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A Meta-Analysis of the Enantioselectvie Dechlorination of Select Chiral Polcychlorinated Biphenyls (PCBs)

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A META-ANALYSIS OF THE ENANTIOSELECTVIE DECHLORINATION OF SELECT CHIRAL POLCYCHLORINATED BIPHENYLS (PCBs)

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ABSTRACT

A meta-analysis was performed to investigate nonracemic enantiomer fraction (EF) levels of chiral polychlorinated biphenyls (PCBs) found in various media across the world. Nonracemic EF levels are those determined to be statistically different than 0.5. The existing body of knowledge from the literature provided the necessary data to determine via meta-analysis a true average EF for five selected congeners, 91, 95, 132, 136, and 149.

A thorough literature search was conducted to find relevant articles that contained sampling data from lab and field studies for chiral PCBs. A database was constructed to organize the sampling data obtained from the literature. SAS statistical software was used to perform a meta-analysis of the dataset. True average EF values for each of the five select congeners were determined in each of four media: air, soil, sediment, and tissue. The p values were determined to distinguish any difference between the calculated EF values and a racemic value. All statistical tests were conducted at the 0.05 significance level.

Nonracemic EF levels indicate that an enantioselective process occurred at some point. Several hypotheses were proposed and tested through the meta-analysis. The first possibility was enantioselective bioaccumulation. Many organisms possess the capability to bioaccumulate one enantiomer of a PCB over the other. All organisms bioaccumulating one preferred enantiomer may be the reason why nonracemic EF levels are observed in soil or sediment. The hypothesis was evaluated by statistically determining the EF levels of chiral PCBs in the tissue of these organisms. The EF levels

of the chiral PCBs in tissue were then reconciled with EF levels in soils and sediments to reveal any significant impact of enantioselective bioaccumulation.

The second possibility that might result in nonracemic EF levels comes from parent/product congener relationships. If a higher chlorinated, chiral PCB is dechlorinated enantioselectively to another chiral PCB, then the lower chlorinated, chiral PCB will show an enrichment of one enantiomer. Currently, the body of data did not allow for any significant analysis of these relationships. Too much remains unknown concerning the elution order of each chiral congener as well as which product enantiomer is produced by the dechlorination of the parent enantiomer.

Enantioselective dechlorination is the last possibility considered. Microorganisms can dechlorinate chiral contaminants and show a preference for to dechlorination of one enantiomer of the contaminant over the other. Lab and field samples from around the world indicated nonracemic levels of chiral PCBs in soil and sediment. Results from the meta-analysis confirmed enantioselective dechlorination has occurred.

The five chiral congeners analyzed in this study, PCBs 91, 95, 132, 136, and 149, all contain a biphenyl ring with two *ortho*-chlorines and one *meta*-chlorine, a 236 ring. The known dechlorination process N removes the *meta*-chlorine on a 236 ring. PCBs 91 and 95 are known to only dechlorinate through process N while the others have the potential to dechlorinate through process N. Results from the meta-analysis provided sufficient evidence that dechlorination process N functions enantioselectively.

Overall this study provided significant evidence for understanding the enantioselective processes that may affect chiral PCBs. Enantioselective bioaccumulation

was observed throughout much of the literature. Organisms typically did not show a trend for selectively bioaccumulating favoring one enantiomer over the other. Parent/product relationships between chiral PCBs were determined to be inconsequential for many of the chiral PCBs in this study. Results indicate that enantioselective dechlorination of the chiral PCBs had occurred and was the most reasonable source of nonracemic EF levels observed in worldwide samples.

DEDICATION

This thesis is dedicated to my teachers. I am humbled when I reflect on the astounding amount of teaching I needed to even be able to understand the most basic concepts of this research. From kindergarten teachers up to professors overseeing my grad courses, all were important and worthy of appreciation. Additionally I would like to dedicate this document to my fiancé. Let my hard work contained herein be a sign of the hard work and commitment I intend to give to you and our future marriage.

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Special thanks to the employees within the EES department at Clemson who do hard work to ensure the success of myself and fellow graduate students. Barbara Smith and Betty J. Cowans in particular are deserving of thanks from me for their behind-the-scenes work. I would also like to thank my bus driver for many of my trips between main campus and Rich Lab, Jay Coker. You provided me with plenty of good conversation and safe commutes.

Finally, I would like to thank my parents for supporting me throughout my time at graduate school. I have appreciated your gentle hand of guidance throughout my academic career that respected the individual decisions I made.

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1.0 INTRODUCTION

1.1 Chirality

Chirality in a molecule typically arises from an sp^3 hybridized carbon atom bonded to four distinct groups. An sp^3 hybridized carbon lies at the center of a tetrahedral conformation with angles between bonds having roughly 109.5 degrees. When an sp^3 hybridized carbon bonds to four distinct groups that carbon atom is said to be a chiral center. The stereochemistry involving the placement of the bonds around the chiral center provides the possibility of producing two different conformations that are non-superimposable mirror images. These two distinct conformations are defined as enantiomers. A molecule with just one chiral center will have an (R) and (S) enantiomer with the only difference between the two molecules being the placement of two groups bonded to the chiral center (Figure 1). For molecules with multiple chiral centers, the (R) or (S) designation is given to each chiral center. Human hands can be thought of as chiral with the palm being the chiral center. Our right and left hands both have five fingers with a pinky, ring finger, middle finger, index finger, and thumb on each hand and in that specific order. However, when one holds their right hand up to a mirror, one will find that the mirror image is non-superimposable. The same holds true for one's left hand.

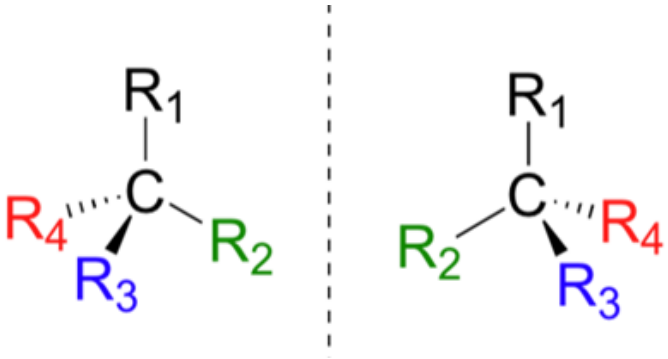

Figure 1. An sp^3 hybridized carbon is the chiral center for this molecule. R_1, R_2, R_3, and R_4 are all unique groups bonded to the carbon atom.

(Adapted from McMurry,2012).

Some molecules, such as PCBs (polychlorinated biphenyls), do not contain any sp^3 hybridized carbons but still possess a chiral nature. For some PCBs the two benzene rings linked by a C-C bond will not both lie flat in the same plane due to the repulsion caused by overlapping electron orbitals of the *ortho*-substituted chlorine atoms. Instead the benzene rings are orthogonal to one another, so one ring lies in the horizontal plane while the other in the vertical plane (Figure 2). The rings' substituents at select, asymmetric locations allow for the molecule to have conformations that are non-superimposable mirror images or enantiomers.

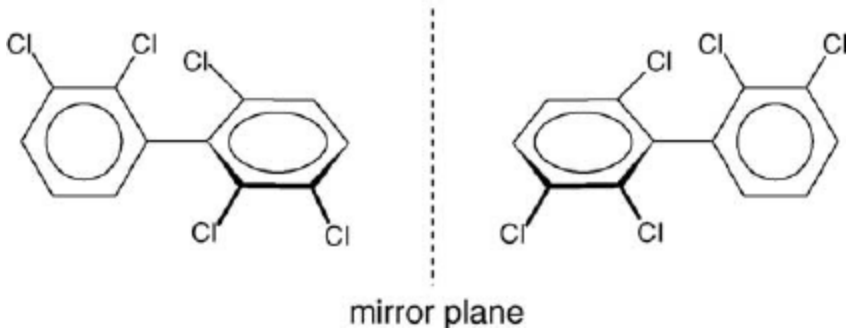

Figure 2. Because of the orthogonality of the benzene rings, select PCBs like PCB 84 shown above can also exist as enantiomers. (Source: Lehlmer et al., 2005).

1.2 Enantiomers

The synthesis of a chiral molecule will likely have followed some nonspecific process that results in a 50/50 ratio of the enantiomers. The equal portion of each enantiomer produces a racemic mixture. A racemic mixture will have an enantiomeric fraction (EF) of 0.5. An enantiomeric fraction is the proportion of one enantiomer divided by the total concentration or peak area of the molecule as represented by Equation 1. Equation 1 is best explained by Harner et al. (2000) who detailed the rise of the emerging EF as it replaced the enantiomer ratio (ER). In Equation 1 A and B are the first and last eluting enantiomers on a chiral chromatographic column. Therefore, the EF of a chiral molecule will range from 0 to 1. In the environment the EF of a chiral molecule may not be 0.5 indicating that one enantiomer has either been removed or converted to the other enantiomer.

$$EF = \frac{A}{A + B} \qquad Equation\ 1$$

Enantiomers can be differentiated from one another either as S or R enantiomers or as (+) or (–) enantiomers if the molecule has one chiral center. Designating an enantiomer as S or R requires one to draw the chiral center with the four bonded groups in space. Priority is given to each group with hydrogen having the lowest priority and a halogen or oxygen species having the highest priority. The chiral center may be connected to other carbon atoms which themselves are lower priority than oxygen. Should the chiral center be bonded to multiple other carbon atoms, priority is assigned based on what those carbon atoms are bonded to. Once the priority has been given to each group, either a 1, 2, 3, or 4, the numbers will be arranged clockwise or counterclockwise while increasing sequentially from 1 to 4. If the configuration has the numbers increasing while going counterclockwise, then the molecule is labeled the S enantiomer. If instead the numbers increase while going clockwise, then the molecule is labeled the R enantiomer (Smith, 2014). However, because PCBs do not have a single carbon atom as the chiral center of the molecule, R and S designations cannot be used. Instead the enantiomers are differentiated by a (+) or (–) label. Labeling the enantiomers this way corresponds to the optical activity of each enantiomer. A (+) enantiomer will rotate the plane of polarized light in a clockwise manner while a (–) enantiomer will rotate the same plane of polarized light in a counterclockwise manner (Smith, 2014).

1.3 Polychlorinated Biphenyls

For PCBs the substituents on the two benzene rings will either be chlorine or hydrogen atoms. To accurately classify each of the 209 PCB congeners, the nomenclature follows specific rules designated by the International Union of Pure and Applied Chemistry (IUPAC). The first carbon on each benzene ring is the carbon bonded to the neighboring ring. Therefore, there will never be a chlorine at the 1 position. The 2 and 6 positions on the ring contain the *ortho*-chlorines while the 3 and 5 positions are taken up by the *meta*-chlorines. The 4 position corresponds to a *para*-chlorine. If a ring only has one *ortho*-chlorine, then it is determined to be at the 2 position instead of the 6 position. In general the lowest numbered positions are occupied first. To differentiate between the substituents on either ring an apostrophe is used. For example the first ring may have one *ortho*-chlorine along with one *para*-chlorine. If the other ring has the same substituents, the congener would be called 2,2',4,4' –tetrachlorobiphenyl or congener number 47. IUPAC numbers the PCB congeners based on their number of chlorine substituents. PCB congener 1 has only one *ortho*-chlorine while PCB 209 has 10 chlorine atoms occupying all available carbons. Therefore, the IUPAC number increases with increasing chlorination.

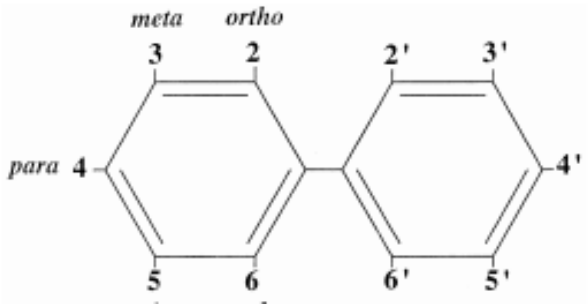

Figure 3. The IUPAC method of numbering the possible chlorine positions on a PCB. Figure adapted from Wiegel and Wu (2000).

The chlorine substituents can often be categorized as flanked, doubly flanked, or unflanked. An unflanked *meta*-chlorine will have no neighboring chlorine atoms bonded at the 2 or 4 positions. A doubly flanked *meta*-chlorine would have both an adjacent *ortho*-chlorine as well as an adjacent *para*-chlorine.

Of the 209 possible congeners of PCBs, 78 possess a chiral nature. However, only 19 of these chiral congeners (Table 1) are stable enough to exist as separate enantiomers (Vetter, 2016). The remaining 59 congeners can freely rotate around the C-C bond connecting the phenyl rings allowing them to fluctuate between either conformation. The 19 stable chiral congeners have three or four *ortho*-chlorines preventing the rotation around the central C-C bond under ambient temperature and ambient temperature conditions used for gas chromatography (GC).

Table 1. The 19 stable chiral PCB congeners (Vetter, 2016).

Congener	Chlorination Pattern
45	236-2
84	236-23
88	2346-2
91	236-24
95	236-25
131	2346-23
132	236-234
135	236-235
136	236-236
139	2346-24
144	2346-25
149	236-245
171	2346-234
174	236-2345
175	2346-235
176	236-2346
183	2346-245
196	2346-2345
197	2346-2346

The emphasis placed on the chirality of PCBs can be attributed to the tendency of biological organisms to interact uniquely with each enantiomer of any chiral compound. For example, the common drug ibuprofen is a chiral molecule that exists as a racemic mixture when manufactured and consumed. Only the S enantiomer is biologically active (Davies, 1998). The R enantiomer does not have any significant, observable effects when compared to the S enantiomer and usually is expelled from the body. Therefore, one enantiomer actively accomplishes a positive result while the other has no effect.

Enantiomers can also have opposite biological effects. In an extreme case with thalidomide in the 1950s and 1960s, one enantiomer acted in a similar role to aspirin whereas the other enantiomer could cause birth defects (Vianna et al., 2017).

Chiral PCBs are not pharmaceuticals, but they have been shown to produce different biological effects (Rodman et al., 1991). Additionally, chiral PCBs have the potential to be dechlorinated enantioselectively (Pakdeesusuk et al., 2003a) and bioaccumulated enantioselectively (Warner et al., 2005).

PCBs were produced commercially in the USA under the tradename Aroclor (Frame et al., 1996a). However, PCBs are not currently being produced in the US as of 1977 (U.S. EPA, 1979), and the initial directive for PCB use in Europe was replaced in 1996 (EU, 1996). Aroclors were distinguished from one another by the weight percent of chlorine in the mixture which is generally shown as the last two digits in the Aroclor number. Aroclor 1254 would have 54% chlorine by weight while Aroclor 1242 had 42% chlorine by weight. The exception is Aroclor 1016 which has 41% chlorine by weight (Mayes et al., 1998). These different Aroclors contained varying distributions of congeners. Frame et al. (1996b) examined the distribution of congeners within Aroclors and found there to be significant variability even within the same Aroclor. However, general weight percent data were determined for the major congeners represented in each Aroclor (Frame et al., 1996b). Some chiral congeners were major components of higher weight percent Aroclors.

The literature review yielded substantial data for only five of the 19 chiral PCBs. These PCBs were congeners 91, 95, 132, 136, and 149 (Figures 4 and 5). Their

prevalence in the literature can be attributed to their substantial presence in many Aroclors. In particular PCBs 95, 132, and 149 were well represented in higher weight percent Aroclors such as 1254 and 1260 (Table 2). The other chiral congeners outside of the five selected for this study had little to nothing reported about them in the literature. Therefore, only these five congeners were analyzed.

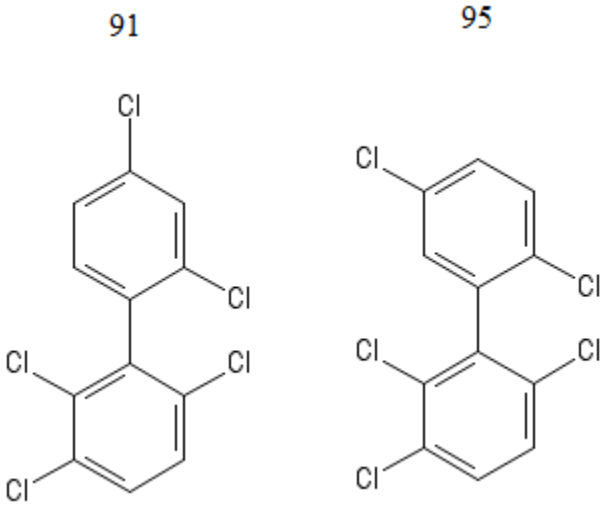

Figure 4. The pentachlorinated PCB congeners analyzed in this study, PCB 91 (left) and PCB 95 (right).

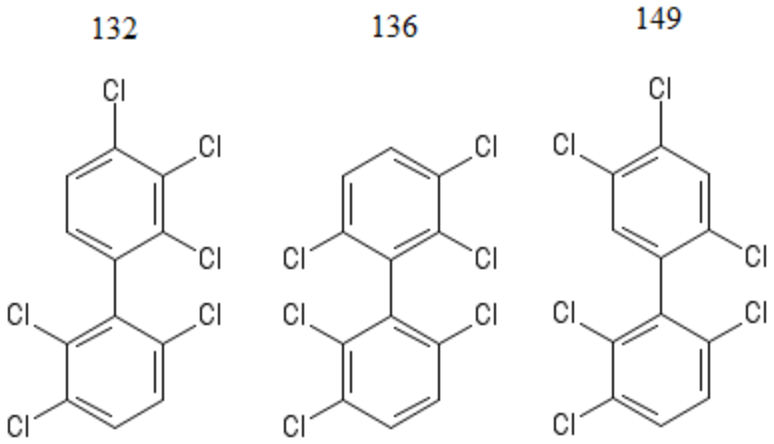

Figure 5. The hexachlorinated PCB congeners used in this study, PCB 132 (left), PCB 136 (center), and PCB 149 (right).

The process of producing PCBs whether individually or a part of an Aroclor is thought to not be enantioselective. Dang et al. (2013) reported racemic EF values of PCBs 91, 95, and 149 in a 1:1:1 mixture of Aroclors 1016, 1254, and 1260. The EF ranged from 0.5±5%. Pakdeesusuk et al. (2003a) found the EF of PCBs 84 and 91 standards to be racemic, 0.5±0.005. Therefore, for this study, all releases of PCB to the environment were assumed to be racemic amounts of chiral PCBs.

Extracting chiral PCBs from all media considered is assumed to not be enantioselective. PCBs from soils and sediments are extracted using a variety of methods such as sonication in an organic phase (Pakdeesusuk et al., 2003b) or by using Soxhlet extraction (Chen et al., 2014). The study by Pakdeesusuk et al. (2003a) extracted chiral PCBs from several microcosms. The PCBs extracted from the sediment of the live microcosms had nonracemic EFs which suggests enantioselective microbial activity.

However, the PCBs extracted from the sediment of the control microcosm showed racemic levels of all chiral PCBs. This result provides evidence that extraction techniques are not enantioselective. Additionally, no evidence was found during the literature review to support the idea that PCBs can be enantioselectively extracted from any media.

Table 2. Weight percentage in some commercial Aroclor mixtures of chiral PCBs analyzed in this study.[a]

Congener	Chlorination Pattern	A1016[b]	A1242[c]	A1248a[d]	A1248g[e]	A1254a[f]	A1254g[g]	A1260[h]
91	236-24	0.06	0.21	0.63	0.56	0.53	0.93	0.01
95	236-25	0.31	0.61	1.96	1.43	1.84	6.25	2.45
132	236-234		0.04	0.15	0.14	1.5	2.29	2.9
136	236-236			0.05	0.06	0.24	0.7	1.46
149	236-245		0.06	0.24	0.33	1.82	3.65	8.75

(Frame et al., 1996b)

[a] Blank values indicate not detected; all values are fractions represented in weight percent.
[b] A1016 = Lot A2.
[c] A1242 = Mean 3 lots.
[d] A1248a = Lot A3.5.
[e] A1248g = Lot G3.5.
[f] A1254a = Lot A4.
[g] A1254g = Lot G4.
[h] A1260 = Mean 3 lots.

1.4 Dechlorination Processes

Decgradation of PCBs happens through anaerobic or aerobic processes. Both process types can be carried out by biological organisms (Passatore et al., 2014). However, the majority of the literature examines the anaerobic reductive dechlorination of PCBs. Reductive dechlorination proceeds by an electron donor, such as hydrogen (Zanaroli et al., 2012), interacting with an electron acceptor, the PCB. Reductive dechlorination of PCBs can be achieved by an ultrasound-assisted chemical process (Chen et al. 2013) and photocatalytic processes (Ghosh et al., 2012). Both processes produce a hydrocarbon radical which reacts with the PCB to ultimately remove a chlorine substituent and create a lower chlorinated PCB radical. The PCB radical will then react to remove a hydrogen atom from solution or another molecule (Figure 6).

$$[t-\mathrm{BuOOBu}-t]+\mathrm{Fe}^{+2}\xrightarrow[55^{\circ}\mathrm{C}]{k_1}[t-\mathrm{BuO}\cdot]+[t-\mathrm{BuO}^{-}\mathrm{Fe}^{+3}] \quad (1)$$

$$[t-\mathrm{BuO}\cdot]+(\mathrm{CH_3})_2\mathrm{CHOH}\xrightarrow{k_2} t-\mathrm{BuOH}+(\mathrm{CH_3})_2\mathrm{C}\cdot\mathrm{OH} \quad (2)$$

$$(\mathrm{CH_3})_2\mathrm{C}\cdot\mathrm{OH}+\mathrm{OH}^{-}\underset{k_{-3}}{\overset{k_3}{\leftrightarrow}}(\mathrm{CH_3})_2\mathrm{CO}^{\cdot-}+\mathrm{H_2O} \quad (3)$$

$$[(\mathrm{CH_3})_2\mathrm{CO}]^{\cdot-}+\mathrm{ArCl}\xrightarrow{k_4}(\mathrm{CH_3})_2\mathrm{CO}+\mathrm{Ar}\cdot+\mathrm{Cl}^{-} \quad (4)$$

$$\mathrm{Ar}\cdot+(\mathrm{CH_3})_2\mathrm{CHOH}\xrightarrow{k_5}(\mathrm{CH_3})_2\mathrm{C}\cdot\mathrm{OH}+\mathrm{ArH} \quad (5)$$

$$2(\mathrm{CH_3})_2\mathrm{C}\cdot\mathrm{OH}\xrightarrow{k_6}\text{termination products} \quad (6)$$

$$(\mathrm{CH_3})_2\mathrm{C}\cdot\mathrm{OH}+[(\mathrm{CH_3})_2\mathrm{CO}]^{\cdot-}\xrightarrow{k_7}\text{termination products} \quad (7)$$

$$2[(\mathrm{CH_3})_2\mathrm{CO}]^{\cdot-}\xrightarrow{k_8}\text{termination products} \quad (8)$$

Figure 6. A detailed reaction mechanism for the reductive dechlorination of PCBs using the ultrasound-assisted chemical process. Figure 6 was adapted from the original by Chen et al. (2013).

Biological reductive dechlorination is the most common dechlorination process represented in the literature. If PCBs are present in large enough concentrations, the PCBs will induce dechlorination (Vasilyeva & Strijakova, 2007). Once dechlorination has been initiated, tracking the reaction as it proceeds is easily done by analyzing PCB concentrations over time (Pakdeesusuk et al., 2003a). Increasing biological reductive dechlorination of PCBs can be done through a few methods. Zanaroli et al. (2012) list two methods for increasing dechlorination activity of microbial communities. Spiking sediments with halogenated compounds promotes the growth of the native microbial

population capable of dechlorination. However, the method is often impractical since halogenated compounds are often contaminants themselves. Zanaroli et al. (2012) also discuss adding electron donors to the biological system. Electron donors such as hydrogen and lactate have been shown to stimulate PCB dechlorination (Matturo et al., 2016; Zanaroli et al., 2012).

Biological reductive dechlorination has been linked to many different bacterial strains thus far including *Dehalobium chlorocoercia* and *Dehalococcoides mccartyi* (Wang et al., 2014). Reductive dechlorination is not limited to these bacterial strains, however. Research suggests most, if not all, environments contain microbial communities capable of reductive dechlorination of PCBs (Passatore et al., 2014). Like other biologically mediated reactions, enzymes are employed to catalyze the redox reaction. Wang et al. (2014) used quantitative polymerase chain reaction (qPCR) to identify a dominant group of genes in three *Dehalococcoides mccartyi* strains, *rdhA*, which was responsible for the degradation of numerous PCB congeners in Aroclor 1260. The functional site of the protein encoded by the rdhA gene is unknown. Therefore, further research needs to be done to determine the reaction mechanism employed by the dechlorinating *rdhA*. Specialized dechlorinase genes, pcbA1, pcbA4, and pcbA5, in some other strains of *Dehalococcoides mccartyi* are also known to dechlorinate PCBs (Matturo et al., 2016).

White-rot fungus, a strain of *Phanerochaete chrysosporium*, can also dechlorinate PCBs (De et al., 2006). Under aerobic conditions, the nitrate reductase gene mediates the

dechlorination. Dechlorination was promoted by bivalent ions and inhibited by tungsten (De et al., 2006). Their research suggested PCBs do not induce the nitrate reductase gene.

Bedard and Quensen (1995) discuss the dechlorination of PCBs. The authors understood that the dechlorination was a result of the work of microbial communities although no specific microorganism had been identified at the time. Additionally, the authors recognized that no abiotic mechanism can dechlorinate PCBs under environmental conditions.

Dechlorination of PCBs proceeds through a number of different processes outlined by Bedard and Quensen (1995). These processes are arbitrary ways to categorize the removal of *meta*- or *para*-chlorines. *Ortho*-chlorines are exceptionally difficult, but not impossible, to remove due to the steric hindrance associated with their position on either ring. Each process targets a specific type of chlorine, such as an unflanked *meta*-chlorine or a doubly flanked *para*-chlorine. Overlap between the processes has been observed in field samples from Lake Hartwell (Bzdusek et al. 2006) and multiple sites considered by Bedard and Quensen (1995). However, these processes are useful in predicting the pathway a PCB congener will take as it is dechlorinated. Judging from the reactivity of the chlorine groups on each ring one can determine which chlorine atom will be lost next. Dechlorination process N exists as one of a few major pathways (Table 3). Process N proceeds by removing the singly or doubly flanked *meta*-chlorine on a 34, 234, 245, or 236 ring (Bedard and Quensen, 1995). All of the chiral PCB congeners considered in this study contain at least one 236 ring; PCB 136 has two.

Table 3. Dechlorination processes and their general information (Bedard and Quensen, 1995).

Dechlorination Process	Targeted Chlorine	Reactive Groups
M	Flanked and unflanked *meta* chlorines	23, 25, 34, 234, 236
Q	Flanked and unflanked *para* chlorines, meta chlorine of a 23 group	4, 23, 34, 245
H'	Flanked and doubly flanked *para* chlorines, meta chlorine on a 23 or 234 ring	23, 34, 245, 234
H	Flanked and doubly flanked *para* chlorines, meta chlorine on a 234 ring	34, 245, 2345, 23456, 234
P	Flanked and doubly flanked *para* chlorines	34, 234, 245, 2345, 2346, 23456
N	Flanked and doubly flanked *meta* chlorines	34, 234, 236, 245, 2345, 2346, 23456

Differentiating between processes can be difficult because there is potential overlap. Processes M, N, and P can potentially dechlorinate a 234 ring. However, processes M and N will remove the *meta*-chlorine leaving the product as a 24 ring while process P will remove the *para*-chlorine leaving a 23 ring. Additionally, processes M and N can both dechlorinate the *meta*-chlorine on a 236 ring. However, process M targets lower chlorinated congeners while process N has been observed for higher chlorinated

congeners. Using the scheme developed by Bedard and Quensen (1995) one can determine all the potential dechlorination pathways for each specific congener.

Bzdusek et al. (2006) identified major pathways for the dechlorination of PCBs in Lake Hartwell, South Carolina. Processes M and Q were found to dominate the dechlorination. The processes assigned corresponded to the dechlorination exhibited by the entire suite of PCBs instead of just the chiral congeners which represent a small portion of the bulk PCBs. However, Bedard and Quensen (1995) have reported two chiral congeners, PCB 91 and 95, as not being dechlorinated by any process besides N. Because the work by Bzdusek et al. (2006) did not focus on the chiral congeners, one can build on the foundation to determine if any of the major processes are enantioselective by nature.

A study by Pakdeesusuk et al. (2003a) considered the enantioselective dechlorination of four PCB congeners. PCBs 91 and 95 showed preferential dechlorination of one enantiomer. Both PCB 95 and 91 lost the *meta*-chlorine on their 236 ring following process N. Indeed this is the only process PCBs 91 and 95 can undergo according to Bedard and Quensen (1995) which suggests the possibility of process N being an enantioselective dechlorination pathway.

Of the 19 stable, chiral congeners, 12 contain a 34, 234, 245, or 236 ring (Table 1) which are N targets. Therefore, it would be expected that these 12 congeners (45, 84, 91, 95, 132, 135, 136, 149, 171, 174, 176, and 183) would have the possibility of being enantioselectively dechlorinated as was observed by Pakdeesusuk et al. (2003a). While microorganisms can dechlorinate these congeners through process N because of their 34, 234, 245, or 236 ring, many are not limited to dechlorination through only process N.

Such is the case with PCB 149 in the study done by Pakdeesusuk et al. (2003a). PCB 149 remained racemic during the incubation with microbial communities taken from sediment with high levels of PCB contamination in South Carolina's Lake Hartwell. The study lasted 140 days for the first microcosm and 258 days for the second. The study showed PCB 149 going through process H to lose the *para*-chlorine on its 245 ring instead of process N where the *meta*-chlorine on the 236 ring would have been lost. The same study also analyzed the dechlorination of PCB 132. Like PCB 149, PCB 132 remained at racemic levels throughout the duration of the study. Data from the study show PCB 132 losing the *meta*-chlorine on its 234 ring by following process H or N. The results from the dechlorination of PCB 149 indicate that process H was not enantioselective. Results from the dechlorination of PCB 132 suggest several things about the different processes. If process H was active in the microcosms, then the results indicate process H was not enantioselective. If process N was active in the microcosm, then it suggests that process N is not enantioselective under all circumstances.

Process N may work enantioselectively only when it specifically involves the *meta*-chlorine on a 236 ring. While determining the elution sequences of chiral PCBs, Haglund and Wiberg (1996) observed that congeners with a 236 ring could be separated on a Chirasil-Dex column while those chiral congeners that lacked a 236 ring were unable to be separated on the column. The authors concluded that a 236 ring on a chiral PCB seemed to be required for any chiral interactions. However, Wong and Garrison (2000) were able to separate all 19 chiral congeners on at least one of seven chiral columns, which implies a 236 ring is not necessary for chiral interactions. The 236 ring may not be

required for chiral interactions as shown by Wong and Garrison (2000), but the literature suggests that it is be essential to enantioselective dechlorination. The results from the study by Pakdeesusuk et al. (2003a) measured nonracemic EFs for chiral PCBs only after removal of the *meta*-chlorine on a 236 ring.

If process N does function enantioselectively, then the next question is to what extent. Will the dechlorination exclusively work on one enantiomer, or will one enantiomer simply be more likely to dechlorinate than the other? If process N is exclusive, then over time the EF levels of congeners affected by it will move towards 0 or 1 signifying complete elimination of one enantiomer. Judgements on the extent of enantioselective dechlorination can be made when analyzing the EF levels of samples from the same location over a long period of time.

1.5 EF influencers

Examining the enantiomer fraction of chiral PCBs only gives one a snapshot of what has actually happening. Several factors may affect why a congener is present in nonracemic proportions.

The most obvious factor is dechlorination of the congener in question. However, relationships between the parent and product congener may have a large impact. For example PCB 149 can be dechlorinated to PCB 91, 95, or 102 (Figure 7).

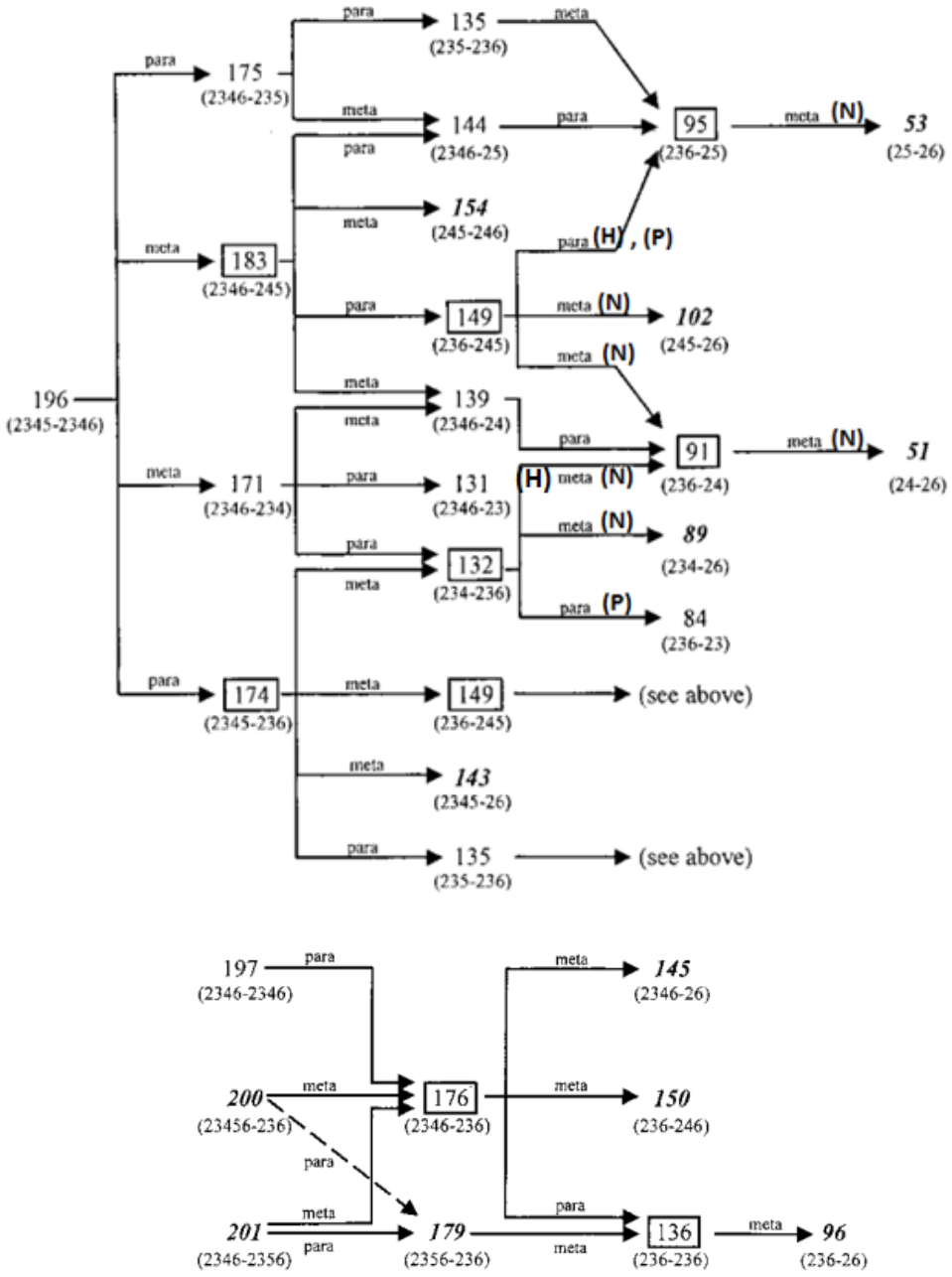

Figure 7. Some of the parent/product relationships that include the five target chiral PCBs in his study. The dechlorination process that acted on the parent congener is given in parentheses next to the chlorine that was removed. Achiral congeners are bolded and in italics. Figure 7 was adapted from Wong et al. (2001).

Therefore, if PCB 149 is enantioselectively dechlorinated to one of these products, the product may also show signs of enantioselective dechlorination. The product congener may not dechlorinate enantioselectively, but, because of the parent congener's enantioselective dechlorination, the product congener may exist at nonracemic levels. Therefore, these relationships must be understood so as not to improperly attribute enrichment of one enantiomer to enantioselective dechlorination.

Currently the data set gathered from the literature review cannot provide any support for the dechlorination of parent congeners affecting the EF of product congeners. Sampling data would need to show the concentrations and EFs of all possible parent congeners as well as product congeners over an extensive time period (greater than one year). Past sampling data typically only include concentration and EF of a few chiral PCBs at one location at one time.

Getting a complete picture of the complexity of dechlorination would require analyzing samples for multitudes of congeners at different times. Bzdusek et al. (2006) used data collected from sediments of Lake Hartwell, South Carolina, at two different times to model likely dechlorination pathways. However, this was the only example found during the literature review. Data from the literature seldom report what product congener is formed from dechlorination of a chiral congener. Likewise the parent congener of the chiral congener is generally unknown. Determining which parent is dechlorinated to which product is difficult for field studies. However, lab studies by Pakdeesusuk et al. (2003a) and Brothersen (2011) have been able to track the dechlorination of chiral PCBs and their chiral products over time. Advanced studies need

to be made before the impacts of enantioselective parent/product relationships can be confidently identified in field settings.

In addition to the parent product relationship, the EF may be affected by bioaccumulation. Bioaccumulation is a complex process that involves direct partitioning of a contaminant between the organism and the medium it lives in as well as ingestion of contaminants from the diet (Schwarzenbach et al., 2003). Once an organism has ingested or made contact with a contaminant, that contaminant may persist in the organism or be excreted. Organic contaminants may accumulate in the lipids, proteins, or lignin among other biomolecules (Schwarzenbach et al., 2003). Proteins are chiral molecules themselves being composed of chiral amino acids. Components found in membranes such as phospholipids, glycolipilds, and sterols are also chiral molecules (Eghiaian, 2015).

Chiral PCB congeners are assumed to have differing levels of interaction with the chiral biomolecules. The difference in interaction may lead to an excess of one enantiomer within an organism. If there are enough organisms selectively accumulating one enantiomer, then less of that enantiomer will remain in the environment. The elimination of one enantiomer will give the impression that the PCB congener is being selectively dechlorinated. This hypothesis is challenging to test, but if all organisms in an ecosystem enantioselectively bioaccumulate the same enantiomer of a chiral congener, then it may point to the possibility that the chiral congener is not being dechlorinated enantioselectively. Instead, the nonracemic EF observed in the environment would be attributed to the universal bioaccumulation of the preferred enantiomer. However, providing evidence for such a claim would require a large body of data from multiple

organisms showing significant enrichment of one enantiomer across the board. Data from the literature review does not provide evidence to support enantioselective bioaccumulation as the driving force for the presence of nonracemic PCBs in environmental systems.

Enantioselective metabolism is not considered a factor in this study. Wu et al. (2014) found that less than 1% of PCB 136 was metabolized in human liver microsomes. The same study found that less than 3% of PCB 136 was metabolized by liver microsomes from male guinea pigs, hamsters, monkeys, mice, rabbits, or female dogs. Kania-Korwel et al. (2015) found that all the metabolites of PCB 95 accounted for only 0.02% of the total dose given to female mice. While these studies found the PCBs to be enantioselectively metabolized, the overall effect of metabolism was neglected for this study because of the small fraction of the total dose of PCBs.

Correlating the EF of chiral PCBs with soil or sediment parameters has been attempted by researchers with no clear answer. Multiple papers by Carlsson et al. (2016), Schuster et al. (2011), and Cui et al. (2011) have shown that concentration is not significantly correlated with EF. Carlsson et al. (2016) studied the EF of PCBs 95, 132, and 149 in addition to other persistent organic pollutants in soil samples from the Czech Republic. The enantiomeric shifts observed in the EFs of the chiral PCBs from 2005 to 2008 were correlated with such soil parameters as total organic carbon (TOC) and nitrogen content. No correlation was found between the change in EF from 2005 to 2008 and concentration of the congener.

Schuster et al. (2011) analyzed background soils from the UK and Norway. Sampling locations were the same as a study done 10 years earlier. The authors tried to find correlations between soil properties and the changes in the various persistent organic pollutants analyzed. The authors analyzed the change in EF for some chiral PCBs and found there to be no correlation between the enantioselectivity observed in the soil and concentration or soil organic matter.

Cui et al. (2011) sampled 23 locations in Jinan, China. Results showed that soil organic matter was significantly correlated to EF deviations for PCBs 95 and 132 but not PCB 149. No correlation between the EFs of any of the PCBs and concentration was found. The authors concluded that soil organic matter had an effect on the EF of the chiral PCBs because the soil organic matter affected the microbial community in the soil.

Total organic carbon and soil organic matter have been shown to be significantly correlated to EF by Carlsson et al (2016) and Cui et al. (2011). However, the soil organic matter was shown to not be significantly correlated with a change in EF by Schuster et al. (2011). Soil parameter data across the board are severely lacking, so making any universal statements at this point would be unreasonable. Much of the data in the literature includes only the EF and the concentration. Since concentration has been shown to not have any significant effect on the EF, concentration data remain unhelpful in analyses of chiral PCBs. To significantly correlate any parameter with the EF of any chiral PCB, improvements need to be made in how many measured parameters are reported alongside sampling data from the same location over a longer timescale. Carlsson et al. (2016) reported many different parameters of the soil from sampling

events done in 2005 and 2008. Adapting a similar plan that measures the soil parameters they did would be beneficial.

1.6 Meta-analysis

A meta-analysis seeks to compile literature data on a subject to find a true effect. It is "the combination of results from multiple independent studies" (Sutton 2007). Meta-analyses are often conducted on medical trials to determine the true effect of a new drug or therapy on patients, but they can be applied to any body of knowledge.

The literature search is the first step in performing a meta-analysis. Articles must be found that contain relevant data. Data then need to be extracted from the articles and compiled in a usable format. Statistical software can then be used to analyze the data and produce results to test hypotheses. For example, results of the meta-analyses have been used to show the decline of organic contaminants over time (Meng et al., 2016), the impact of a contaminant on cancer (Leng et al, 2016), or what affects pollutant concentrations in organisms (Hitchcock et al., 2017).

Meta-analyses are able to incorporate large amounts of data, but are limited in their performance by the quality of that data. Meng et al. (2016) collected 30 years of data on organic contaminants in sludge from the literature. Data for 35 classes of organic contaminants were taken from studies (n = 159) done in China. Results from the meta-analysis provided the authors with evidence to determine that the concentration of

polycyclic aromatic hydrocarbons in sludge had been in decline. The decline was attributed to the switch from domestic coal and biomass to natural gas and petroleum.

Leng et al. (2016) studied the effects of PCBs on breast cancer. Of the 2530 articles that were identified during the searching process, only 16 were specific enough to include in the meta-analysis. Results of the meta-analysis indicated that PCBs 99, 183, and 187 increase the risk of breast cancer. The authors were able to suggest mechanism for the PCBs' influence on breast cancer.

Analyzing chiral PCBs presents a challenge as literature data for chiral PCBs are often severely lacking compared to other fields of study to which meta-analyses have been applied. As with every aspect of statistics, an increased sample size gives the user greater confidence in the calculated results and predictions that stem from those results. Meta-analyses combine the data from multiple studies to increase the sample size. Therefore, by combining the small amount of studies that analyzed chiral PCBs, a larger sample size is created allowing for more confidence in the results.

Meta-analyses providing insight to various fields have been conducted with limited data. For example, Hitchcock et al. (2017) proceeded with a meta-analysis on pinnipeds (seals) in which few studies had eligible data. Data were collected on PCBs and mercury in mother-pup pairs, in addition to non-paired female-juveniles. Only eight studies contained data for PCBs in mother-pup pairs. However, relationships between contaminant concentrations and life history factors were still assessed. Therefore, it has been shown that meta-analyses can be done with small data sets.

Performing a meta-analysis provides an opportunity for researchers to understand the current body of knowledge. Those researchers have a better understanding of what data have been collected. Additionally, those researchers have an even better understanding of the holes that exist in the data. Meng et al. (2017) observed a lack of representative sampling in sludge nationwide. Leng et al. (2016) identified major factors such as weight loss that were not evaluated in the epidemiological studies they analyzed. Hitchcock et al. (2017) found there to be a significant lack of data included with the articles that they surveyed. Identifying the weaknesses of a data set will allow for future research to correct those problems.

By combining the results from different studies done around the world, a meta-analysis of EF values can explore whether a dechlorination process acts enantioselectively. The meta-analysis determines the variance within the data set as well as calculating the average EF value. These results can be used to determine if the true, average EF of a chiral PCB is racemic or not.

The meta-analysis will provide the average EF in addition to the standard error associated with that calculation. The standard error is the standard deviation divided by the square root of the sample size. A t statistic (Ott and Longnecker, 2010) can be calculated from the results of the meta-analysis (Equation 2).

$$t = \frac{y - \mu_o}{s/\sqrt{n}} \qquad Equation\ 2$$

where y is the calculated average EF, μ_o is the value we are comparing with the average EF, s is the standard deviation, and n is the sample size.

The t statistic then can be compared to a computed t value with the corresponding degrees of freedom and level of significance. T tables are available which contain computed t values for degrees of freedom and levels of significance. Using a wide variety of software, p values can be calculated from t statistics to show "the strength of evidence in the data against the null hypothesis" (Halsey et al., 2015).

2.0 RESEARCH OBJECTIVES

The goals of this research are to test two hypotheses:

1. Dechlorination process N is enantioselective.

This hypothesis will be tested by conducting t-tests:

- to determine the true average EF of PCBs 91 and 95 in soils and sediments.
- to determine the true average EF of PCBs 132, 136, and 149 in soils and sediments, as well as confirm which dechlorination process acted on each congener.

2. Enantioselective bioaccumulation occurs, but is not the reason for observing nonracemic EFs of chiral PCBs in soils and sediments.

This hypothesis will be assessed by the following:

- Analyze the enantioselective bioaccumulation of the chiral PCBs to determine any pattern in the data.
- Analyze the PCB concentration data within organisms if patterns reveal universal enrichment of one enantiomer.

3.0 EXPERIMENTAL PROCEDURE

A meta-analysis of enantiomeric fraction (EF) data for five PCB congeners was carried out with the SAS statistical software program. Data came from worldwide studies analyzing PCB contamination in water, soil, sediment, air, and organisms. A smaller portion of the data came from laboratory studies investigated bioaccumulation or dechlorination of the five selected PCBs. The appendix (Tables A.1-A.5) contains all the data gathered during the beginning stage of the meta-analysis.

All accessible data was used to compare and contrast the possible enantioselective dechlorination processes of the five selected stable chiral PCBs. Hypothesis tests were conducted to answer the following inquiries:

- if dechlorination process N is acts enantioselectively.
- if other known dechlorination processes can function enantioselectively.
- if the reported enantiomeric fraction of a PCB in a sample in the literature can be shown to statistically differ from 0.5 indicating the activity of any enantioselective forces.
- if the variance of the EF for a congener in a specified medium is equal to zero. All hypothesis tests were carried out using a statistical significance level of 0.05 following standard procedures (Cox, 2016).

EF results of the meta-analysis were determined to be nonracemic if smaller than 0.495 or larger than 0.505, which is consistent with the racemic composition used by Pakdeesusuk et al. (2003a). Determining if the calculated EFs are nonracemic was done

by using the resulting p value from a t-test. A p value lower than 0.05 signified a significantly nonracemic result. Nonracemic EFs could then be attributed to enantioselective bioaccumulation or dechlorination.

To provide the data for the meta-analysis, the literature was searched for keywords to find applicable journal articles. Keywords such as 'PCB', 'chiral', 'EF', 'soil', 'sediment', and 'dechlorination' were used. A total of 39 relevant articles were identified. Of these 39 articles, three contained only data duplicated from other publications. Furthermore, 15 articles did not contain all of the critical information for the meta-analysis. Contact was attempted with corresponding authors for each article with inaccessible data. Two authors responded to the request for data from three articles which left 12 articles with inaccessible data. The criteria for the data to be included in the meta-analysis were an average EF with a standard deviation and a reported sample size. The 12 articles with inaccessible data contained only one or two of the necessary three types of information, which disqualified them from being used in the meta-analysis. The resulting 24 articles (Figure 8) formed the sources of data. Every congener was not represented in every publication. Therefore, some congeners have more available data with which to work. PCB 95 was most represented in the literature while PCBs 132 had the least representation.

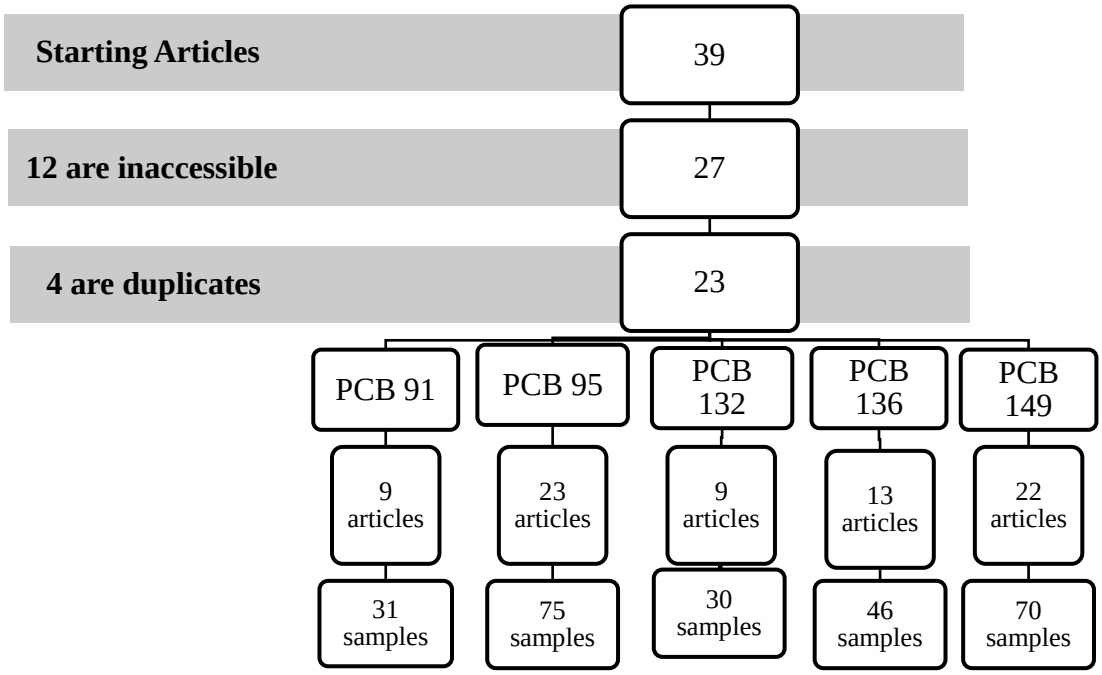

Figure 8. The total amount of sampling data from applicable articles for each of the target congeners. Individual sample data can be found in the appendix.

Data were extracted from the literature articles or their supplementary information. The average EF, standard deviation, and sample size were recorded and organized in a spreadsheet. Some supplementary information contained every data point from the sampling event. For these cases the average EF and standard deviation were calculated in Excel. Data also came from direct contact with Dr. Cindy Lee of Clemson University, Dr. Charles S. Wong of the University of Winnipeg, and Dr. Pernilla Carlsson of the Norwegian Institute for Water Research. All data that were extracted or received were

organized in spreadsheets. The entirety of the data used in this study is included in the appendix (Tables A.1-A.5). Some articles contained data from many different sites or organisms. Lowercase letters in tables A.1-A.5 were used to distinguish the sampling locations or organisms. The specific site name or organism from which the sample came can be identified in the individual articles.

Data were organized by the media in which the PCB was found. The media analyzed were air, soil, sediment, and tissue. These four media were chosen because of their significance and availability of data. There were not enough PCB data from the water column to make any significant judgements. PCBs were commonly analyzed in soil and sediments. These groups were analyzed separately due to the general likelihood of soils being a more oxic environment while sediments are typically anoxic environments. Differences have been noted between aerobic degradation and reductive dechlorination of PCBs (Wiegel and Wu, 2000). Aerobic degradation typically only affect lower chlorinated congeners while reductive dechlorination can affect highly chlorinated PCB congeners (Wiegel and Wu, 2000). Additionally, oxic environments contain different microbial communities than anoxic environments. Different microbial communities may utilize different dechlorination processes as they dechlorinate PCBs. Therefore, because of the difference in microbial communities and the difference in aerobic degradation and anaerobic dechlorination, a distinction was recognized between the two media.

The PCB data from animal or plant tissue were grouped together. This grouping was done to determine if the biological life exposed to PCBs bioaccumulated the same enantiomer. Further research may focus on any similarities or differences in

enantioselective bioaccumulation of similar organisms. However, for the purposes of this study, all data from organisms were combined.

The spreadsheet containing the data for the five select congeners had one tab for each congener to conform to the standards required by the SAS program. The SAS code (Appendix B) was run for each congener. The SAS code is provided to allow for ease of future analysis. Should more research be done with chiral PCBs, that data need only be added in the proper format to the existing data set (Appendix A, Tables A.1-A.5).

Results from SAS included an estimation of the variance for each congener in each medium, the estimated true average EF for each congener in each medium, and the standard error and 95% confidence interval for the EF. T-tests and p value determinations using the results of the SAS program were done in Excel.

4 RESULTS AND DISCUSSION

4.1 Variance within Media

The meta-analysis provided a description of the variance within each data set. The result accounts for the variance within each study as well as the variance between studies. Since the data were grouped according to the corresponding media, the variance within a particular media for each congener can be examined. A p value was used to determine if the variance is greater than 0 (Table 4). Having a variance greater than zero amongst the data sets implies that there was heterogeneity in the samples, which may result from a variety of causes such as differences in sampling or analysis technique. The significance level of 0.05 that governed the hypothesis tests also determined the significance of the variance findings. Determining a variance for PCB 91 in air and soil was not possible because only one source reported data from air and no sources reported data from soil.

Table 4. P values that govern the determination of zero variance. No p value is given for PCB 91 in air and soil because of the lack of data. Significant p values (< 0.05) in bold. The strength of the findings increases with color (yellow<orange<red).

PCB	91	95	132	136	149
	p	*p*	*p*	*p*	*p*
Air		0.0741	0.2526	0.1412	**0.0416**
Soil		**0.0028**	**0.0437**	**0.0281**	**0.0058**
Sediment	**0.0348**	**0.0042**	0.1284	**0.0215**	**0.0043**
Tissue	**0.0062**	**0.0002**	**0.0461**	**0.0083**	**0.0003**

The data for all target PCBs in air except for PCB 149 displayed variances determined to be zero ($p > 0.05$). All soil, sediment, and tissue samples except for PCB 132 in sediment showed variances greater than zero ($p < 0.05$). Because of this distinction in variances among the media, it is unlikely that the variances in the soil, sediment, and tissue samples were caused by experimental.

The lack of variance within air samples was expected. Chiral signatures of PCBs in air generally are racemic everywhere except for close to the Earth's surface where revolatilization of nonracemic PCBs could possibly occur (Harrad et al., 2006). Changes in EF occur when enantioselective bioaccumulation or dechlorination has taken place. Since neither of these processes happen in the atmosphere, air samples should have mostly uniform, racemic EF levels.

All congeners in soil and sediment have variances greater than zero except for PCB 132 in sediment. As seen from a study by Abraham et al. (2002), differences in microbial communities can lead to discrepancies in the degree of enantioselective dechlorination. The variance confirmed by the meta-analysis within the data sets for soil and sediment may be attributed to the varying microbial communities found therein. Other environmental conditions may be suggested to account for the variance in the EF of the data sets. TOC and total nitrogen levels have been proposed as affecting the EF of chiral PCBs (Carlsson et al., 2016). However, pinpointing a correlation between these or similar factors is not possible with the collected data. Most studies only included the concentration and EF values, so soil characteristics were largely unknown. Therefore, the results suggest the variance was nonzero in the data sets for soil and sediment because of

the differences in microbial communities. These results concur with results produced by Singer et al. (2002). They studied the degradation of four chiral PCBs by five different bacteria. Significant differences were seen in the selectivity of the bacteria when degrading the chiral PCBs.

All tissue samples showed variance greater than zero. From the literature review it was determined that enantioselective bioaccumulation may result from differences in the interactions of chiral biomolecules with the chiral PCBs. Organisms contain a wide variety of different proteins and lipid content (Schwarzenbach et al., 2003), so enantioselective bioaccumulation varying among organisms was not surprising. The results confirm the variability that exists within chiral biomolecules of biological organisms.

4.2 Racemic determination - Overview

The EF value for each congener in each media is shown in Table 5. For all five congeners in the four types of media, only seven combinations (bolded) resulted in nonracemic determinations. All congeners were racemic within air. Only PCB 95 was significantly nonracemic in soil. Three congeners, PCBs 95, 132, and 136 were significantly nonracemic within sediment. Only two congeners, 91 and 149, showed significantly nonracemic EFs in tissue.

Table 5. Average EF values and p values that govern nonracemic determinations. Bolded values represent statistically significant nonracemic EFs. No soil data and only one air sample for PCB 91 were found in the literature. Therefore, no p values could be calculated for PCB 91 in air and soil. P values less than 0.05 are significant (bold). The strength of the findings increases with color (yellow<orange<red).

	91		95		132		136		149	
	EF	p	EF	p	EF	p	EF	p	EF	p
Air	0.4920	-	0.4959	0.8430	0.4654	0.1853	0.4998	1.0000	0.4988	0.9790
Soil	-	-	0.4522	**0.0001**	0.4738	0.1690	0.4968	0.5660	0.5062	0.4300
Sediment	0.4721	0.0580	0.4250	**0.0010**	0.5222	**0.0030**	0.5391	**0.0140**	0.4689	0.2130
Tissue	0.3927	**0.0380**	0.5488	0.0580	0.4998	0.6250	0.4681	0.1090	0.4524	**0.0240**

Table 6 shows the p values associated with the significant differences in the average EF of the PCBs in differing media. Bolded p values indicate that there was a significant difference. For example, if the EF of a congener was significantly different between the air and soil, then there is evidence that the EF in the soil may not have resulted by simply deposition from the air. Some enantioselective force must have acted upon it if the EF is different from its assumed source.

Table 6. P values determining significant differences between the same congener in different media. P values less than 0.05 (bolded) indicate a significant difference in the EF in between the media. The strength of the findings increases with color (yellow<orange<red).

Differences in Media					
PCB	91	95	132	136	149
	p	p	p	p	p
Air/soil		**0.0007**	0.4087	0.3927	0.1583
Air/sediment	0.0883	**0.0038**	0.1078	**0.0135**	0.1896
Air/tissue	**0.0423**	**0.0455**	0.1955	0.09	**0.0295**
soil/sediment		0.1067	**0.0262**	**0.0193**	0.1353
soil/tissue		**0.0017**	0.1692	0.1273	**0.0125**
sediment/tissue	0.0899	**0.0009**	0.0915	**0.0078**	0.3349

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4.3 PCB 91

PCB 91 had the second least amount of usable data out of the five selected PCBs with 31 data points. Only one study reported results for EF values in air samples, and no

studies were found that reported EF values for PCB 91 in soil samples. Sufficient data were available from sediment and tissue samples. The one air sample on record indicates a racemic EF having an average of 0.492 (Asher et al., 2007). However, no statistical analysis can be done on the data from just one study.

Results indicated that congener 91 was racemic in sediment samples. However, the p value of 0.058 associated with PCB 91 in sediment was close to the rejection value of 0.05. Figure 9 shows the data from the literature. Much of the sampling data shows enrichment of the second eluting enantiomer. Eight of the 13 data points show EF values below 0.5. The remaining five data points were equal to 0.5 (three points) or above 0.5 (two points).

Figure 9 displays the data in a forest plot. Lowercase letters are used in the plots to distinguish between data taken from the same study with different sampling locations or different organisms. Forest plots are commonly used in meta-analyses to display the data from all sources as well as the results of the meta-analysis. The average EF of the studies is displayed with the 95% confidence interval as the error bars. Points that are missing error bars represent data that consisted of just one sample. The average EF and 95% confidence interval from the calculation derived from the meta-analysis is displayed last and in red to distinguish it from the literature data. The purpose of the forest plots is to show how well the original raw data corresponds to the calculated value.

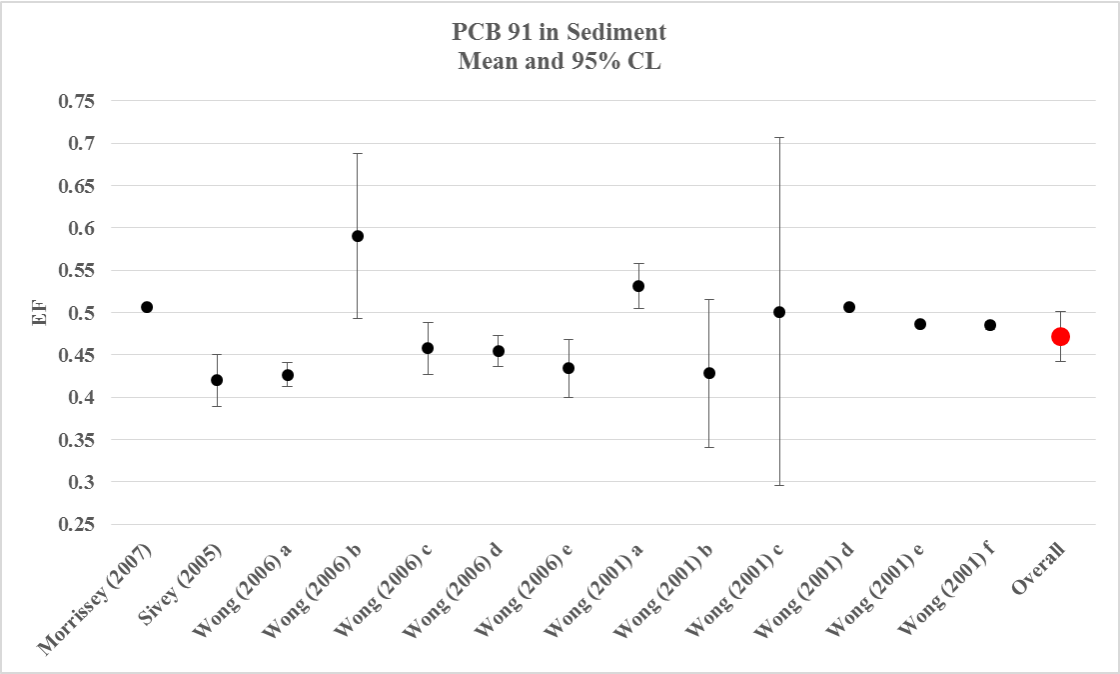

Figure 9. Forest plot of PCB 91 sampling data from sediment. Raw data can be found in Table A.1. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis. Large error bars occurred for Wong (2006) b and Wong (2001) c because of the large standard deviation and small sample size (n = 2), respectively.

As with most of the congeners, tissue data for PCB 91 (Figure 7) did not follow a recognizable trend. The overall EF of tissue data was significantly nonracemic with a value of 0.393. However, organisms did not universally bioaccumulate one enantiomer. The data showed dispersion across the board with some organisms preferentially bioaccumulating the first eluting enantiomer while others preferred the second. While there are some differences in the enantiomer bioaccumulated, ten of the 15 data points show average EFs less than 0.5. The result supports the hypothesis that any nonracemic

levels of PCB 91 in soils and sediments were not a consequence of enantioselective bioaccumulation.

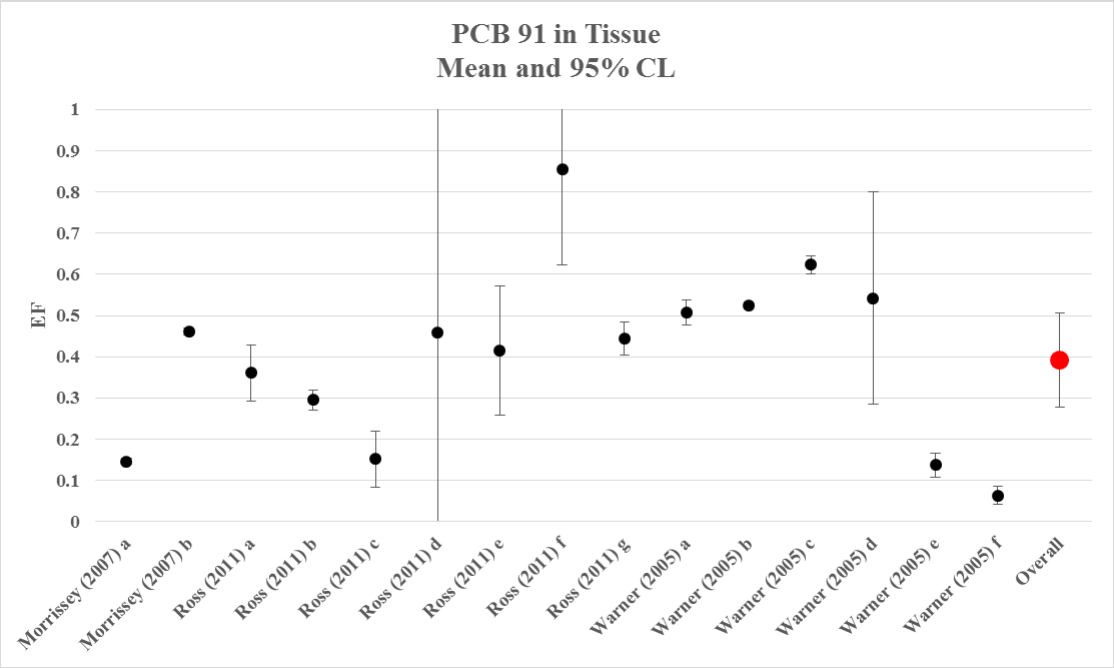

Figure 10. Forest plot of PCB 91 sampling data from tissue. Raw data can be found in Table A.1. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis. Large error bars occurred for Ross (2011) d and Warner(2005) d because of the small sample size (n = 2) and large standard deviation, respectively.

Data from sediment as well as tissue on average indicated elimination of the first eluting enantiomer. No difference was found between the EF values of PCB 91 in the sediment and in tissue (p=0.0899). Enrichment of the same enantiomer in the sediment and tissue implied a depletion of the other enantiomer. Data from the soil were not

available for PCB 91, and only one study by Dang et al. (2013) reported EF values for PCB 91 in water. The reported average EF value of PCB 91 in water was 0.659 which showed enrichment of the first eluting enantiomer. The reported EF from water showed enrichment of the opposite enantiomer than the EFs in sediment and tissue. One possibility may be that the first eluting enantiomer dominates in the soil or water. If one enantiomer dominates in the water and soil and the other enantiomer dominates in the sediment and tissue, differences in microbial communities due to oxic or anoxic environments may be responsible for the discrepancy in the enantioselectivity. However, data were not available to test the idea. With no data from soil samples for PCB 91 and only one study reporting EF values of PCB 91 in water, evidence was lacking concerning the difference in EF when comparing oxic and anoxic environments. The significant lack of the first eluting enantiomer suggests that PCB 91 undergoes enantioselective dechlorination or the enrichment of the second eluting enantiomer was a product of enantioselective dechlorination of a chiral parent PCB.

Because the elution order of PCB 91 is not known, it is not possible to compare the potential parent/product relationship with the other chiral congeners (PCB 132, 139, and 149) for which PCB 91 could be a product unless samples were taken over a period of time and produced EF data for both the parent and product congener. Also it is unclear whether a (+) parent enantiomer will dechlorinate to a (+) or (-) product enantiomer. Therefore, currently it is not possible to assess the impacts of the potential enantioselective dechlorination of chiral parent congeners on the EF of PCB 91 for most samples.

Two studies by Pakdeesusuk et al. (2003a) and Brothersen (2011) analyzed the enantioselective dechlorination of PCBs 132 and 91 by microorganisms from sediment in Lake Hartwell, South Carolina. Both studies showed PCB 132 was not enantioselectively dechlorinated, but PCB 91 was. These studies do not provide any evidence on whether or not a parent congener being enantioselectively dechlorinated will affect the EF of the product congener because PCB 132, the parent congener, was not enantioselectively dechlorinated. However, the type of experiments performed would have allowed for such an analysis had the parent congener been enantioselectively dechlorinated.

PCB 91 is only known to dechlorinate by process N (Bedard and Quensen, 1995). Since one enantiomer of PCB 91 is not universally bioaccumulated, enantioselective bioaccumulation does not explain the nonracemic levels of PCB 91 in soils and sediments. Parent/product relationships between PCB 91 and other chiral congeners lack the necessary information to make any judgements concerning their impact. Thus, evidence from the meta-analysis suggested that PCB 91 was dechlorinated enantioselectively. Since PCB 91 is only known to dechlorinate through process N, results from the meta-analysis support the hypothesis that dechlorination process N functions enantioselectively.

4.4 PCB95

Of the five congeners PCB 95 was the most represented in the literature with 75 data points. Statistics revealed PCB 95 to be nonracemic in both the soil and sediment but

not air or tissue. Racemic EF values in the air were to be expected as seen in Figure 11. The EF value for tissue samples (Table 3) was close to being considered nonracemic with an EF value of 0.5488 (p=0.0588).

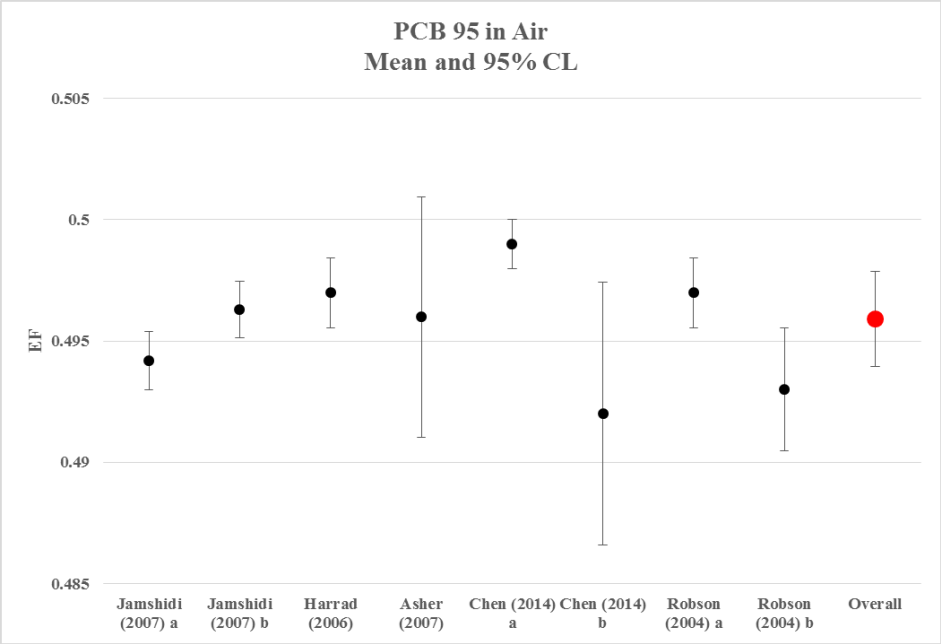

Figure 11. Forest plot of PCB 95 sampling data from air. Raw data can be found in Table A.2. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis. Note the narrow scale of the y axis, indicating the data as clustered around a core value.

A p value of 0.058 was found for the nonracemic determination of PCB 95 in tissue; therefore, overall the EF values measured in tissues for PCB 95 were not significantly different from racemic. The p value may be explained by the error assigned to some of the sampling data as seen in Figure 12.

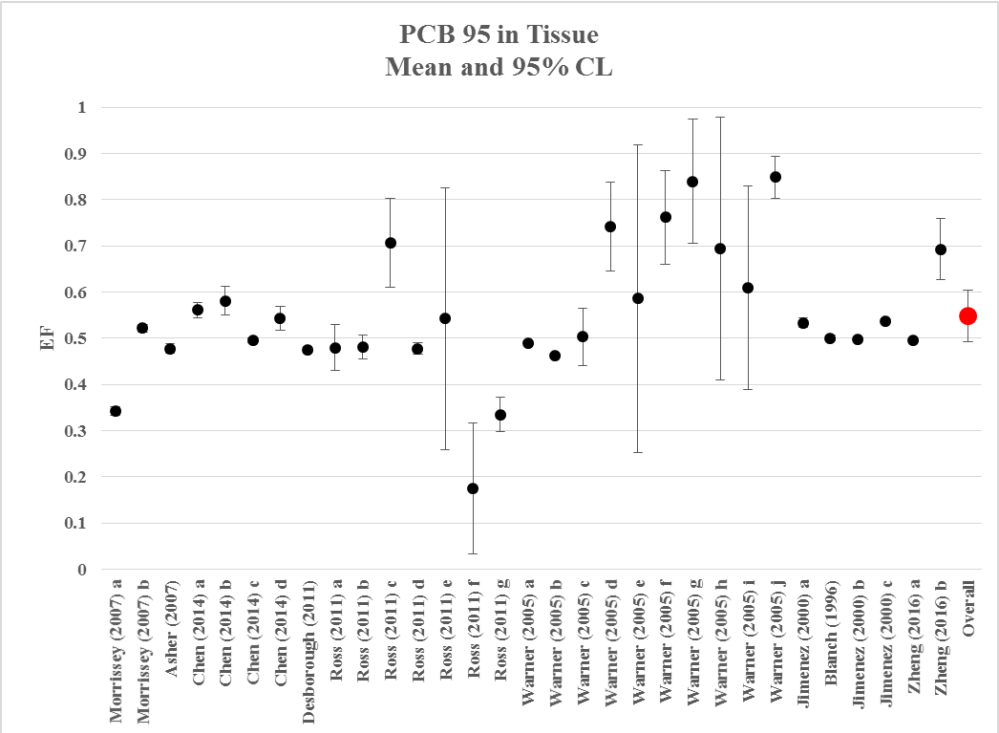

Figure 12. Forest plot of PCB 95 sampling data from tissue. Raw data can be found in Table A.2. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis. Large error bars are given throughout the data points due to large standard deviations, small sample sizes, or a combination of both.

-

However, from the forest plot it is apparent that enantioselective bioaccumulation takes place in a wide variety of organisms. The average EF value then was not found to be racemic due to the sporadic nature of the bioaccumulation. Some organisms preferentially accumulated the first eluting enantiomer while others preferred the second. With more data the plot would likely remain just as noisy. This conglomeration of

differences in enantioselective bioaccumulation is vital. The current data set from the literature review showed there was no universally preferentially bioaccumulated enantiomer of PCB 95. Therefore, it remains unlikely that the nonracemic EF values measured in the soil and sediment were a result of enantioselective bioaccumulation.

The sediment (Figure 13) and soil (Figure 14) data indicated that the first eluting enantiomer of PCB 95 was preferentially dechlorinated. Both estimated average EF values were significantly nonracemic and showed enrichment of the second eluting enantiomer. Only two observations by Wong et al. (2009) and Zheng et al. (2014) reported EF values above 0.5.

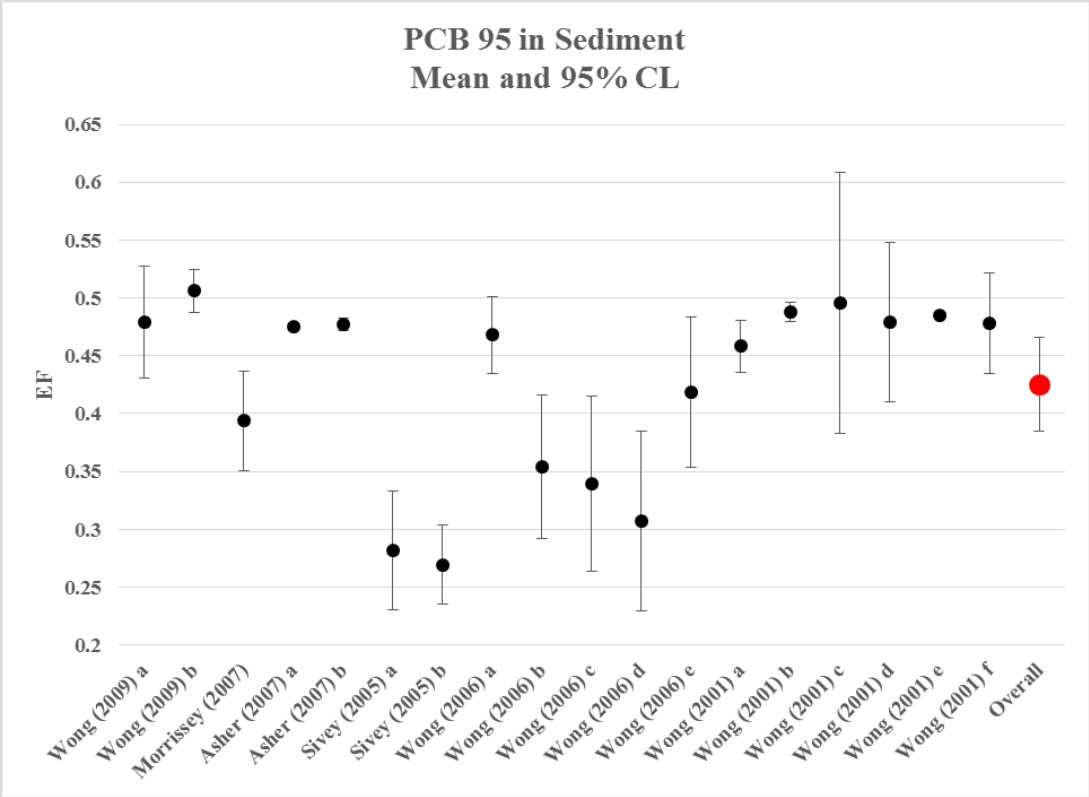

Figure 13. Forest plot of PCB 95 sampling data from sediment. Raw data can be found in Table A.2. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis. The data point for Wong (2001) c has the largest error bars due to its small sample size (n =2).

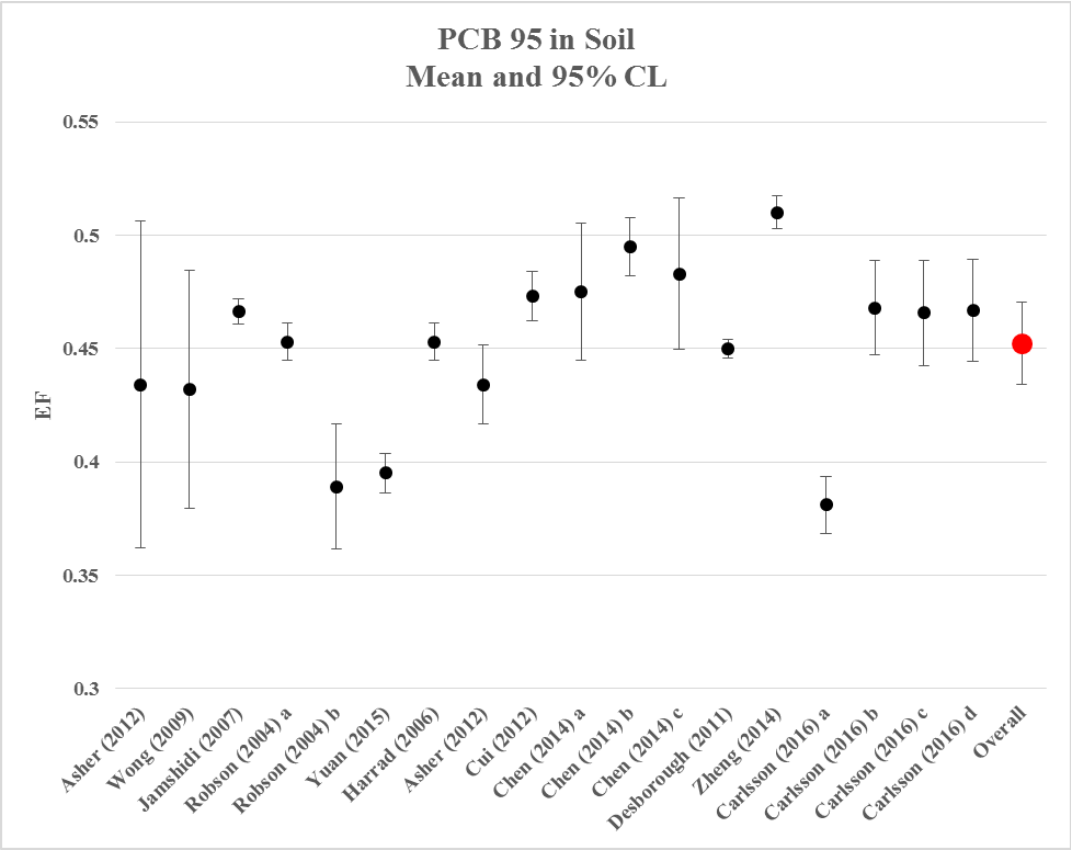

Figure 14. Forest plot of PCB 95 sampling data from soil. Raw data can be found in Table A.2. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis. The data from Asher (2012) has a large standard deviation which increases the confidence interval.

The elution order of PCB 95 is currently unknown. Therefore, parent/product relationships cannot be properly analyzed. Additionally, data for all the possible parent congeners of PCB 95 except PCB 149 were especially limited. The literature review yielded significant results for only congener 149. PCB 149 was racemic in soil and

sediment. Therefore, since PCB 149 was racemic in those media, there was not any evidence to suggest PCB 149 was being enantioselectively dechlorinated. Since the parent congener was not enantioselectively dechlorinated, there was no evidence to support enantioselective dechlorination of a chiral parent congener affecting the EF of the chiral product congener.

Tissue data for PCB 95 revealed sporadic enantioselective bioaccumulation and an overall racemic EF value. Parent/product relationships are currently unavailable for consideration for PCB 95. Therefore, enantioselective dechlorination remains the most reasonable explanation for the nonracemic EFs observed in soils and sediments. PCB 95 has been reported to only dechlorinate through dechlorination process N (Bedard and Quensen, 1995). Consequentially, the results of the meta-analysis suggest that dechlorination process N acts enantioselectively.

4.5 PCB 132

PCB 132 constituted the least amount of data of the five selected PCBs with 30 data points. PCB 132 was determined to be only significantly nonracemic in sediments (Table 4). Data from the literature showed unusual sampling trends from air as well as organisms compared to the other congeners studied. In air samples, PCB 132 had an average EF value of 0.4654, which although not significant ($p = 0.1853$) was the lowest EF of the five chiral congeners. In tissue samples, the average EF was 0.4998, which

again was not found to be significant ($p = 0.625$), but was the closest to racemic of any of the studied congeners.

The only air data were from one study done in southern China by Chen et al. (2014). The study reported racemic EF values for other chiral PCBs in the air such as 95, 136, and 149. In addition, PCB 84, which is also a chiral congener but not included in my study, was reported as having a nonracemic EF in the air. PCBs 84 and 132 from air samples were analyzed using a BGB 172 column while the other chiral congeners, PCBs 95, 136, and 149, were analyzed using a ChiraSil-Dex column. Every other air sample used in the meta-analysis for the five target chiral PCBs was analyzed using a ChiraSil-Dex column (Chen et al., 2014, Robson and Harrad, 2004, Jamshidi et al., 2007, Asher et al., 2007, and Harrad et al., 2006). PCB 132 from sediment samples has even been shown to be separated on a ChiraSil-Dex column (Wong et al., 2007). It was not clear from the article by Chen et al. (2014) or from the supporting information why the BGB 172 column was used instead of the ChiraSil-Dex. Regardless, the meta-analysis determined there were insufficient amounts of data to determine if the EF of PCB 132 in the air was significantly nonracemic. It may be possible that the waste site that was sampled in southern China did have some volatilization of enantioselectively dechlorinated PCB 132 from the soil. However, the samples from soil for the area reported racemic levels of PCB 132. The organisms measured at the site also appeared to be enriched in the second eluting enantiomer (average EF = 0.4685) as was reported in the air samples. The discrepancy presents a conundrum as it appears the second eluting enantiomer has been removed as it is not in the soil, organisms, or air at the southern China site. With the

current lack of knowledge on any parent/product relationships, these sampling data for the air may be viewed as an outlier. All other air data for every congener analyzed in the meta-analysis reported racemic EF levels. I would suggest that the BGB 172 column did not correctly separate the enantiomers of PCB 132 from air samples which resulted in EF values that appear to be nonracemic.

Results suggested that congener 132 resists enantioselective bioaccumulation (Figure 15). Results from the meta-analysis indicated that PCB 132 was racemic within tissues. The average EF for PCB 132 in organisms was racemic because the data showed mostly racemic values. Racemic EF values for PCB 132 in tissue denies the possibility of any nonracemic EF values in the soil or sediment being attributed to some enantioselective bioaccumulation.

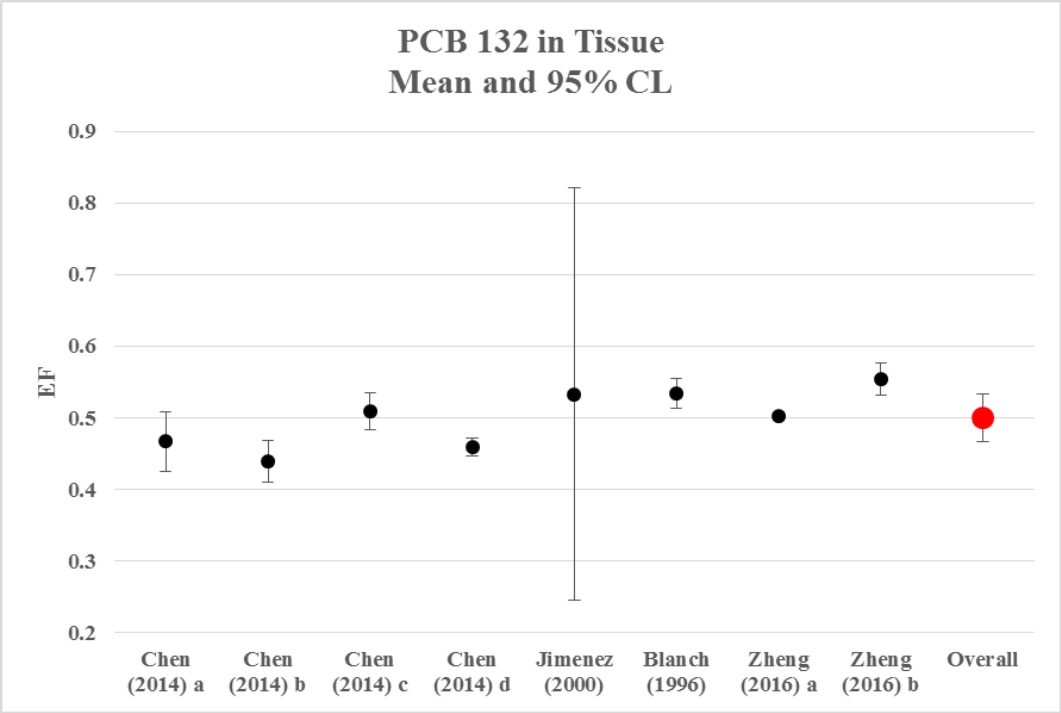

Figure 15. Forest plot of PCB 132 sampling data from tissue. Raw data can be found in Table A.3. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis. The large error bar for the data from Jimenez (2000) was attributed to the small sample size (n = 2).

The average EF of PCB 132 in sediment (EF = 0.5222) was nonracemic (p =0.0030) while the average EF of PCB 132 in soil (EF = 0.4738) was racemic (p = 0.1690). The EF values from the two media are oppositely enriched and significantly different from one another (p = 0.0262). The soil samples (Figure 17) showed an enrichment of the second eluting enantiomer while the sediment samples (Figure 16) showed enrichment of the first eluting enantiomer. The difference in the enriched

enantiomer provides evidence for the different capabilities of dechlorinating microorganisms as has been discussed.

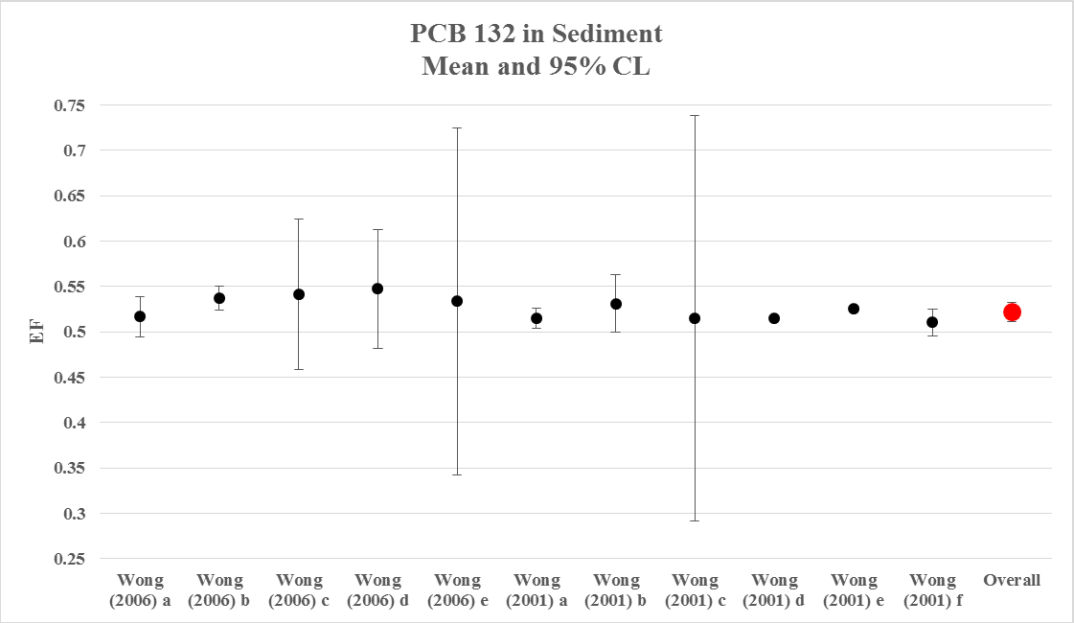

Figure 16. Forest plot of PCB 132 sampling data from sediment. Raw data can be found in Table A.3. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis. Large error bars occurred for Wong (2006) e and Wong (2001) c because of the small sample size (n = 2 for both).

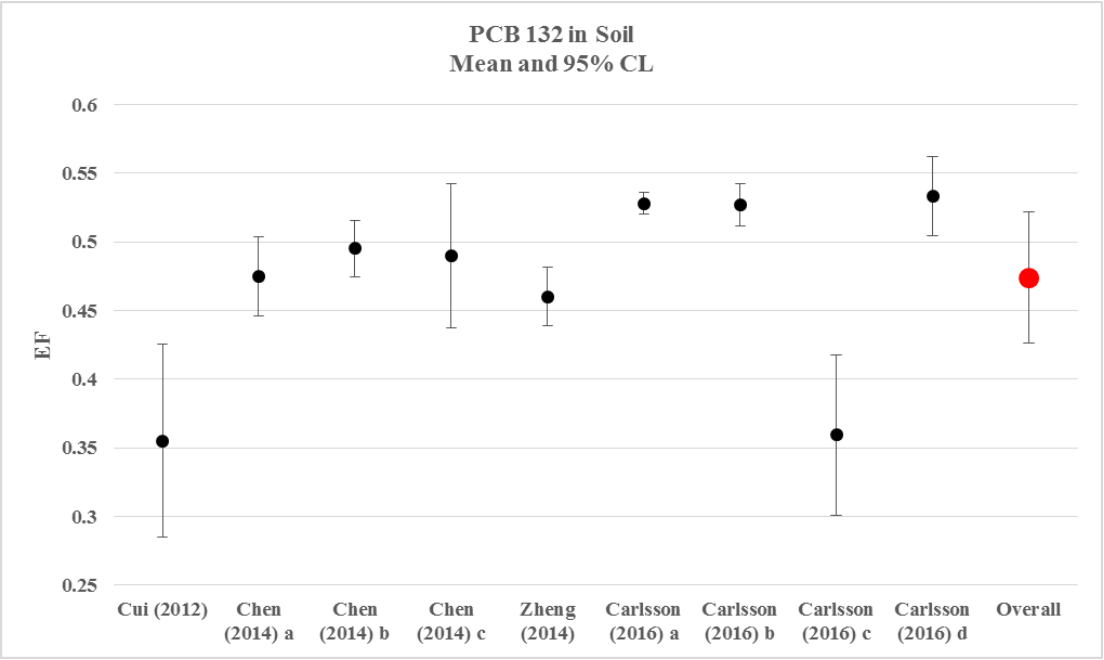

Figure 17. Forest plot of PCB 132 sampling data from soil. Raw data can be found in Table A.3. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis. Large error bars are given to data points Cui (2012) as well as Carlsson (2016) c due to their large standard deviations.

Enantioselective bioaccumulation seemingly did not occur to any great extent for PCB 132. As a result any nonracemic EFs observed in soils or sediments cannot be attributed to organisms universally bioaccumulating one enantiomer. Likewise, reasons surrounding the unknowns of parent/product relationships prohibit their correlation with any nonracemic EFs of PCB 132. Therefore, the nonracemic EFs observed for PCB 132 in sediments can be attributed to enantioselective dechlorination.

PCB 132 has been reported to dechlorinate through processes N, P, or H (Bedard and Quensen, 1995). If the product congener that is produced by the dechlorination of PCB 132 remains unknown, determining which process was active is not possible. Some studies done by Pakdeesusuk et al. (2003a) and Brothersen (2011) analyzed laboratory incubations of PCB 132 as it was dechlorinated by microorganisms from sediments taken from Lake Hartwell, SC. Both studies showed PCB 132 lost the *meta*-chlorine on the 234 ring to produce PCB 91. Process N and process H both can act to remove that *meta*-chlorine (Bedard and Quensen, 1995). The two studies showed PCB 132 was not enantioselectively dechlorinated implying that one or both processes do not dechlorinate enantioselectively. Process N can also act on PCB 132 to remove the chlorine on the 236 ring. If process N was active for these two studies, results may instead suggest that process N is only enantioselective when acting on the 236 ring.

Results suggested PCB 132 was enantioselectively dechlorinated in sediments but not in soils, which implies that not all dechlorination processes acting on PCB 132 are enantioselective. If only one process (N, H, or P) was active in all samples, then results imply that the active process was not enantioselective all the time. Due to the three potential processes that can act on PCB 132, these results cannot supply the evidence for the enantioselective dechlorination of process N.

4.6 PCB 136

A total of 46 data points were found for PCB 136 in the literature review. Meta-analysis results indicated that PCB 136 existed at nonracemic levels in only sediments (Table 4). PCB 136 was racemic in all other media studied. Average EF levels in the air were racemic (Figure 18) which was to be expected.

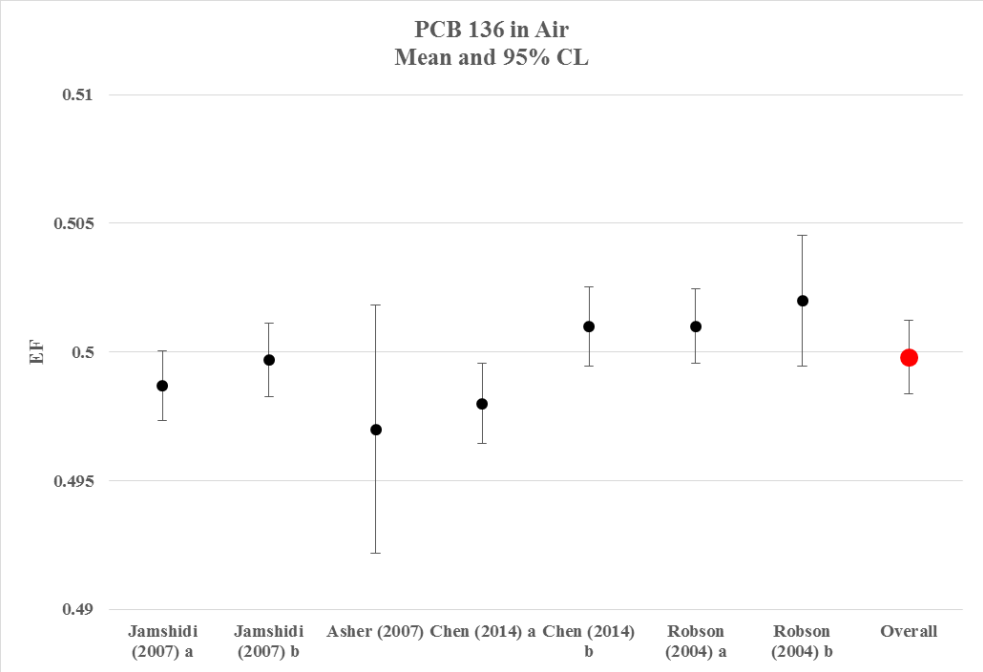

Figure 18. Forest plot of PCB 136 sampling data from air. Raw data can be found in Table A.4. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis. Larger error bars from the study by Asher et al. (2007) were due to the large standard deviation observed in the smaller sample size (n = 13).

Results indicated that PCB 136 existed at racemic levels in soil (Figure 19). Six of the nine data points showed near racemic EFs. The three data points that seemingly deviate from racemic levels were not uniformly enriched. Data from Yuan et al. (2015) showed enrichment of the (+) enantiomer, and data from Robson and Harrad (2004) showed enrichment of the (-) enantiomer. The discrepancy between the studies can best be explained by potential differences in microbial communities (Abraham et al., 2002).

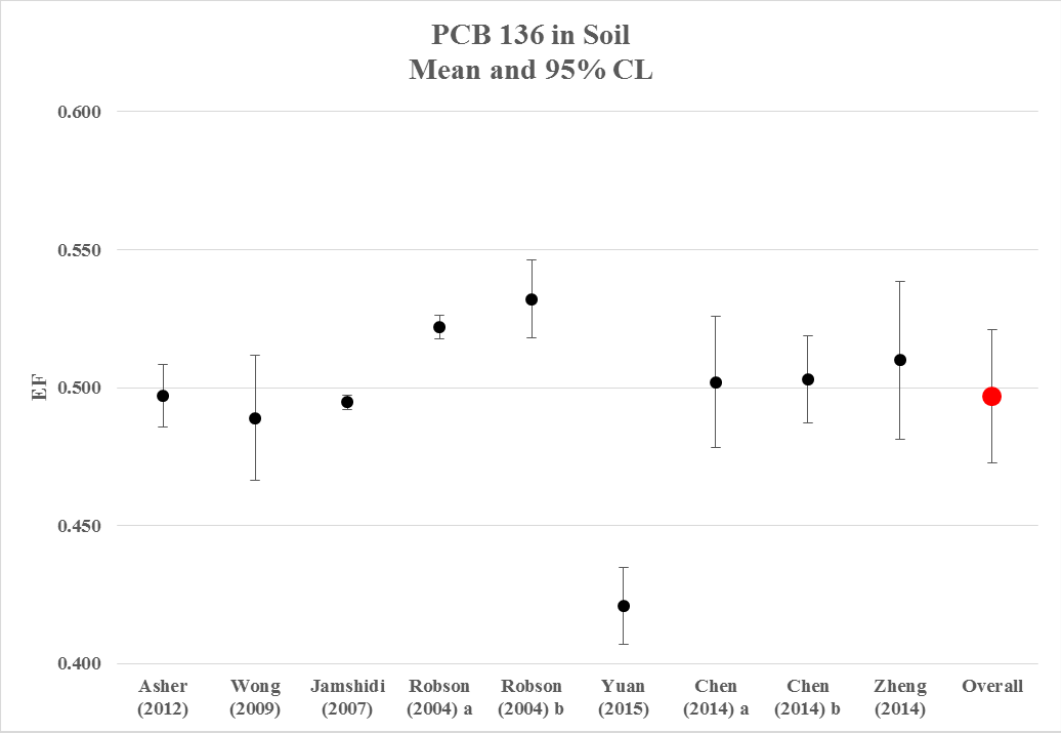

Figure 19. Forest plot of PCB 136 sampling data from soil. Raw data can be found in A.4 of the appendix. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis.

The average EF of congener 136 in sediment was nonracemic with an enrichment of the (+) enantiomer (Figure 20). Only data from one location in one study (Wong et al., 2001) suggested enrichment of the (-) enantiomer (EF = 0.4565). This abnormal result came from one sample taken at one location which does not provide much confidence in the true EF value for that location. Statistical analysis revealed the EFs of sediment and soil to be significantly different from one another ($p = 0.0193$). Again the difference between the two media highlights potential differences between aerobic and anaerobic microorganisms and their preferences for degradation of enantiomers.

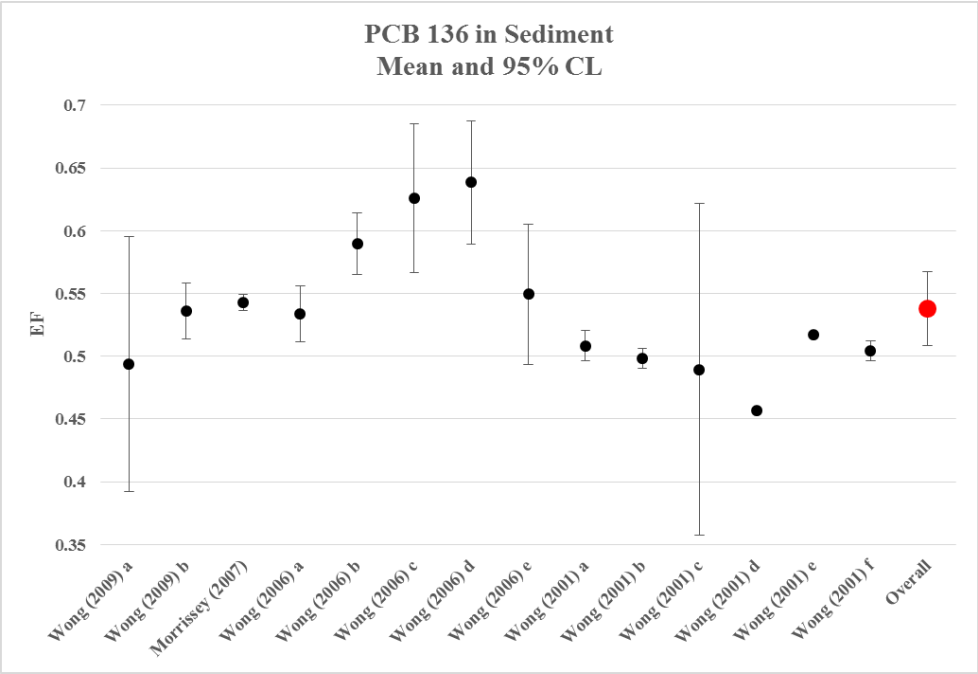

Figure 20. Forest plot of PCB 136 sampling data from sediment. Raw data can be found in Table A.4. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis. The large error bars seen for Wong (2001) c were due to the small sample size (n = 2).

Data from organisms appear to show a general lack of significant enrichment of any enantiomer of PCB 136 (Figure 21), and the data contained insufficient grounds for any nonracemic determination ($p = 0.1090$). Thirteen of the 16 data points showed near racemic values while the other three showed enrichment of the (-) enantiomer. The general randomness of the EF values observed in tissue provided further evidence for the variability that exists within the chiral molecules of cells.

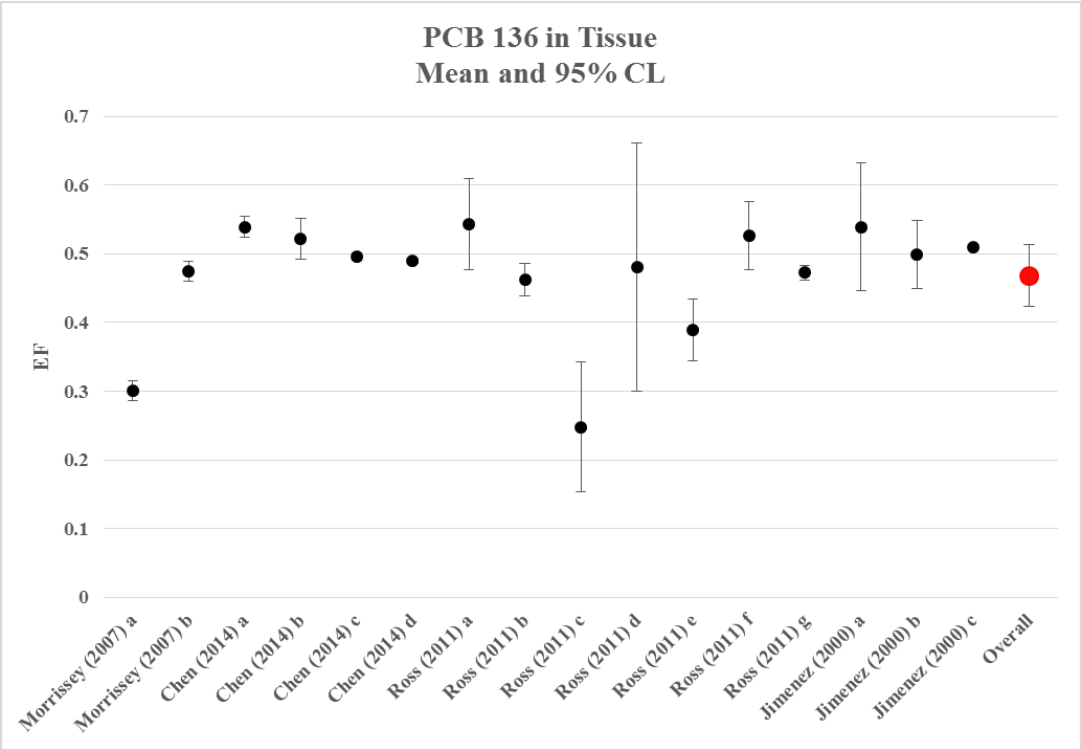

Figure 21. Forest plot of PCB 136 sampling data from tissue. Raw data can be found in A.4 of the appendix. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis. Large error bars are given to the data point Ross (2011) d because of the small sample size (n = 2).

Because the meta-analysis did not determine the average EF in tissue to be nonracemic, the enrichment of the first eluting enantiomer in sediment cannot be attributed to bioaccumulation of the second enantiomer. Neglecting parent/product relationships, enantioselective dechlorination remains as the most reasonable explanation.

PCB 136 was not reported as being dechlorinated by any process. Because it contains two 236 rings, it is likely that process N can act upon it. However, without any

confirmation of which processes can dechlorinate PCB 136, the results of the meta-analysis cannot provide evidence for any claim made about process N being enantioselective.

4.7 PCB 149

PCB 149 had the second largest amount of available data from the literature with 70 data points. Results from the meta-analysis indicated PCB 149 existed at racemic levels in all media (Table 4) except for the tissue of organisms (Figure 22). The EF of PCB 149 in tissue was similarly random like the EF of the other PCBs in tissue. Because the meta-analysis determined the EF in tissue to be nonracemic, any nonracemic EFs of PCB 149 in soil or sediment cannot be caused by enantioselective bioaccumulation.

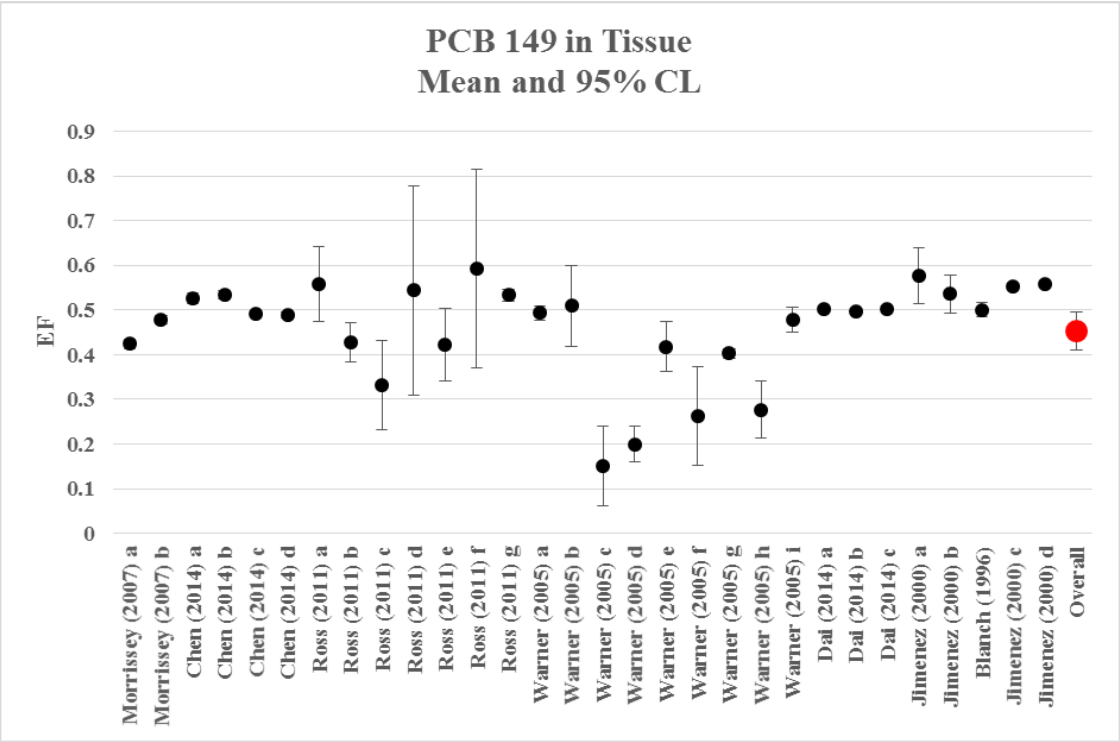

Figure 22. Forest plot of PCB 149 sampling data from tissue. Raw data can be found in Table A.5. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis.

Of the five congeners studied, PCB 149 was the only one to be found at racemic EFs in both soil (Figure 23) and sediment (Figure 24). The forest plots give an interesting picture. EF levels in soil may deviate slightly from the racemic value, but clearly the true average EF in soil was racemic as supported by the meta-analysis. The data for PCB 149 in sediment contained three data points that appear to be nonracemic. Two data points from Sivey (2005) show significant enrichment of the second eluting enantiomer while the other data point from Morrissey et al. (2007) shows slight enrichment of the first eluting enantiomer. Instead of these data being considered outliers, they support the

varying capabilities of microbial communities. These nonracemic data points may instead be attributed to different processes acting on PCB 149. PCB 149 can dechlorinate through process N, H, or P (Bedard and Quensen, 1995). One or two of these processes may be enantioselective which would explain why very few data points show nonracemic EFs.

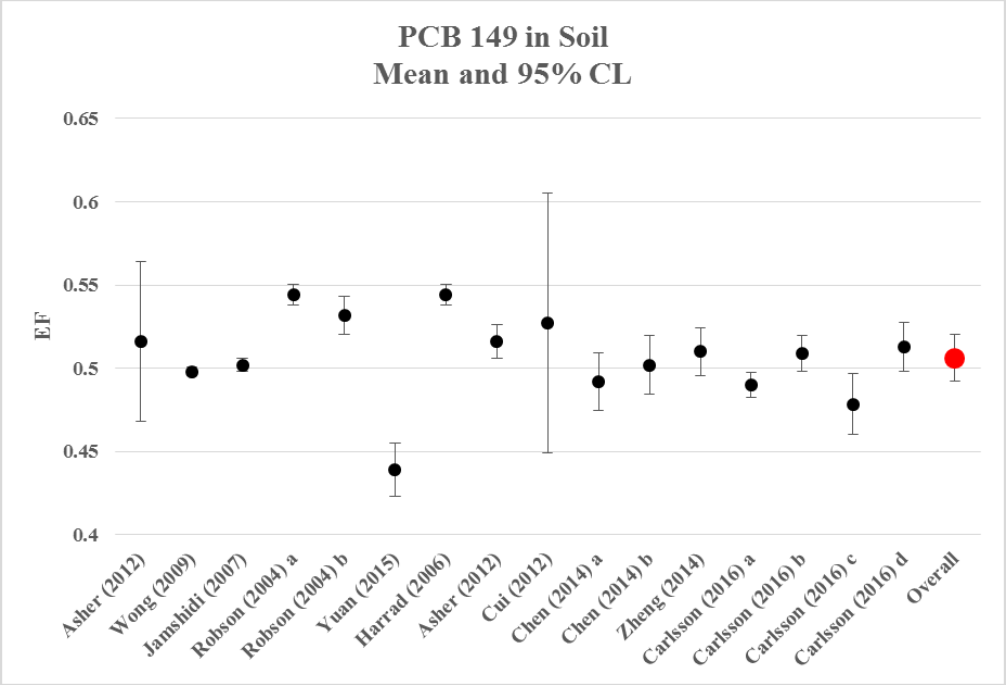

Figure 23. Forest plot of PCB 149 sampling data from soil. Raw data can be found in Table A.5. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis. Significant error bars occurred for the data from Cui (2012) because of their large standard deviation.

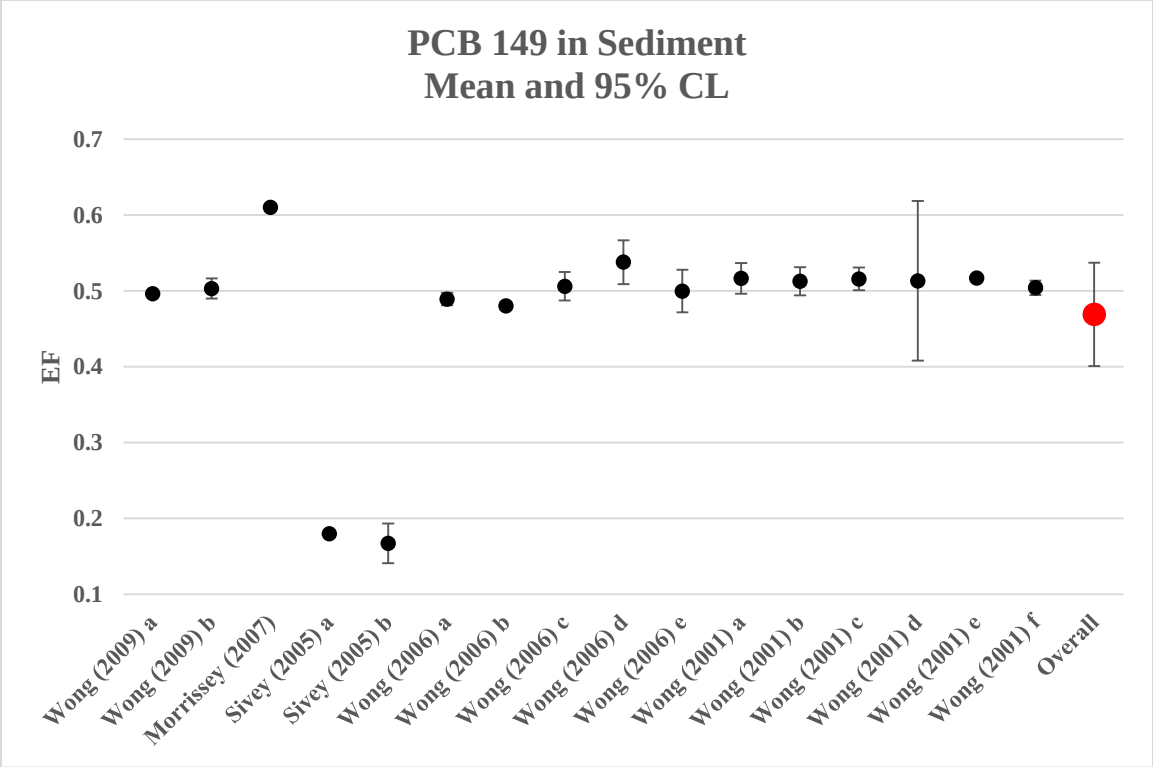

Figure 24. Forest plot of PCB 149 sampling data from sediment. Raw data can be found in Table A.5. Each point on the plot is the average EF. Error bars show the 95% confidence interval. The red dot displays the true average EF suggested by the meta-analysis.

PCB 149 can be dechlorinated through process N, P, or H (Bedard and Quensen, 1995). As seen with PCB 132 and 136, the results from the meta-analysis cannot give support to the idea that process N does or does not act enantioselectively due to the multiple possible dechlorination pathways.

PCB 149 can be dechlorinated to PCB 91 or 95. The overall average EF showed that congener 149 exists at racemic levels in the soil and sediment. Both media

containing racemic levels of PCB 149 suggests that any enantiomeric enrichment of PCB 91 or 95 cannot be attributed to the enantioselective dechlorination of PCB 149. However, this is only one of many possible parent/product relationships that can potentially influence the EF of PCB 91 and 95.

A study in 2005 by Sivey examined chiral PCBs in sediment in Lake Hartwell, SC, USA. Sediment cores were taken from the same two locations as other studies (Pakdeesusuk et al., 2003b; Wong et al., 2007). The data showed PCB 149 and 95 both at highly nonracemic levels which may cause one to speculate that the enantioselective dechlorination of PCB 149 was producing an enrichment of one PCB 95 enantiomer. Since it is unknown which enantiomer ((+) or (-)) of PCB 95 is formed by the dechlorination of the (+) or (-) enantiomer of PCB 149, it remains impossible to determine if the enantioselective dechlorination of PCB 149 affected the EF of PCB 95.

4.8 Extent of Enantioselective Dechlorination

Determining the extent of enantioselective dechlorination is imperative. Using concentrations and EF data from the same sampling locations at different times allows the determination of which enantiomer is being dechlorinated. Wong et al. (2007) has shown that the EF of a chiral PCB changes as time passes. Interestingly the direction and magnitude of the change is not uniform. Evidence suggests that strict enantioselective dechlorination that will only dechlorinate one enantiomer is possible.

The data from the study by Wong et al. in 2007 analyzed EF levels in sediments from Lake Hartwell, South Carolina from 1987 and 1998. EF levels were statistically determined to be significantly different from one another for multiple locations and congeners. Some PCBs showed increasing enrichment of one enantiomer while other PCBs saw a return to racemic over time. However, concentration data were not included with the EF data. Therefore, it was not possible to determine how much of each enantiomer was dechlorinated during the time and, by extension, the extent to which enantioselective dechlorination acted.

Carlsson et al. (2016) also analyzed chiral PCB EF data from soil samples taken in 2005 and again in 2008. Concentration data was included with the EF data for their study. Using equation 1 the concentrations of each enantiomer can be determined. For example, the average concentration of PCB 132 in samples from 2005 was 0.095 ng/kg. The average EF from the samples was 0.3592.

$$(1)\quad EF = \frac{A}{A+B} = 0.3592 = \frac{A}{0.095\frac{ng}{kg}}$$

$$(2)\quad A = 0.3592 * 0.095\frac{ng}{kg} = 0.0341\frac{ng}{kg}$$

$$(3)\quad B = 0.095\frac{ng}{kg} - 0.0341\frac{ng}{kg} = 0.0609\frac{ng}{kg}$$

The same calculation can be used to find the concentrations of the first (-) and second (+) eluting enantiomers from the samples taken in 2008. Table 7 summarizes the

results. From just this one study strict enantioselective dechlorination is implied. It appeared that none of the (-) enantiomer was dechlorinated over the three years. The data revealed that only the (+) enantiomer was selectively dechlorinated.

Table 7. Results of enantioselective dechlorination of PCB 132 from 2005 to 2008.

PCB 132	Concentration, ng/kg		EF
	(-)	(+)	
2005	0.0341	0.0609	0.3592
2008	0.0346	0.0304	0.5329

Carlsson et al. (2016) also reported data for PCB 149 at the same location three years apart. Data for PCB 149 showed dechlorination of both enantiomers but again a larger decrease in the (+) enantiomer (Table 8).

Table 8. Results of enantioselective dechlorination of PCB 149from 2005 to 2008.

PCB 149	Concentration, ng/kg		EF
	(-)	(+)	
2005	0.1344	0.1466	0.4783
2008	0.1190	0.1130	0.5129

Data such as these broadens the understanding of enantioselective dechlorination processes. The dechlorination of PCB 132 was particularly interesting. In 2005 the EF in the soil was 0.3592, which was significantly nonracemic and suggested the (-) enantiomer

was being preferentially dechlorinated. However, between 2005 and 2008 the (-) enantiomer was entirely untouched, and the (+) enantiomer was dechlorinated. These changes in which enantiomer is preferentially dechlorinated support the variance results of the meta-analysis. Differing microbial communities possess the capability to dechlorinate different enantiomers of chiral PCBs.

Ultimately these results from 2005 and 2008 gave no indication as to the enantioselective capabilities of dechlorination process N. Because the product congener was not identified, it is not possible to determine which dechlorination process was acting on PCB 132 in the years leading up to 2005 and between 2005 and 2008. However, the results do provide evidence that enantioselective dechlorination processes can act to varying degrees.

Results of the meta-analysis for PCBs 91 and 95 gave evidence that process N acts enantioselectively. However, it is unknown to what extent the dechlorination occured. It appeared process N proceeds by preferentially dechlorinating the first eluting enantiomer of PCBs 91 and 95. However, there were no studies available that analyze the dechlorination of each enantiomer over time as Carlsson et al. (2016) did with PCBs 132 and 149. This is a shortcoming in the studies of the dechlorination of chiral PCBs.

5 CONCLUSIONS AND RECOMMENDATIONS

The purpose of my study was to test the hypothesis that process N enantioselectively dechlorinated chiral PCBs. A meta-analysis produced statistics to test this hypothesis and also a second hypothesis that enantioselective bioaccumulation was the driving force behind nonracemic EFs in soils and sediments. The results of the meta-analysis statistical study provided insight into enantioselective processes.

Variance results from the meta-analysis confirm that microbial communities impact the EF levels of chiral PCBs. Microbial communities may prefer degrading a specific enantiomer, or they may exclusively dechlorinate a specific enantiomer. Evidence of this idea was supported by Wong et al. (2007) and Carlsson et al. (2016) who analyzed the change in EF over time at the same location.

The meta-analysis indicated that as a whole enantioselective bioaccumulation was not a factor when determining nonracemic EF levels in soils and sediments. Even where opposite enrichment of enantiomers was seen between organisms and soils or sediments, low concentrations in the organisms make it unlikely that enantioselective bioaccumulation drives nonracemic EF levels in the soil and sediment.

Results from the meta-analysis were unable to offer any guidance on the impacts of parent/product relationships because most field studies did not include data from the same location over time. The actual parent/product relationship needs to be researched. It is currently unknown if a (+) enantiomer of a chiral PCB will dechlorinate to a (+) enantiomer of a lower chlorinated PCB. Until more data exist on the subject of

parent/product relationships, little can be done to assess the impacts that parent/product relationships have on the EF levels of chiral PCBs in all media.

Evidence for enantioselective dechlorination of chiral PCBs occurring was provided by the results of the meta-analysis. Sufficient evidence exists to suggest that process N acts enantioselectively. To confirm the extent by which process N acts enantioselectively, additional sampling over time from the same locations would be most useful. As done by Wong et al. (2007) and Carlsson et al. (2016), resampling the same locations allows for the determination of the extent of enantioselective dechlorination. Observing how the EF changes with time will aid in determining how enantioselective any dechlorination process is. If dechlorination process N is exclusively enantioselective and favors degrading the first eluting enantiomer, future sampling events that follow the same analytical procedure as those in the meta-analysis will show the true average EF of PCBs 91 and 95 decreasing as the first eluting enantiomer is depleted.

Future sampling events also need to prioritize analyzing the possible product congeners to determine which dechlorination process is active. The microcosm study done by Pakdeesusuk et al. (2003a) provided an example of how to determine which product congener is being produced by dechlorination processes. For field samples such an analysis will be more difficult. In the field there may be hundreds of congeners present in a sample. However, measuring the chiral congeners and their product congeners is necessary to fully understand the dechlorination process under field conditions. Combining this approach with sampling the same locations periodically will allow a more complete picture of how any enantioselective dechlorination is proceeding.

In addition to encouraging repeated sampling events of locations, recording more chemical parameters of the sampling site will aid in determining major soil or sediment properties that affect the EF. Currently very few properties have been shown to be significantly correlated with EF levels. Correlations that have been made typically come from a small number of studies. Powerful correlations may provide insight if the necessary data be recorded for future sampling events. Recommended soil and sediment properties to record are those that are provided in the supplementary material from the work done by Carlsson et al. (2016). Additionally, analysis of the microbial communities would help the task of associating groups of microorganisms with a particular dechlorination process. Recommended parameters for tissue samples would include lipid and protein content.

My study using meta-analysis has universally confirmed that differing microbial communities are capable of degrading chiral PCBs enantioselectively and to different extents. Additionally, the results provide evidence that nonracemic EFs in soils and sediments are not the result of enantioselective bioaccumulation but rather enantioselective dechlorination. Perhaps most importantly, this study has confirmed the idea that dechlorination process N possesses the ability to act enantioselectively.

APPENDICES

Table A.1
Raw Sampling Data for PCB 91

Study	N	EF	Sdev	Media
Asher (2007)	12	0.492	0.011	air
Morrissey (2007)	4	0.507	0.001	sediment
Sivey (2005)	12	0.4201	0.0485	sediment
Wong (2006) a	6	0.4266	0.0135	sediment
Wong (2006) b	14	0.5907	0.1685	sediment
Wong (2006) c	12	0.4577	0.0476	sediment
Wong (2006) d	11	0.4546	0.028	sediment
Wong (2006) e	4	0.434	0.0215	sediment
Wong (2001) a	5	0.531	0.0215	sediment
Wong (2001) b	6	0.4286	0.0833	sediment
Wong (2001) c	2	0.501	0.02289	sediment
Wong (2001) d	1	0.507		sediment
Wong (2001) e	1	0.487		sediment
Wong (2001) f	3	0.485	0.002	sediment
Overall		0.4721		
Asher (2012)	9	0.498	0.014	soil
Morrissey (2007) a	6	0.146	0.007	tissue
Morrissey (2007) b	6	0.461	0.009	tissue
Ross (2011) a	15	0.3606	0.12188	tissue
Ross (2011) b	14	0.29575	0.04218	tissue
Ross (2011) c	14	0.15175	0.11895	tissue
Ross (2011) d	2	0.45962	0.06939	tissue
Ross (2011) e	5	0.41555	0.12636	tissue
Ross (2011) f	6	0.85599	0.22329	tissue
Ross (2011) g	21	0.44455	0.08702	tissue
Warner (2005) a	3	0.507	0.012	tissue
Warner (2005) b	2	0.524	0.001	tissue
Warner (2005) c	3	0.623	0.009	tissue
Warner (2005) d	4	0.542	0.162	tissue
Warner (2005) e	5	0.137	0.024	tissue
Warner (2005) f	12	0.063	0.034	tissue

Overall		0.3927		
Dang (2013)	15	0.659	0.01831	water

Table A.2
Raw Sampling Data for PCB 95

Study	N	EF	Sdev	Media
Jamshidi (2007) a	100	0.4942	0.0061	air
Jamshidi (2007) b	31	0.4963	0.0032	air
Harrad (2006)	32	0.497	0.004	air
Asher (2007)	25	0.496	0.012	air
Chen (2014) a	60	0.499	0.004	air
Chen (2014) b	60	0.492	0.021	air
Robson (2004) a	32	0.497	0.004	air
Robson (2004) b	12	0.493	0.004	air
Overall		0.4959		
Wong (2009) a	5	0.479	0.0392	sediment
Wong (2009) b	9	0.506	0.0242	sediment
Morrissey (2007)	4	0.394	0.027	sediment
Asher (2007) a	4	0.475	0.002	sediment
Asher (2007) b	26	0.477	0.014	sediment
Sivey (2005) a	17	0.2817	0.0998	sediment
Sivey (2005) b	16	0.2692	0.0642	sediment
Wong (2006) a	6	0.4679	0.0315	sediment
Wong (2006) b	14	0.3541	0.1068	sediment
Wong (2006) c	12	0.3398	0.1191	sediment
Wong (2006) d	11	0.3075	0.1157	sediment
Wong (2006) e	4	0.4184	0.0408	sediment
Wong (2001) a	5	0.45839	0.01798	sediment
Wong (2001) b	8	0.488	0.00983	sediment
Wong (2001) c	2	0.49606	0.01257	sediment
Wong (2001) d	2	0.47911	0.00767	sediment
Wong (2001) e	1	0.48454		sediment
Wong (2001) f	3	0.478	0.0176	sediment
Overall		0.425		
Asher (2012)	17	0.434	0.14019	soil
Wong (2009)	7	0.432	0.0567	soil
Jamshidi (2007)	108	0.4663	0.0295	soil
Robson (2004) a	32	0.453	0.023	soil
Robson (2004) b	11	0.389	0.041	soil

Yuan (2015)	41	0.395	0.0278	soil
Harrad (2006)	32	0.453	0.023	soil
Asher (2012)	17	0.434	0.034	soil
Cui (2012)	23	0.473	0.025	soil
Chen (2014) a	4	0.475	0.019	soil
Chen (2014) b	4	0.495	0.008	soil
Chen (2014) c	4	0.483	0.021	soil
Desborough (2011)	14	0.45	0.007	soil
Zheng (2014)	10	0.51	0.01	soil
Carlsson (2016) a	10	0.381	0.0174	soil
Carlsson (2016) b	10	0.468	0.029	soil
Carlsson (2016) c	12	0.4658	0.0365	soil
Carlsson (2016) d	9	0.467	0.0292	soil
Overall		0.4522		
Morrissey (2007) a	6	0.343	0.009	tissue
Morrissey (2007) b	6	0.522	0.008	tissue
Asher (2007)	6	0.478	0.01	tissue
Chen (2014) a	30	0.561	0.045	tissue
Chen (2014) b	30	0.581	0.083	tissue
Chen (2014) c	30	0.496	0.013	tissue
Chen (2014) d	30	0.543	0.069	tissue
Desborough (2011)	14	0.475	0.011	tissue
Ross (2011) a	15	0.4796	0.08909	tissue
Ross (2011) b	13	0.48076	0.04212	tissue
Ross (2011) c	14	0.70686	0.16625	tissue
Ross (2011) d	2	0.47766	0.00141	tissue
Ross (2011) e	5	0.54304	0.22835	tissue
Ross (2011) f	7	0.1748	0.15289	tissue
Ross (2011) g	21	0.33497	0.08048	tissue
Warner (2005) a	3	0.49	0.001	tissue
Warner (2005) b	3	0.463	0.001	tissue
Warner (2005) c	2	0.503	0.007	tissue
Warner (2005) d	5	0.742	0.078	tissue
Warner (2005) e	2	0.586	0.037	tissue
Warner (2005) f	3	0.762	0.041	tissue
Warner (2005) g	2	0.84	0.015	tissue
Warner (2005) h	4	0.694	0.179	tissue

Warner (2005) i	5	0.609	0.177	tissue
Warner (2005) j	12	0.849	0.072	tissue
Jimenez (2000) a	4	0.5334	0.0067	tissue
Blanch (1996)	4	0.5007	0.0043	tissue
Jimenez (2000) b	1	0.4975		tissue
Jimenez (2000) c	1	0.5376		tissue
Zheng (2016) a	29	0.495	0.01	tissue
Zheng (2016) b	29	0.693	0.172	tissue
Overall		0.5488		
Asher (2007)	11	0.472	0.015	water
Dang (2013)	15	0.507	0.01163	water

Table A.3
Raw Sampling Data for PCB 132

Study	N	EF	Sdev	Media
Chen (2014) a	60	0.484	0.022	air
Chen (2014) b	60	0.445	0.069	air
Wong (2006) a	5	0.5168	0.0177	sediment
Wong (2006) b	14	0.5372	0.0236	sediment
Wong (2006) c	9	0.5416	0.1081	sediment
Wong (2006) d	7	0.5476	0.0707	sediment
Wong (2006) e	2	0.5338	0.0213	sediment
Wong (2001) a	5	0.51537	0.00898	sediment
Wong (2001) b	5	0.53115	0.02546	sediment
Wong (2001) c	2	0.5151	0.02491	sediment
Wong (2001) d	1	0.51456		sediment
Wong (2001) e	1	0.52607		sediment
Wong (2001) f	3	0.5106	0.006	sediment
Overall		0.5222		
Cui (2012)	19	0.355	0.146	soil
Chen (2014) a	4	0.475	0.018	soil
Chen (2014) b	4	0.495	0.013	soil
Chen (2014) c	4	0.49	0.033	soil
Zheng (2014)	10	0.46	0.03	soil
Carlsson (2016) a	10	0.528	0.011	soil
Carlsson (2016) b	10	0.527	0.0215	soil
Carlsson (2016) c	12	0.3593	0.0922	soil
Carlsson (2016) d	9	0.5329	0.0375	soil
Overall		0.4738		
Chen (2014) a	30	0.467	0.111	tissue
Chen (2014) b	30	0.439	0.078	tissue
Chen (2014) c	30	0.509	0.068	tissue
Chen (2014) d	30	0.459	0.033	tissue
Jimenez (2000)	2	0.5329	0.0321	tissue
Blanch (1996)	6	0.5339	0.0196	tissue
Zheng (2016) a	29	0.503	0.01	tissue
Zheng (2016) b	29	0.554	0.058	tissue
Overall		0.4998		

Table A.4
Raw Sampling Data for PCB 136

Study	N	EF	Sdev	Media
Jamshidi (2007) a	94	0.4987	0.0066	air
Jamshidi (2007) b	17	0.4997	0.0028	air
Asher (2007)	13	0.497	0.008	air
Chen (2014) a	60	0.498	0.006	air
Chen (2014) b	60	0.501	0.006	air
Robson (2004) a	32	0.501	0.004	air
Robson (2004) b	12	0.502	0.004	air
Overall		0.4998		
Wong (2009) a	2	0.494	0.01131	sediment
Wong (2009) b	7	0.536	0.02429	sediment
Morrissey (2007)	4	0.543	0.004	sediment
Wong (2006) a	6	0.5337	0.0212	sediment
Wong (2006) b	14	0.5897	0.043	sediment
Wong (2006) c	12	0.6261	0.0931	sediment
Wong (2006) d	11	0.6385	0.073	sediment
Wong (2006) e	4	0.5495	0.0352	sediment
Wong (2001) a	4	0.50851	0.00751	sediment
Wong (2001) b	7	0.49844	0.00853	sediment
Wong (2001) c	2	0.48958	0.01473	sediment
Wong (2001) d	1	0.45652		sediment
Wong (2001) e	1	0.51691		sediment
Wong (2001) f	3	0.5044	0.0033	sediment
Overall		0.5381		
Asher (2012)	12	0.497	0.018	soil
Wong (2009)	7	0.489	0.02452	soil
Jamshidi (2007)	97	0.495	0.0125	soil
Robson (2004) a	32	0.522	0.012	soil
Robson (2004) b	11	0.532	0.021	soil
Yuan (2015)	35	0.421	0.0406	soil
Chen (2014) a	4	0.502	0.015	soil
Chen (2014) b	4	0.503	0.01	soil
Zheng (2014)	10	0.510	0.04	soil
Overall		0.497		
Morrissey (2007) a	6	0.301	0.014	tissue

Morrissey (2007) b	6	0.475	0.014	tissue
Chen (2014) a	30	0.539	0.04	tissue
Chen (2014) b	30	0.522	0.079	tissue
Chen (2014) c	30	0.496	0.014	tissue
Chen (2014) d	30	0.49	0.011	tissue
Ross (2011) a	10	0.54313	0.09232	tissue
Ross (2011) b	14	0.46193	0.04055	tissue
Ross (2011) c	12	0.24799	0.14939	tissue
Ross (2011) d	2	0.48116	0.02013	tissue
Ross (2011) e	4	0.38895	0.02822	tissue
Ross (2011) f	4	0.52642	0.03095	tissue
Ross (2011) g	21	0.47264	0.02344	tissue
Jimenez (2000) a	2	0.5392	0.0103	tissue
Jimenez (2000) b	3	0.4989	0.0198	tissue
Jimenez (2000) c	1	0.5102		tissue
Overall		0.4681		

Table A.5
Raw Sampling Data for PCB 149

Study	N	EF	Sdev	Media
Jamshidi (2007) a	97	0.4972	0.0049	air
Jamshidi (2007) b	20	0.5003	0.0036	air
Harrad (2006)	32	0.502	0.0030	air
Asher (2007)	22	0.503	0.0140	air
Chen (2014) a	60	0.496	0.007	air
Chen (2014) b	60	0.49	0.008	air
Robson (2004) a	32	0.502	0.0030	air
Robson (2004) b	12	0.501	0.0040	air
overall		0.4988		
Wong (2009) a	6	0.496	0.0038	sediment
Wong (2009) b	9	0.503	0.0173	sediment
Morrissey (2007)	1	0.61	0.0030	sediment
Sivey (2005) a	1	0.1797	0.0304	sediment
Sivey (2005) b	24	0.167	0.0621	sediment
Wong (2006) a	6	0.4892	0.0078	sediment
Wong (2006) b	14	0.4803	0.0096	sediment
Wong (2006) c	12	0.5061	0.0296	sediment
Wong (2006) d	11	0.5378	0.0431	sediment
Wong (2006) e	4	0.4997	0.0176	sediment
Wong (2001) a	5	0.51646	0.0163	sediment
Wong (2001) b	7	0.51279	0.0200	sediment
Wong (2001) c	2	0.51574	0.0017	sediment
Wong (2001) d	2	0.51324	0.0117	sediment
Wong (2001) e	1	0.51691		sediment
Wong (2001) f	3	0.5041	0.0038	sediment
Overall		0.4689		
Asher (2012)	22	0.516	0.1079	soil
Wong (2009)	7	0.498	0.0028	soil
Jamshidi (2007)	109	0.5019	0.0216	soil
Robson (2004) a	32	0.544	0.0170	soil
Robson (2004) b	11	0.532	0.0170	soil
Yuan (2015)	26	0.439	0.0394	soil
Harrad (2006)	32	0.544	0.0170	soil
Asher (2012)	22	0.516	0.0230	soil

Cui (2012)	20	0.527	0.1670	soil
Chen (2014) a	4	0.492	0.0110	soil
Chen (2014) b	4	0.502	0.0110	soil
Zheng (2014)	10	0.51	0.02	soil
Carlsson (2016) a	10	0.49	0.0105	soil
Carlsson (2016) b	10	0.509	0.0151	soil
Carlsson (2016) c	12	0.4783	0.0289	soil
Carlsson (2016) d	10	0.5129	0.0204	soil
Overall		0.5062		
Morrissey (2007) a	6	0.424	0.0110	tissue
Morrissey (2007) b	6	0.479	0.0090	tissue
Chen (2014) a	30	0.527	0.0310	tissue
Chen (2014) b	30	0.534	0.0240	tissue
Chen (2014) c	30	0.492	0.0180	tissue
Chen (2014) d	30	0.489	0.0150	tissue
Ross (2011) a	14	0.55762	0.1444	tissue
Ross (2011) b	14	0.42861	0.0765	tissue
Ross (2011) c	14	0.33247	0.1723	tissue
Ross (2011) d	2	0.54413	0.0261	tissue
Ross (2011) e	5	0.42276	0.0652	tissue
Ross (2011) f	6	0.59273	0.2117	tissue
Ross (2011) g	21	0.53292	0.0283	tissue
Warner (2005) a	3	0.493	0.0060	tissue
Warner (2005) b	3	0.509	0.0360	tissue
Warner (2005) c	5	0.151	0.0710	tissue
Warner (2005) d	5	0.2	0.0320	tissue
Warner (2005) e	5	0.418	0.0450	tissue
Warner (2005) f	5	0.262	0.0890	tissue
Warner (2005) g	5	0.403	0.0080	tissue
Warner (2005) h	5	0.277	0.0520	tissue
Warner (2005) i	13	0.478	0.0450	tissue
Dai (2014) a	4	0.503	0.001	tissue
Dai (2014) b	4	0.496	0.001	tissue
Dai (2014) c	4	0.501	0.002	tissue
Jimenez (2000) a	2	0.5764	0.007	tissue
Jimenez (2000) b	4	0.5357	0.0269	tissue
Blanch (1996)	5	0.5008	0.0132	tissue

Jimenez (2000) c	1	0.5525		tissue
Jimenez (2000) d	1	0.5587		tissue
Overall		0.4524		
Asher (2007)	4	0.504	0.017	water

Appendix B
SAS Code used for the meta-analysis.

```
proc import datafile='/home/eboley0/Data/Researchworksheet.xlsx' out=META
dbms=xlsx replace;
sheet='PCB 149';
run;

Data META;
set META;
SE=Sdev/SQRT(N);
WGT=1/SE**2;
proc print;
proc sort; by MEDIA;
proc mixed covtest;by media;
Class study;
weight WGT;
model ef= ;
random study;
repeated ;
Parms (.1) (1) / EQCONS=2;
estimate 'EF' INT 1 / CL;* DF=10000;
Title1 Results for PCB 149;
Run;Quit;
```

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