher lipid content in the fish. Mayflies had higher PCB concentrations than PCB concentrations in clams at all four sites, suggesting that time may play a role in bioaccumulation of PCBs and reach to steady state or equilibrium. Sediments indicated the lowest PCB concentrations at all four sites inferring a low content of organic matter in the sediment samples. In addition, the total levels of PCBs concentration showed a more consistent pattern in the aquatic species than the riparian species. The finding might be derived from a variety of riparian species or trophic level of species in the riparian food web compared to the aquatic food web.

A similarity of PCB homologs distribution in each species, in which tetra-, penta-, and hexa-chlorobiphenyl groups were found most abundantly, suggests that either the bioaccumulation potential for congeners is similar in both food webs or that the source(s) of PCB contamination to Twelve Mile Creek are similar in congener distribution. It is likely that PCBs contamination in food webs in Twelve Mile Creek is derived from one source - the Sangamo Weston plant. The results also lend support for the hypothesis that

the mechanisms of PCBs biodegradation, accumulation, and biotransformation are dependent on the environmental factors. The data obtained from congener-specific PCBs analysis will be useful for chiral analysis and for tracking PCBs biodegradation in the environment.

4.2. Chiral PCBs analysis

4.2.1. Enantiomeric composition of PCBs in the aquatic food web.

Table 4.5 represents EF values of PCB atropisomers measured in the aquatic samples. No atropisomeric PCBs were found in sediment, but some differences in EF values were found among species. This study showed a wide variation in chiral signatures between species and sites investigated. The EF values for PCBs 95, 91, and 149 were found to be nonracemic at all four sites with EF values less than 0.5 except for the EF values of PCB 95 at site 2, and PCB 149 at site 3. The EF values for PCBs 84 and 136 were also found to be nonracemic, but with EFs greater than 0.5. The EF values for PCB 174 were found to be racemic at all four sites.

Table 4.5 Enantiomeric fractions (EFs) \pm standard deviations for the aquatic food web. Values are the average of any detection in all media. ND: non detection in any samples extracted.

	Site 2	Site 3	Site 4	Site 6
PCB 95	0.50 \pm 0.01	0.17 \pm 0.01	0.28 \pm 0.02	0.31 \pm 0.03
PCB 91	0.37 \pm 0.01	0.25 \pm 0.02	0.25 \pm 0.02	0.25 \pm 0.02
PCB 84	0.75 \pm 0.02	0.64 \pm 0.01	0.55 \pm 0.03	ND
PCB 136	0.78 \pm 0.02	0.70 \pm 0.03	0.80 \pm 0.03	0.80 \pm 0.02
PCB 149	0.26 \pm 0.03	0.51 \pm 0.01	0.35 \pm 0.01	0.36 \pm 0.02
PCB 174	0.51 \pm 0.01	0.51 \pm 0.003	0.51 \pm 0.005	0.51 \pm 0.001

The EF values for PCB 84 were strongly nonracemic in clams and mayflies (Figure 4.13 and 4.14), while the congener was not detected in sediment and fish (Table 4.6). Neither enantiomer of PCB 84 was detected in the fish, suggesting that metabolism in the fish may have occurred, because mayflies are a food source for the fish. Concentrations of PCB 84 were detected in clams at all sites and followed a trend of decreasing concentration with distance from the source (Table 4.6). The concentrations of PCB 84 in clams measured at three sites are above the detection limit for the chiral GC analysis for PCB 84. However, no correlations between EF and concentrations were found in the clam samples. Although Wong et al. (2001b) did not measure PCB 84 in the Lake Hartwell biota or in the biota from other locations, the results from site 2 suggest that fish may metabolize PCB 84 completely. A capability for metabolizing PCB 84 by cytochrome P450 1A and cytochrome P450 2B was described by Kannan et al. (1995).

Table 4.6 Concentration of PCB 84 (236-23) (ng/g wet wt) in the aquatic food web

	Site 2	Site 3	Site 4	Site 6
Sediment	ND	ND	ND	ND
Clams	2.49 ± 1.02	0.73 ± 0.3	0.5 ± 0.1	BQL
Mayflies	2.82 ± 0.9	ND	ND	ND
Fish	ND	ND	ND	ND

Similar to PCB 84, there was a trend of decreasing concentrations of PCB 91 in the clams with distance (Table 4.7). Biomagnification of PCB 91 was indicated from mayflies to fish, because the concentrations in the fish were significantly larger than in mayflies. Chiral analysis showed that EF values for PCB 91 were only measurable in mayflies and fish and exhibited significantly nonracemic values, especially for the fish

samples (Figure 4.14 and 4.15). The measurement of non-racemic EFs for PCB 91 is also similar to previous measurement by Wong et al. (2001b) in fish from Lake Hartwell. Significant differences in the EF values measured for PCB 91 in mayflies and fish suggests metabolism of PCB 91 at higher trophic level. No detection of EF values for PCB 91 was observed in sediment and clams, which differs from nonracemic EF values in National Water Quality Assessment Program (NAWQA) sediments and in bivalves from the NAWQA samples (Wong et al., 2001b). Wong et al. (2001b) presented evidence that the clams metabolized PCB 91. The mechanism of metabolizing PCB 91 can be governed by both cytochrome P450 enzymes (Kannan et al., 1995).

Table 4.7 Concentration of PCB 91 (236-24) (ng/g wet wt) in the aquatic food web

	Site2	Site 3	Site 4	Site 6
Sediments	ND	ND	ND	ND
Clams	0.89 ± 0.23	0.28 ± 0.1	0.21 ± 0.09	BQL
Mayflies	2.99 ± 2.03	ND	BQL	0.72 ± 0.14
Fish	ND	ND	11.60 ± 4.03	13.47 ± 3.38

Measurable concentrations of PCB 95 were found in clams, mayflies, and fish at four different sites (Table 4.8). No significant difference in concentration of PCB 95 was observed among mayflies and fish; therefore, there is no evidence for biomagnifications of PCB 95. No trend in concentrations with distance was observed for PCB 95. However, EFs for PCB 95 were found to be significantly nonracemic in clams (site 6) and mayflies (sites 2 and 4) (Figure 4.13 and 4.14), while EF was found to be racemic in the fish from site 6 (Figure 4.15). PCB 95 was not detected in any of the sediment samples. Wong et al. (2007) reported racemic EF values for PCB 95 in the surface sediment at three locations

(G30, G33, and G46) in Lake Hartwell. Additionally, non-racemic EF values for PCB 95 were observed in most fish and other biota sampled (bass, bluegills, crayfish, *Diporeia*, and mysids) in the Hudson River, Housatonic River, and Lake Superior (Wong et al., 2004). The EF values measured in fish from this research suggest that bioprocessing of PCB 95 occurred in fish; however, it is not clear if the bioprocessing is enantioselective. If the mayflies from sites 3 and 4 can be considered representative and a food source for fish, then the fish may be degrading the opposite enantiomer that the mayflies degrade to bring the EF back to 0.5. Wong et al. (2001b) measured EFs of PCB 95 in clams from rivers throughout the United States between 1992 and 1995. The EF values measured were non-racemic and greater than 0.5. The data also showed evidence that the clams may metabolize PCB 95. According to Kannan et al. (1995), PCB 95 can be metabolized by P450 2B enzymes

Table 4.8 Concentration of PCB 95 (236-25) (ng/g wet wt) in the aquatic food web

	Site2	Site 3	Site 4	Site 6
Sediments	ND	ND	ND	ND
Clams	ND	ND	ND	1.12 ± 0.5
Mayflies	ND	8.84 ± 2.03	17.22 ± 5.23	ND
Fish	12.05 ± 3.33	ND	ND	ND

Concentrations of PCB 136 were detected at all four sites in fish but the data did not follow a pattern with distance (Table 4.9). EF values strongly deviated from a value of 0.5 in clams and fish (Figure 4.13 and 4.15), while neither of the PCB 136 enantiomers was detected in sediment or mayflies (Figure 4.14). Surface sediments sampled at three sites as mentioned previously in Lake Hartwell were, however, found to be racemic for

PCB 136 (Wong et al., 2007). Wong et al. (2001b) also reported that fish species in Lake Hartwell had significantly nonracemic EFs of PCB 136 with an average of 0.295, which is inverse to an average EF of 0.8 in fish studied in Twelve Mile Creek. This is due to a different column used for this analysis. Apparently, PCB 136 does not bioaccumulate to any great extent in mayflies. There is no evidence from the fish data to support metabolism of PCB 136. However, Kannan et al. (1995) indicated that PCB 136 can be metabolized by P450 2B enzymes.

Table 4.9 Concentration of PCB 136 (236-236) (ng/g wet wt) in the aquatic food web

	Site2	Site 3	Site 4	Site 6
Sediments	ND	ND	ND	ND
Clams	0.43 ± 0.11	0.76 ± 0.22	BQL	ND
Mayflies	ND	ND	ND	ND
Fish	2.40 ± 0.5	3.19 ± 0.65	2.25 ± 1.02	2.75 ± 0.94

PCB 149 was detected at all sites for clams and mayflies, and at all sites except site 2 for fish (Table 4.10). Concentrations of PCB 149 were significantly higher in fish than in clams and mayflies. The clams show evidence of decreasing concentration with distance. Chiral analysis of PCB 149 found EFs to deviate strongly from 0.5 in all species (Figures 4.13, 4.14, and 4.15). Clams and fish had EFs that were significantly different than mayflies. This observation is evidence for metabolism of PCB 149 in the aquatic species. Bruhn et al. (1995) also reported that PCB 149 can be metabolized by P450 2B isozyme activity. Wong et al (2001b) showed that the enantioselective preference of PCB 149 was reversed between fish and bivalves in NAWQA samples (EF>0.5 for fish;

EF<0.5 for bivalves). This is due to the use of a different column for the measurement of PCB 149 in fish from Lake Hartwell.

Table 4.10 Concentration of PCB 149 (236-245) (ng/g wet wt) in the aquatic food web

	Site2	Site 3	Site 4	Site 6
Sediments	ND	ND	ND	ND
Clams	2.75 ± 0.25	BQL	1.04 ± 0.44	0.60 ± 0.11
Mayflies	10.62 ± 3.23	BQL	2.45 ± 0.32	7.73 ± 2.21
Fish	ND	49.81 ± 9.45	36.90 ± 7.35	50.29 ± 12.23

Concentrations of PCB 174 (236-245) were measured at all four sites for fish and only one site for mayflies (Table 4.11). Concentrations of PCB 174 were similar among sites in fish showing no trend with distance, but the concentrations in fish were higher than the concentrations in mayflies. Due to the low concentration, chiral analysis of PCB 174 did not provide an EF for mayflies. The EF values in fish were racemic in fish (Figure 4.15). While racemic and non-racemic EF values were observed in the surface sediments in Lake Hartwell and in bivalves collected from rivers throughout the United States, respectively (Wong et al., 2006; Wong et al., 2001b). The observations of EF for the Twelve Mile Creek species provide no evidence of metabolism of PCB 174 for aquatic food web. But Kannan et al. (1995) indicated that PCB 174 can be metabolized by the P450 2B isozymes.

Table 4.11 Concentration of PCB 174 (2345-236) (ng/g wet wt) in the aquatic food web

	Site2	Site 3	Site 4	Site 6
Sediments	ND	ND	ND	ND
Clams	ND	ND	ND	ND
Mayflies	0.86 ± 0.33	ND	ND	ND
Fish	5.42 ± 1.21	6.38 ± 2.14	4.61 ± 0.89	6.48 ± 1.23

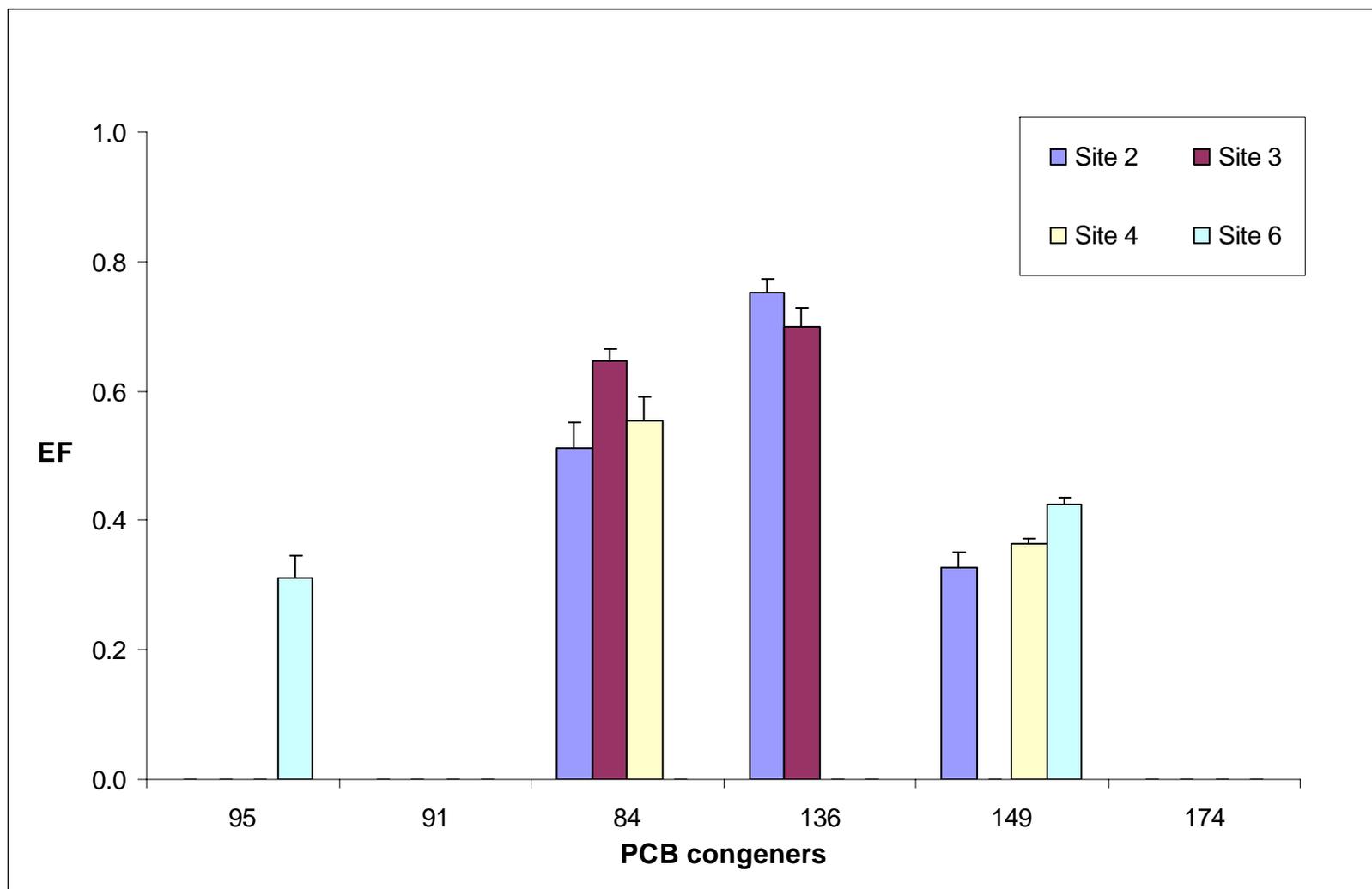


Figure 4.13 Enantiomeric fractions (EFs) of PCB atropisomers in clams; the horizontal line indicates a racemic value of 0.5; error bars present standard deviations

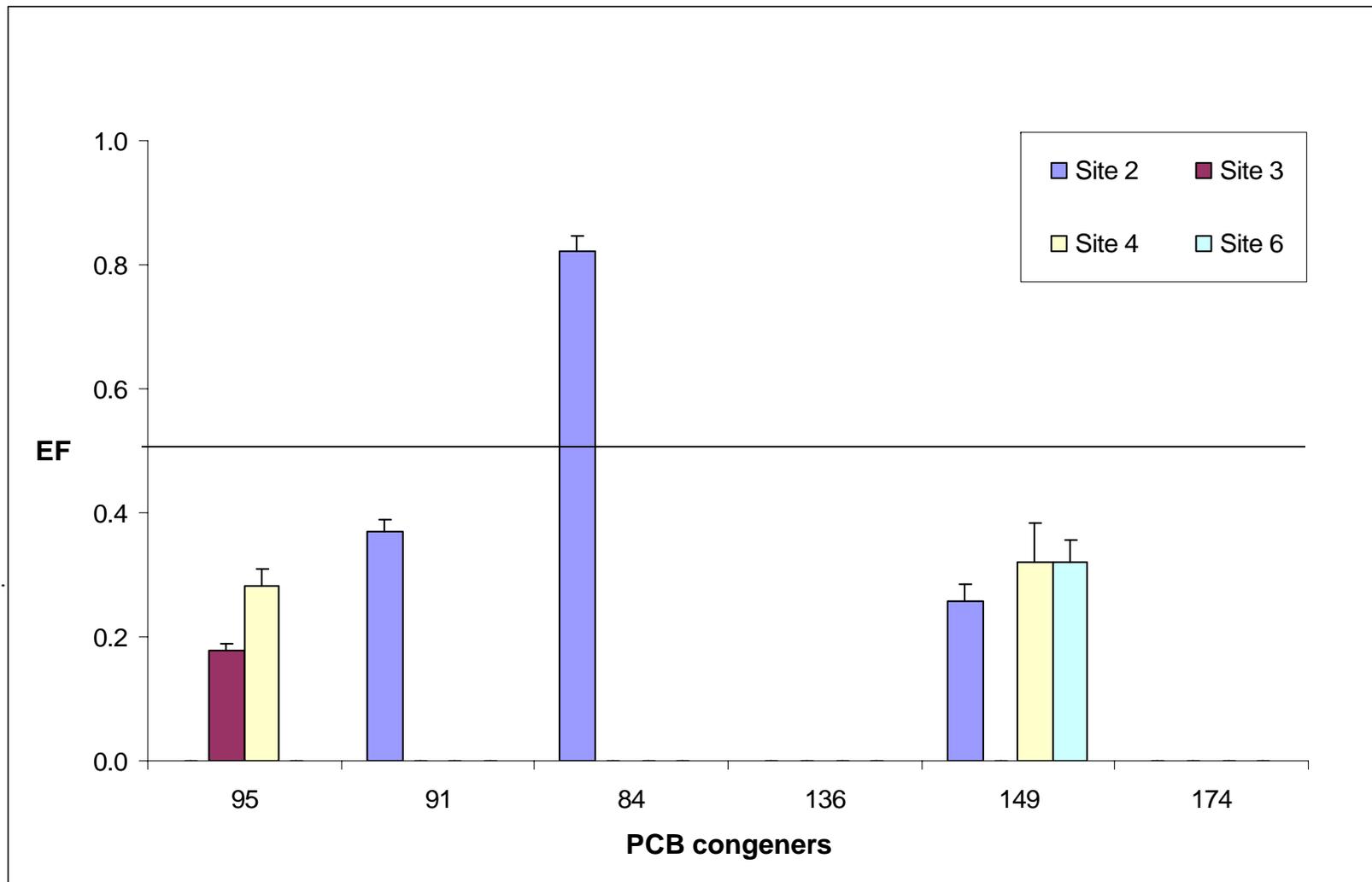


Figure 4.14 EFs of PCB atropisomers in mayflies; the horizontal line indicates a racemic value of 0.5; error bars present standard deviations.

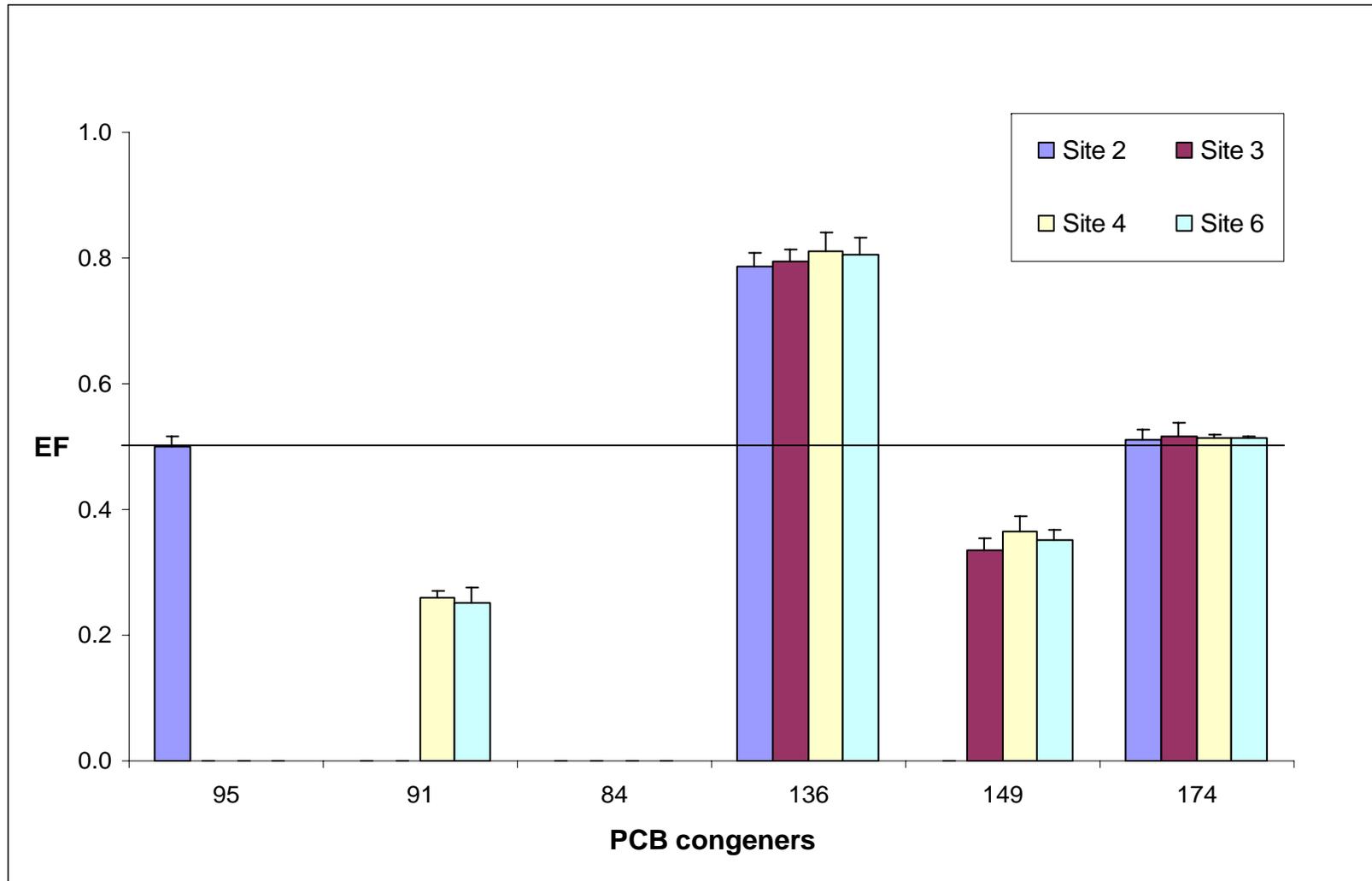


Figure 4.15 EFs of PCB atropisomers in fish; the horizontal line indicates a racemic value of 0.5; error bars present standard deviations.

4.2.2. Enantiomeric composition of PCBs in riparian samples.

Chiral PCB congeners in spider species were measured at four sites (site 1, 2, 3, and 6) in samples collected in Spring, Summer, and Fall 2005. Only PCB 95 and 149 were detected, while the other chiral PCBs were not detected at any of the sites and seasons. Concentrations of PCB 95 and 149 with season are given Table 4.12. The highest concentrations were found in Fall 2005 at all four sites, but no pattern of decreasing concentrations with distance was observed. Compared to the mayflies in the aquatic food web, concentrations of PCB 95 and 149 measured in the spiders were always higher suggesting a greater bioaccumulation.

Table 4.12 Concentrations of PCBs 95 and 149 (ng/g wet wt) in spiders in Spring, Summer, and Fall 2005.

Spring-05				
	Site 1	Site 2	Site 3	Site 6
PCB 95	12.83 ± 4.42	ND	ND	9.57 ± 2.12
PCB 149	16.03 ± 5.02	ND	7.59 ± 4.24	3.23 ± 1.03
Summer-05				
PCB 95	ND	ND	ND	ND
PCB 149	5.56 ± 2.43	ND	2.50 ± 0.46	1.45 ± 0.20
Fall-05				
PCB 95	120.00 ± 24.24	35.58 ± 9.45	28.35 ± 11.34	88.10 ± 33.21
PCB 149	61.09 ± 18.34	29.12 ± 8.34	18.34 ± 6.30	30.79 ± 11.53

The EFs for PCB 95 were similar at sites across all seasons and were nearly racemic (Figure 4.19). Spiders collected typically live for one year and prey sources of spiders typically include insects such as mayflies. The change in EF from mayflies (<0.5) (Figure 4.14) to spiders (~0.5) suggest that metabolism of PCB 95 occurred in spiders.

The EFs for PCB 149 was also similar in all three seasons and were non-racemic (Figure 4.20). Mayflies showed significantly nonracemic EF values for PCB 149 compared to the EFs in spiders, suggesting that bioprocessing for PCB 149 in spiders is possible. However, there has not been any report of EF values for spiders in the literature to compare to this observation.

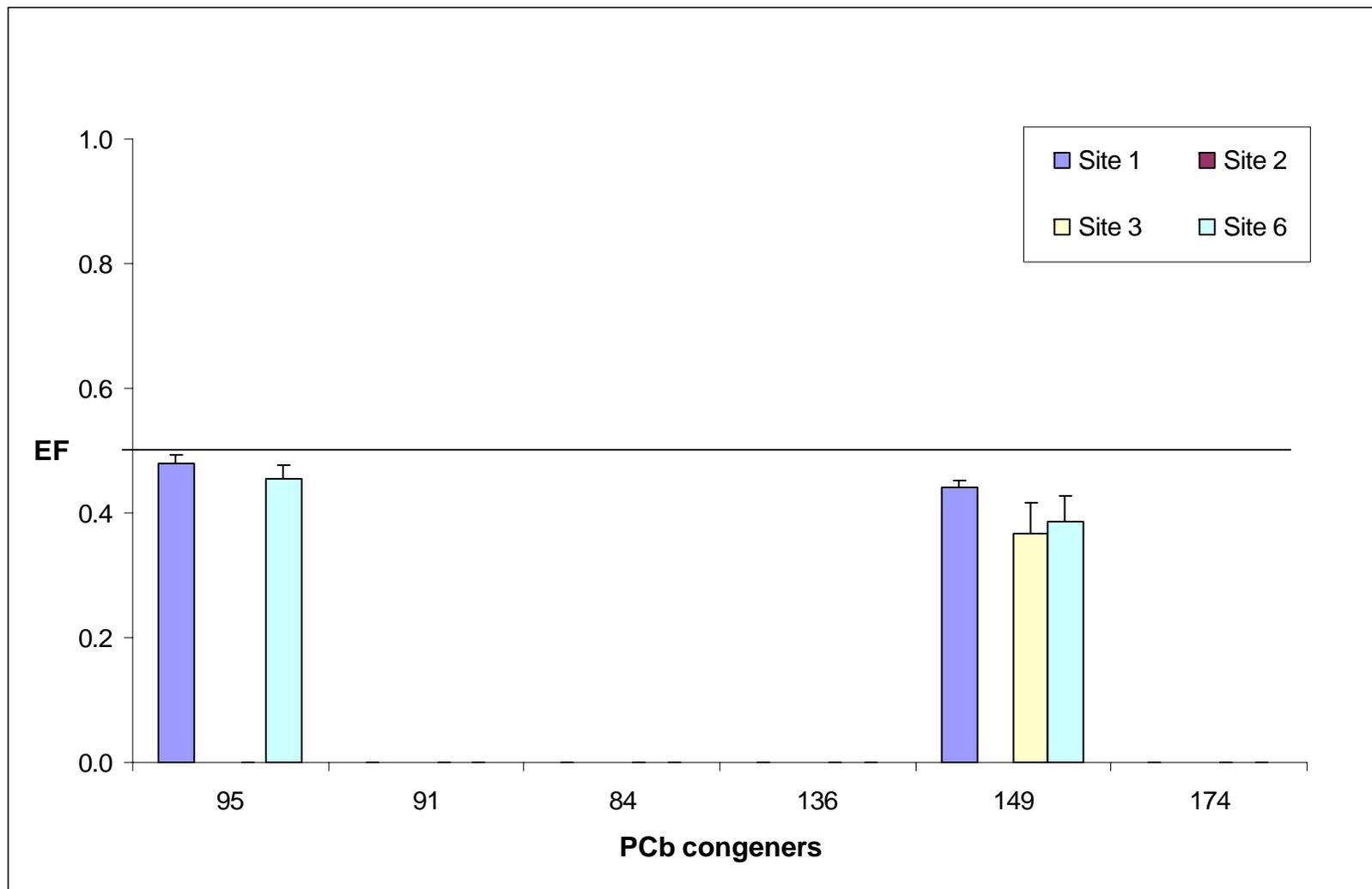


Figure 4.16 EFs of PCB atropisomers in spiders (Spring-05); the horizontal line indicate a racemic value of 0.5; error bars present standard deviations.

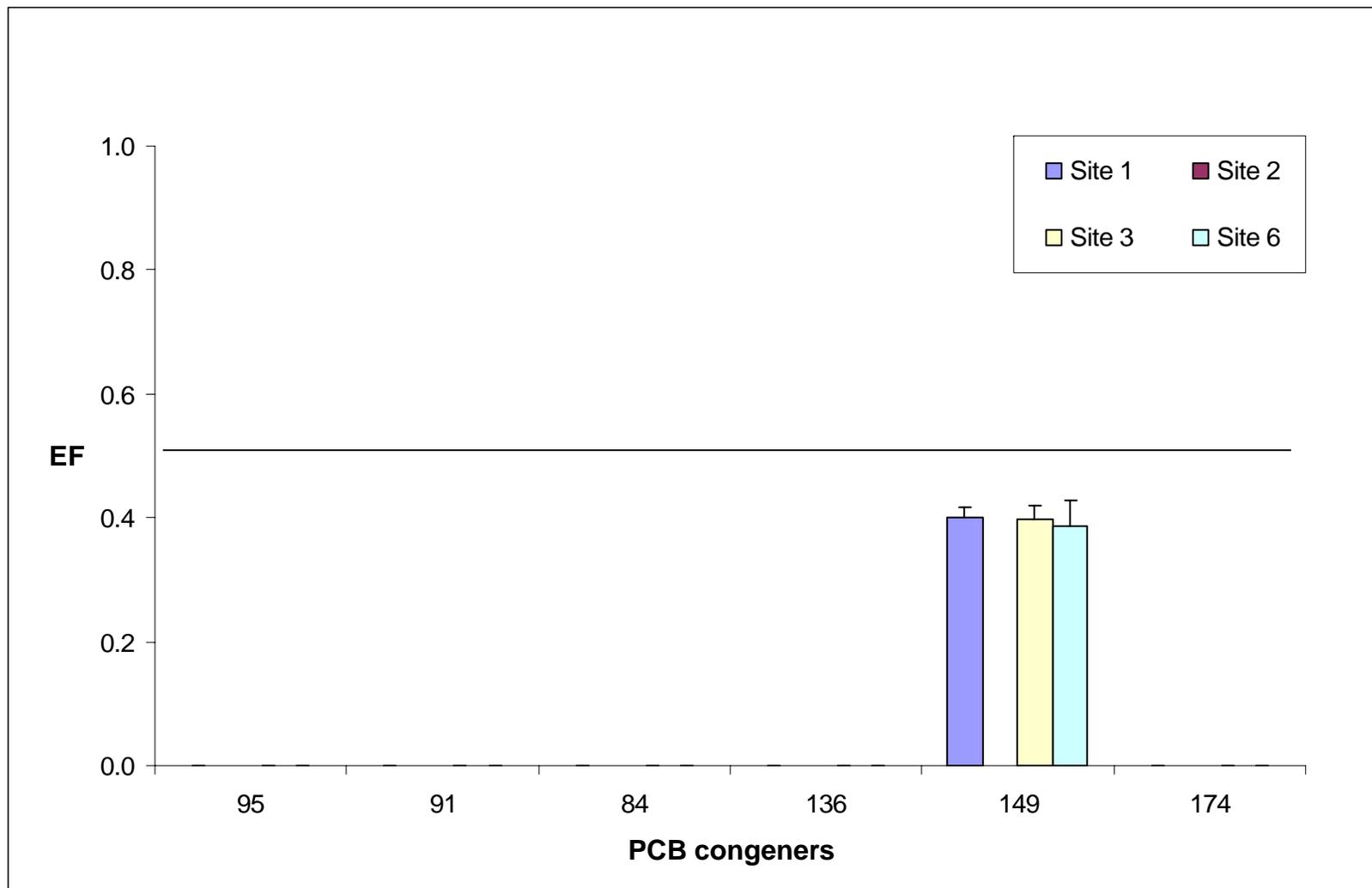


Figure 4.17 EFs of PCB atropisomers in spiders (Summer-05); the horizontal line indicates a racemic value of 0.5; the error bars present standard deviations.

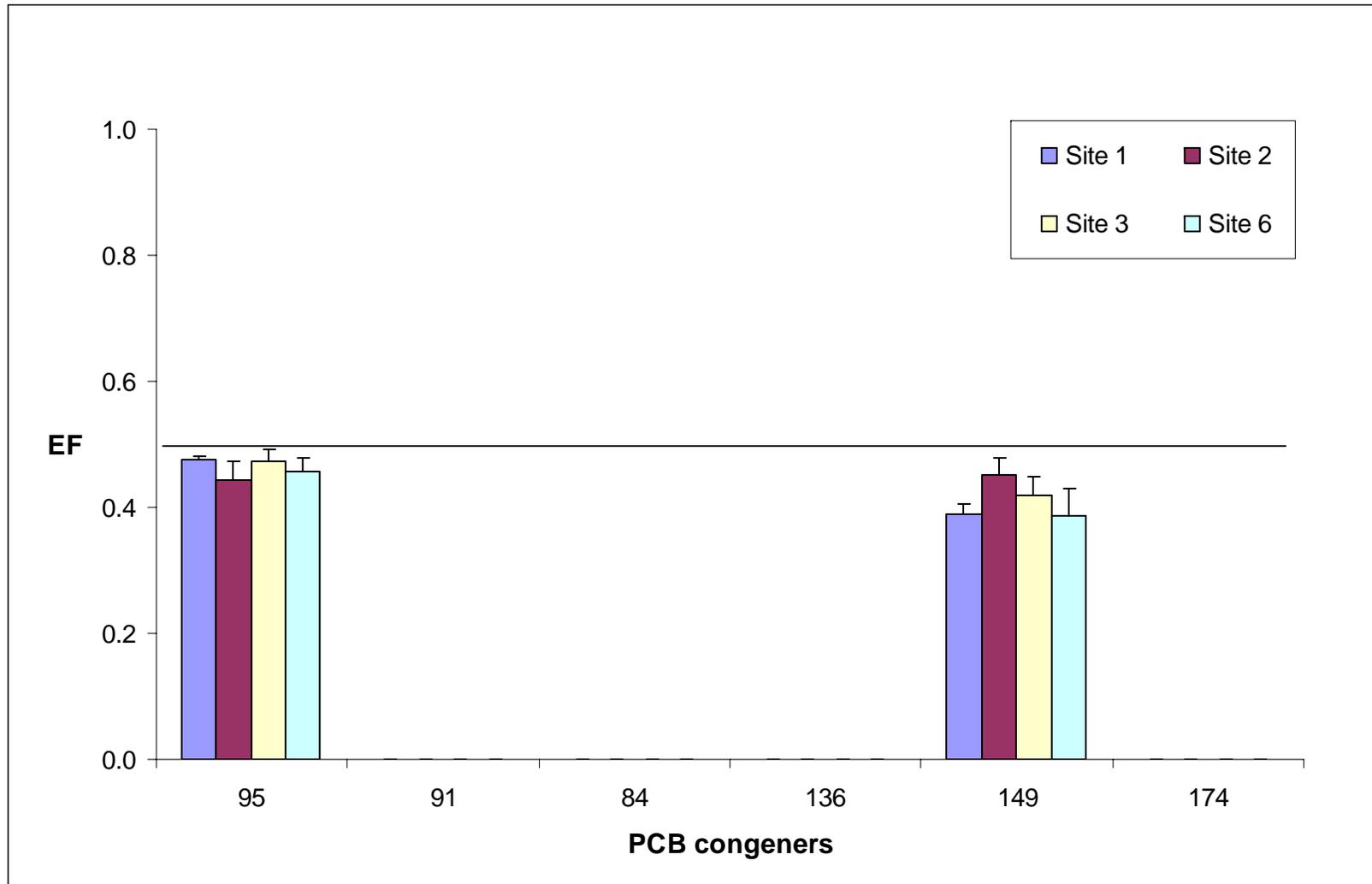


Figure 4.18 EFs of PCB atropisomers in spiders (Fall-05); the horizontal line indicates a racemic value of 0.5; the error bars present standard deviations.

Because of the limited number of amphibian samples, concentrations of chiral congeners were only found for PCBs 95 and 149 at sites 2 and 3 in Fall 2005. Table 4.13 shows the concentrations of PCBs 95 and 149 measured in the amphibians. Similar to spiders, concentration of PCB 95 was higher than concentration of PCB 149 in the amphibians. The concentration of PCB 95 at site 2 was also remarkably higher than the concentration of PCB 149 at sites 2 and 3.

Table 4.13 Concentrations of PCB 95 and 149 (ng/g wet wt) in amphibians (Fall-05)

	Site 2	Site 3
PCB 95	71.07 ± 32.04	ND
PCB 149	10.51 ± 5.23	5.62 ± 2.89

Enantiomeric compositions for amphibian species were only observed at sites 2 and 3 in Fall 2005 and only for PCB 95 and 149 (Figure 4.21). EFs for PCBs 95 and 149 followed a similar trend as was observed in the spider species but were more strongly non-racemic. It has been noted that the food sources of amphibians are variable and likely include spiders. The achiral data also showed that concentrations of PCB 95 were magnified in amphibians compared to the concentrations in spiders. The differences in EFs of PCB 95 and 149 observed between spiders and amphibians support metabolism of PCB atropisomers in amphibian species.

PCB atropisomers 84, 91, 95, 136, 149, and 174 were measured in the aquatic and riparian samples. It has been noticed that congeners with meta, para vicinal hydrogen atoms, which include chiral PCBs 91 (236-24), PCB 95 (236-25), 132 (234-236), 136 (236-236), 149 (236-245), 174 (2345-236), and 176 (2346-236), can be metabolized by

phenobarbital-type cytochrome P-450 2B enzymes (Wong et al., 2001b). However, only PCBs 95 and 149 were likely to be metabolized in the aquatic and riparian species in Twelve Mile Creek given the evidence from changes in EF. The enantiomeric enrichment also occurred more in the aquatic species than in riparian species, suggesting greater biological processes in the aquatic food web although the results did not support the statement comprehensively. The differences in the enantioselectivity of the chiral PCBs investigated among species in the aquatic as well as in riparian species in Twelve Mile Creek can not be explained by any obvious relationship among the time, sites, and species.

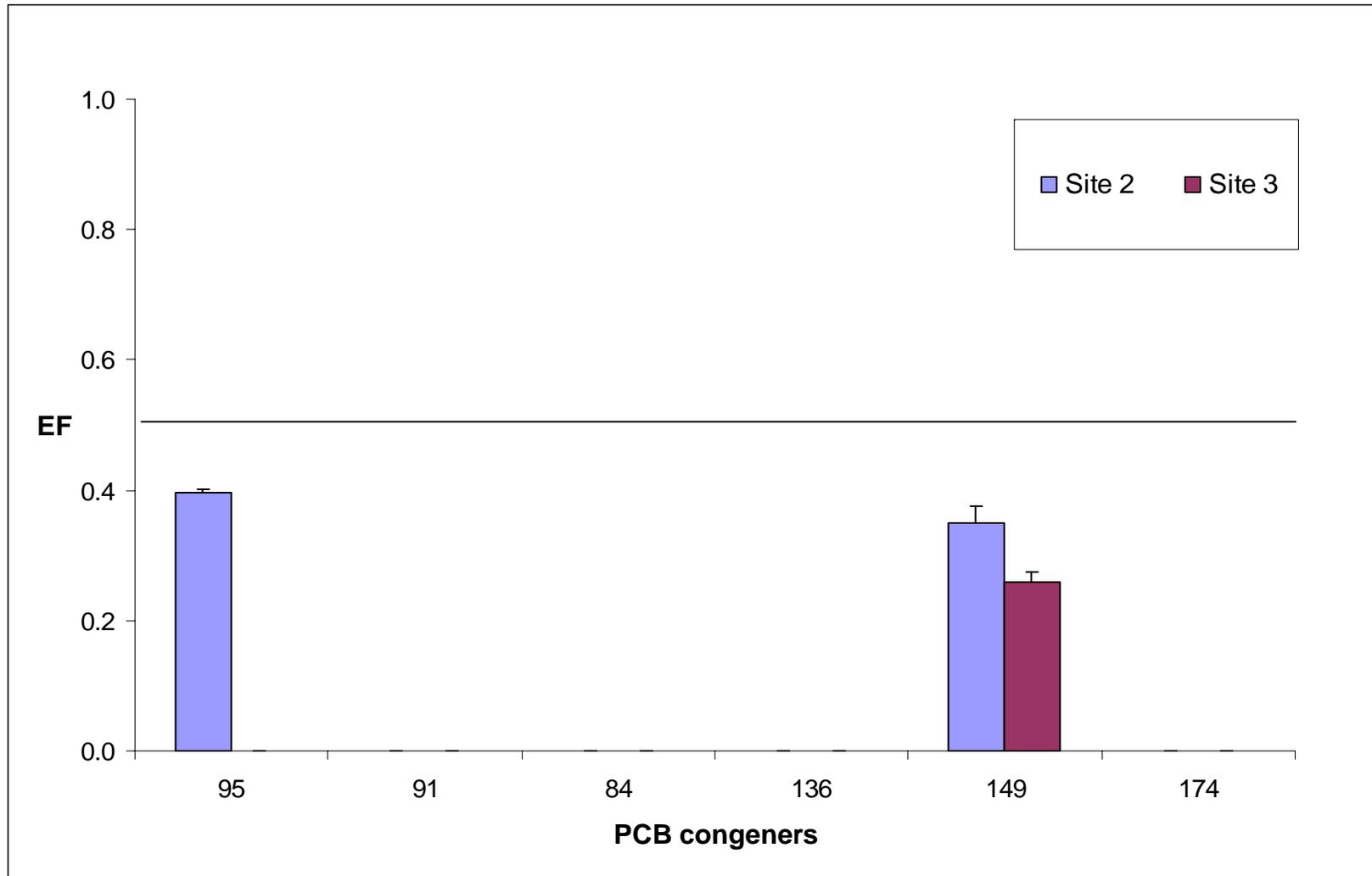


Figure 4.19 EFs of PCB atropisomers in amphibians (Fall-05); the horizontal line indicates a racemic value of 0.5; error bars present standard deviations.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS.

Achiral PCBs analysis in the aquatic food web in Twelve Mile Creek indicated that the highest level of PCBs concentration was present in fish species, while PCB concentrations were the lowest in sediment at all sites studied. This determination leads to the conclusion that biomagnification occurs in the aquatic food web of Twelve Mile Creek. Achiral PCBs analysis in riparian species showed the highest PCBs concentration at site 3 in Spring 2005 and site 6 in Fall 2005. Meanwhile the lowest PCBs concentration was found at site 3 and site 2 in Summer 2005 for spiders and amphibians, respectively. The data may be evidence to support the biomagnification of PCBs in riparian samples.

A wide variation of PCBs concentration with sites and time was observed for riparian species. A species-dependent PCB pattern was observed in the aquatic food web, where PCB concentrations increased from sediments, clams, mayflies to fish. However, this observation was not found in the riparian species suggesting that PCBs biotransformation may be governed preferably by different mechanisms of metabolic enzymes in each riparian species or by different fraction of contaminated prey. However, a similar model of PCB homologues distribution in both aquatic and riparian species was determined, in which tetra-, penta-, and hexa-chloro biphenyls were abundant in all species investigated except for sediment at all sites and clams at site 2. The observation was evidence to support the hypothesis that the distribution of PCBs through the food

web depends on biological and environmental factors such as species, time, and contamination sources.

Enantiomers of PCBs 95, 91, 84, 136, 149, and 174 were found in the aquatic food web but not in sediments. The detections were not consistent from site to site, and with species. Non-racemic values for PCBs 95, 91, and 149 were present in species with an EF less than 0.5 and for PCBs 84 and 149 with an EF greater than 0.5. PCB 174 was present at racemic values at all sites studied. Therefore, an evidence of biological processing of PCBs 95, 91, 84, 136, and 149 in the aquatic species was observed. However, evidence for bioprocessing by the microbial community in the sediment was not found in this research. Alternatively, only PCBs 95 and 149 were detected in the riparian species. The EF values for PCB 95 were nearly racemic, while the EF values for PCB 149 were non-racemic in spiders at all four sites. Non-racemic EFs were observed in amphibians only at 2 sites in Fall 2005. The values of EF were more consistent in spiders than in amphibians. Overall, there was evidence that the higher levels of the food chain (mayflies and fish in the aquatic food web, and riparian species) can metabolize PCBs.

In conclusions, chiral analysis is a useful tool for understanding the mechanism of PCBs biotransformation in food webs. Separation of PCB enantiomers conducted on GC Chirasil-Dex column was found to be effective. Non-racemic EFs are positive proof of bioprocessing in living organisms, since abiotic processes would not affect the enantiomeric composition of a chiral compound.

The results obtained from achiral and chiral PCBs analysis in the aquatic and riparian food web have shed light upon the biotransformation mechanism of PCBs

throughout the food webs. However, there are gaps in the data; therefore, recommendations for further research are provided here. First of all, more species in both food webs should be investigated in order to understand more comprehensively the biotransformations of PCBs through food webs. Especially, lower trophic levels in the riparian food web should be included. Second, the sampling of species needs to be more consistent with all seasons for both food webs and all sites to determine which portions of the food web are mainly governed by site- and time-specific PCBs pattern. Third, the TOC content, if applicable, will provide more evidence on the low level of PCBs concentration measured in the sediment. The sampling of the sediment samples should be to expanded to include depositional areas or areas with high TOC levels. Lastly, chiral PCBs analysis should be conducted on another chiral column to confirm more reliably the data obtained and determine if additional chiral congeners can be resolved.

APPENDIX

Table A.1. Approximate weight percent of PCB homolog groups in Aroclor mixtures 1016, 1254, and 1260 (EPA, 1980)

PCB formulation chlorobiphenyl	A-1016	A-1254	A-1260
	%		
Monochlorobiphenyl	1.0	-	-
Dichlorobiphenyl	20.0	-	-
Trichlorobiphenyl	57.0	-	-
Tetrachlorobiphenyl	21.0	16.0	-
Pentachlorobiphenyl	1.0	60.0	12.0
Hexachlorobiphenyl	Trace	23.0	46.0
Heptachlorobiphenyl	-	1.0	36.0
Octachlorobiphenyl	-	-	6.0
Nonachlorobiphenyl	-	-	-
Decachlorobiphenyl	-	-	-
Total chlorobiphenyls	100	100	100

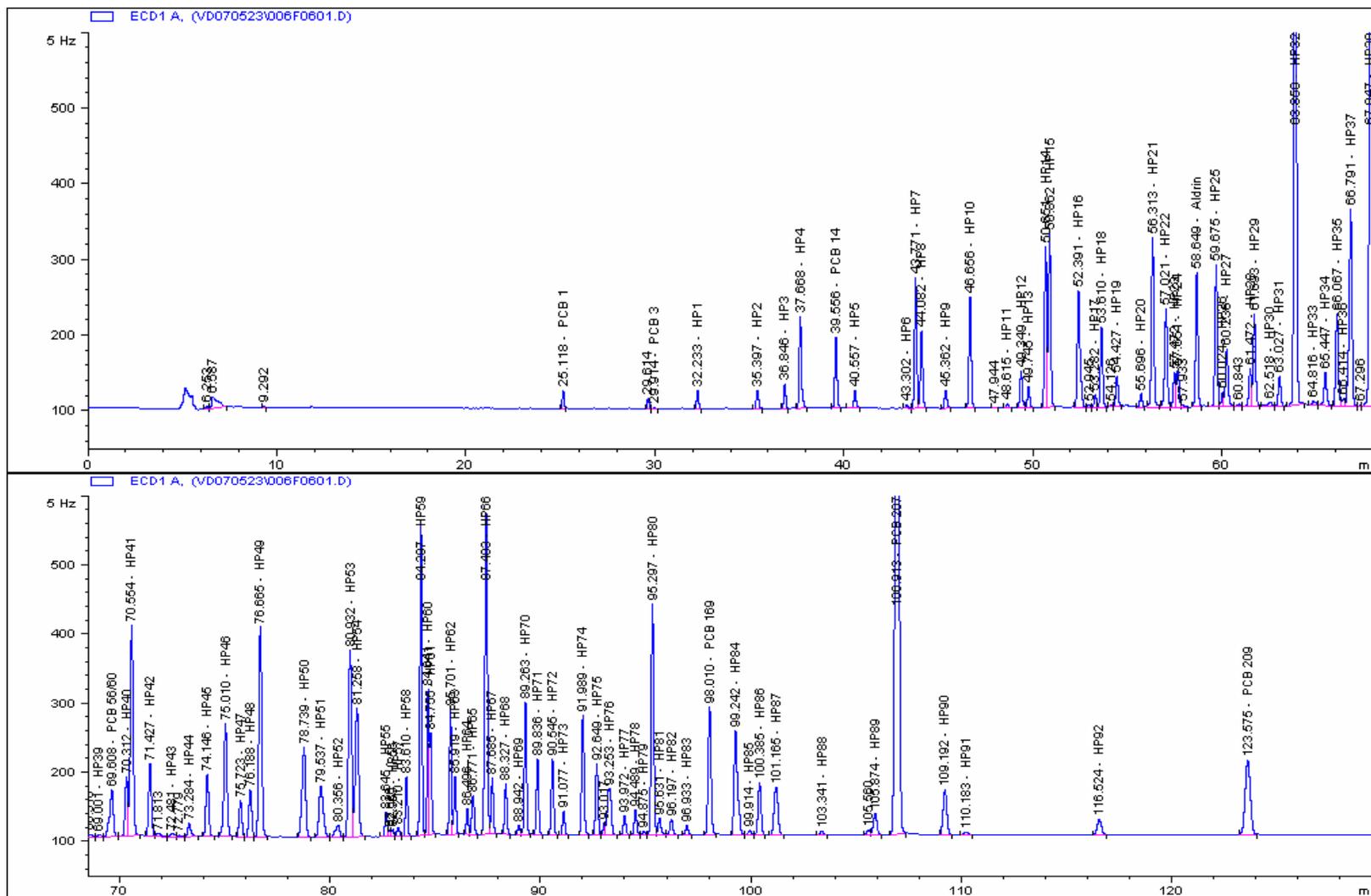


Figure A.1 GC-ECD chromatogram of PCB congeners assigned in a mixture of Aroclors 1016, 1254, and 1260 (1:1:1 w/w/w).

Table A.2 Samples size collected in the aquatic food web (Fall-05). N: number of samples.

	Site 2	Site 3	Site 4	Site 6
Sediment	N=3	N=3	N=3	N=3
Clams	N=12	N=12	N=6	N=6
Mayflies	N=3	N=3	N=3	N=3
Fish	N=3	N=3	N=3	N=3

Table A.3 Samples size collected in riparian predators (Spring, Summer, and Fall-05). N: number of samples.

	Site 1	Site 2	Site 3	Site 6
Dolomedes	N=8	N=6	N=7	N=8
Tetragnathidae	N=9	N=12	N=9	N=12
Garden spider	N=3	N=0	N=3	N=2
Fowler's Toad	N=0	N=0	N=0	N=2
Spring peeper	N=2	N=0	N=0	N=0
Pickerel Frog	N=1	N=0	N=2	N=0
Red spotted newt	N=0	N=4	N=1	N=0
Green Frog	N=0	N=0	N=0	N=2
Northern Cricket Frog	N=0	N=5	N=2	N=0
Anolis carolinses	N=0	N=2	N=1	N=0

Table A.4 Percent of recovery standard PCB 14 in the aquatic and riparian samples

Aquatic samples				
	Site 2	Site 3	Site 4	Site 6
Sediments	81.33 ± 4.72	88.25 ± 3.88	82.16 ± 4.51	81.30 ± 3.88
Clams	69.80 ± 11.60	62.80 ± 5.96	72.10 ± 15.5	73.25 ± 8.48
Mayflies	64.75 ± 6.71	83.80 ± 2.34	74.5 ± 6.06	63.00 ± 6.14
Fish	72.16 ± 5.83	73.00 ± 5.56	69.50 ± 7.07	69.33 ± 8.08
Riparian samples				
	Site 1	Site 2	Site 3	Site 6
Spiders	64.94 ± 4.58	67.50 ± 8.43	65.16 ± 4.59	67.90 ± 4.22
Amphibians	NA	66.30 ± 4.15	61.83 ± 6.69	67.50 ± 7.88

NA: amphibian samples were collected at site 1.

Table A.5 Percent of recovery standard PCB 169 in the aquatic and riparian samples

Aquatic samples				
	Site 2	Site 3	Site 4	Site 6
Sediments	90.50 ± 2.64	89.05 ± 0.70	87.66 ± 1.44	80.33 ± 9.29
Clams	67.16 ± 12.70	74.00 ± 11.57	67.20 ± 7.95	64.75 ± 7.42
Mayflies	45.82 ± 5.33	64.33 ± 6.78	62.33 ± 5.39	43.65 ± 3.56
Fish	42.56 ± 3.58	43.22 ± 1.38	47.22 ± 2.43	41.05 ± 3.05
Riparian samples				
	Site 1	Site 2	Site 3	Site 6
Spiders	57.90 ± 3.92	58.66 ± 4.88	44.16 ± 3.91	44.44 ± 2.76
Amphibians	NA	54.50 ± 4.82	42.31 ± 4.35	49.62 ± 3.58

NA: amphibian samples were collected at site 1.

Table A.6 Concentration of PCB 118 (ng/g wet wt) in the aquatic and riparian samples (Fall-05)

Aquatic samples				
	Site 2	Site 3	Site 4	Site 6
Clams	5.48 ± 0.60	4.01 ± 0.47	4.60 ± .40	3.19 ± 0.67
Mayflies	51.8 ± 2.68	18.63 ± 0.81	33.56 ± 2	32.56 ± 3.75
Fish	168.33 ± 10.26	207 ± 22.60	827.50 ± 24.61	201 ± 2.64
Riparian samples				
	Site 1	Site 2	Site 3	Site 6
Spiders	283.50 ± 87.58	291.57 ± 73.54	109.35 ± 20.56	377 ± 54.86
Amphibians	NA	184.80 ± 45.82	162 ± 2.82	844.5 ± 92.45

NA: amphibian samples were collected at site 1

Table A.7 Concentration of PCB 153 (ng/g wet wt) in the aquatic and riparian samples (Fall-05)

Aquatic samples				
	Site 2	Site 3	Site 4	Site 6
Clams	6.32 ± 0.55	6.06 ± 0.41	3.62 ± 0.39	3.09 ± 1.06
Mayflies	36.7 ± 2.68	4.45 ± 0.84	14.2 ± 2.00	25.8 ± 3.75
Fish	137.67 ± 1.52	183.33 ± 12.66	145.50 ± 14.84	201 ± 7.02
Riparian samples				
	Site 1	Site 2	Site 3	Site 6
Spiders	108.85 ± 39.39	179.62 ± 42.95	73.82 ± 19.47	192.75 ± 40.2
Amphibians	NA	119.83 ± 35.58	108.02 ± 9.75	164.67 ± 28

NA: amphibian samples were collected at site 1

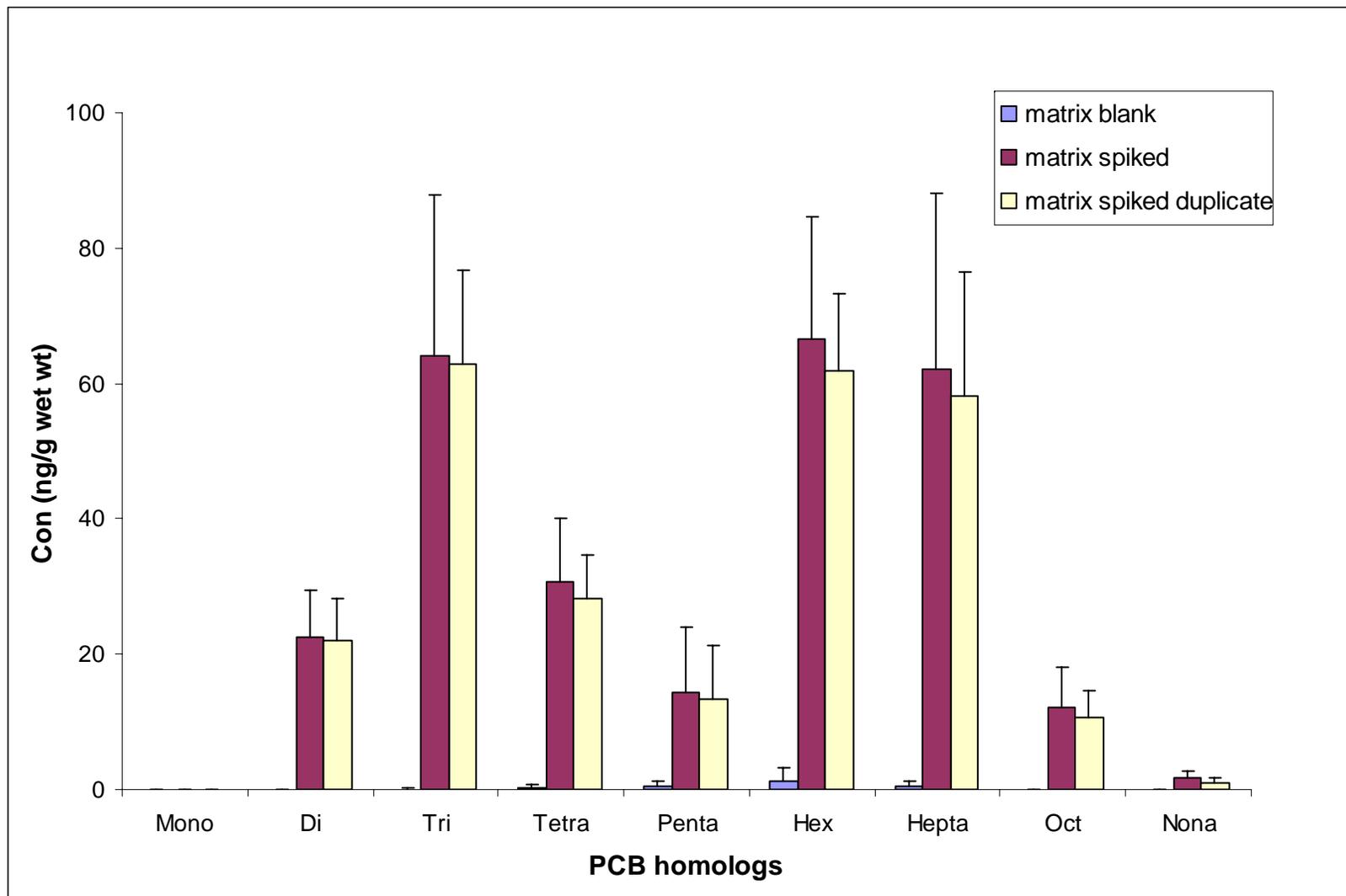


Figure A.2 PCB homologs concentration in QA/QC samples; error bars present standard deviations among triplicate extracts.

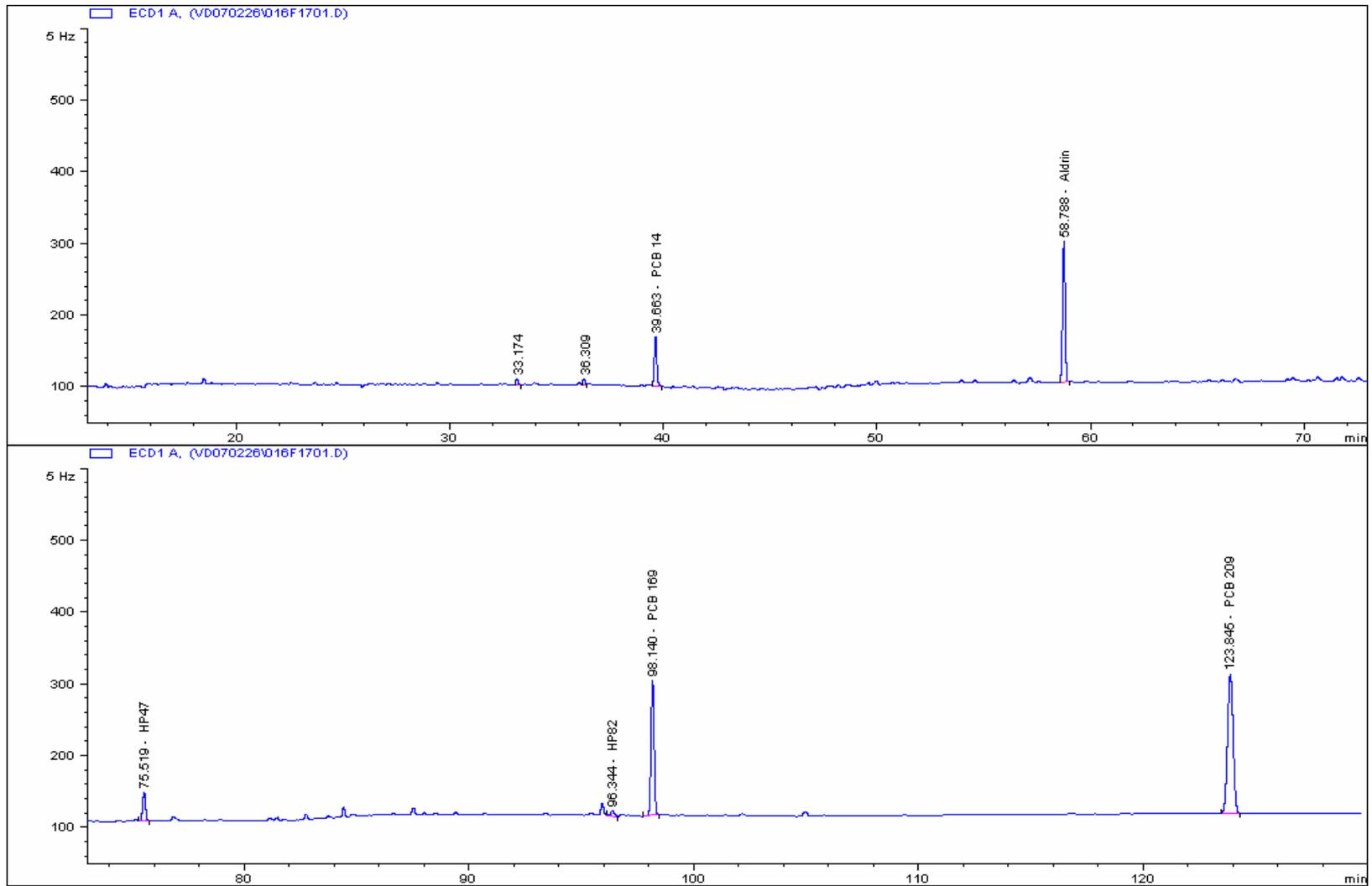


Figure A.3 Chromatogram of matrix blank sample on the 6890 GC-ECD.

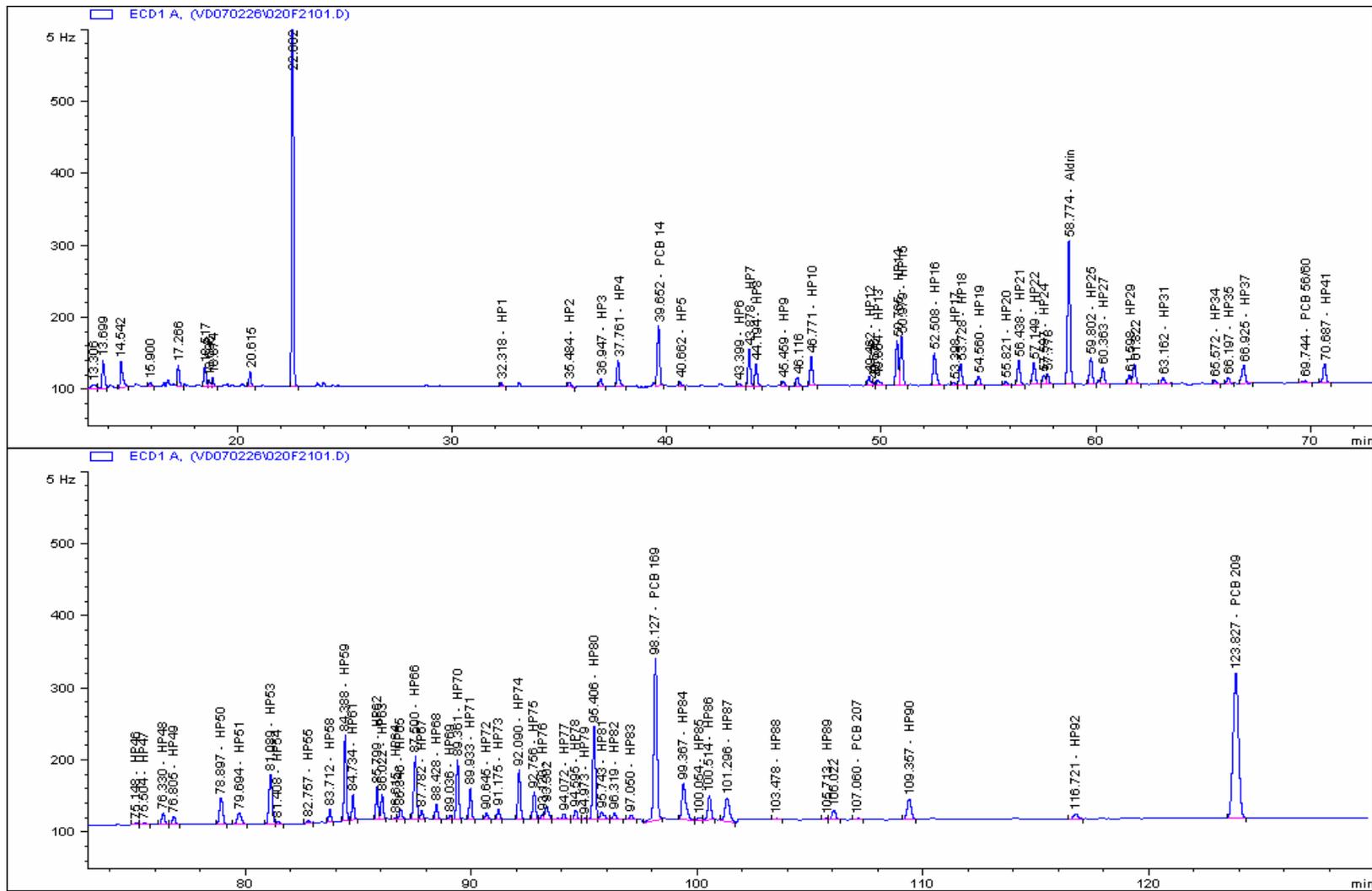


Figure A.4 Chromatogram of matrix spike sample on the 6890 GC-ECD.

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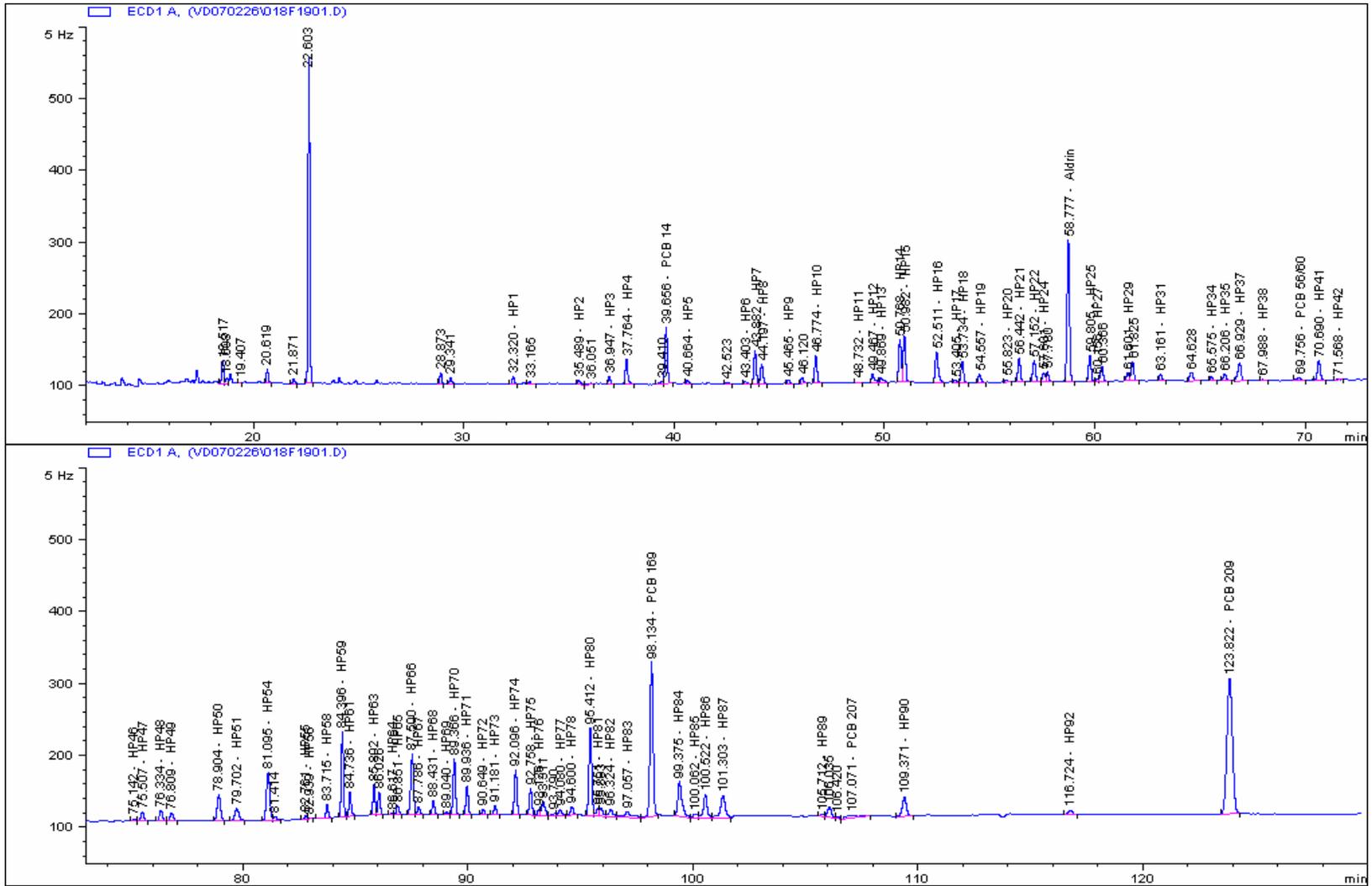


Figure A.5 Chromatogram of matrix spike duplicate sample on the 6850 GC-ECD.

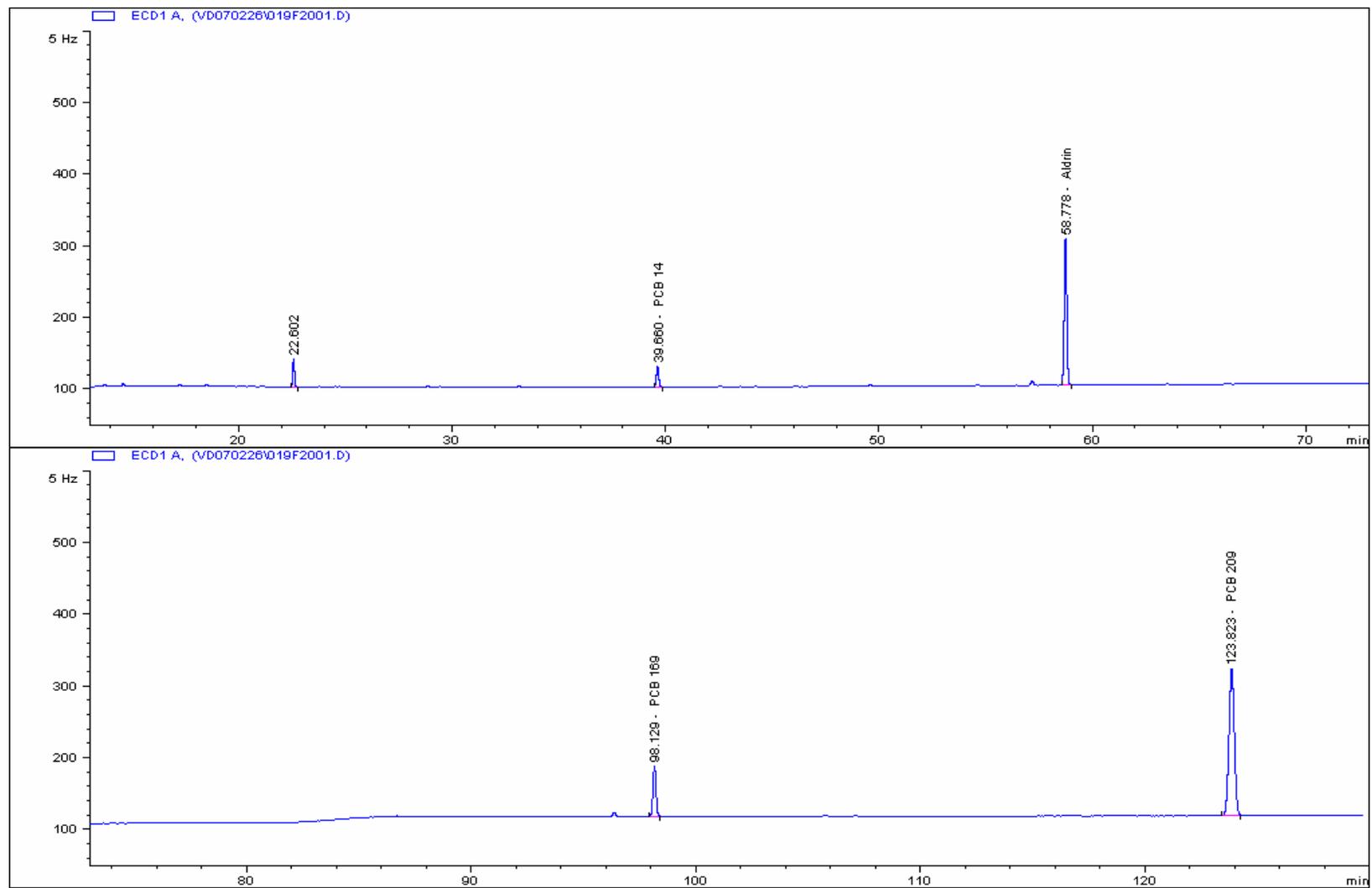


Figure A.6 Chromatogram of reagent blank sample on the 6850 GC-ECD.

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