BEHAVIOURAL CHARACTERISTICS OF POLYBROMINATED DIPHENYL ETHERS IN SOIL

by

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ABSTRACT

This thesis work focused on the determination of behavioral characteristics of polybrominated diphenyl ethers (PBDEs) in a soil setting. As emerging persistent organic pollutants (EPOPs), PBDEs have been shown to impact the health of ecosystems and living organisms in a negative way. PBDEs have a particular affinity towards the organic carbon content found within soil particles, thus understanding their fate and transport in this setting is important information needed for effective and efficient remedial efforts. In this thesis, the adsorption behavior of all 209 PBDE congeners is determined through 3D-QSAR techniques by estimating each of their organic carbon-water partition coefficient (K_{OC}) values. In addition, the biodegradability of commonly occurring PBDE congeners is studied through considering unique enzymes that may be readily available in soil while conducting molecular docking. The research outputs indicated that the degree of bromination plays a significant role in how PBDEs behave in soil due to compound stability and molecular geometry. Moving forward, the findings help to advance the knowledge on PBDE behaviors in soil and facilitate the environmental engineering operations by means of creating more efficient and effective ways of remediating PBDEs out of a soil environment.

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LIST OF SYMBOLS AND ABBREVIATIONS

ABBREVIATIONS

2D	two dimensional
3D	three dimensional
4D	four dimensional
ABS	acrylonitrile butadiene styrene
BDE	brominated diphenyl ether
BFR	brominated flame retardant
CDOCKER	CHARMM-based DOCKER
CHARMM	Chemistry at Harvard Macromolecular Mechanics
CoMFA	comparative molecular field analysis
CoMSIA	comparative molecular similarity indices analysis
cSDEP	cross-validated standard error of predictions
DOCKER	molecular docking method
E.U.	European Union
EPI	estimation program interface
EPOP	emerging persistent organic pollutant
FPUF	flexible polyurethane foam
GA	genetic algorithm
GLU	glutamic acid
HBCD	hexabromocyclododecane
IC	incremental construction
LYS	lysine
MA	matching algorithm
MC	Monte Carlo simulation
MCIs	molecular connectivity indices
MCSS	multiple copy simultaneous search
MD	molecular dynamics
MOL2	chemical table file

PAH	polycyclic aromatic hydrocarbon
PBDD	polybrominated dibenzo-p-dioxin
PBDE	polybrominated diphenyl ether
PBDF	polybrominated dibenzofuran
PCB	polychlorinated biphenyl
PCE	tetrachloroethylene
PDB	Protein Data Bank
PHE	phenylalanine
PLS	partial least squares
POP	persistent organic pollutant
PRO	proline
QSAR	quantitative structure-activity relationship
RMS	root mean square
RMSD	root mean square deviation
SAR	structure-activity relationship
SDF	spatial data file
SEE	standard error of estimates
SEP	standard error of predictions
SER	serine
TBBPA	tetrabromobisphenol-A
TCE	trichloroethylene
TEST	Toxicity Estimation Software Tool
TYR	tryptophan
U.S. EPA	United States Environment Protection Agency
VAL	valine

SYMBOLS

$[A]$	concentration of substance A
$[A]_0$	initial concentration of substance A
$[B]$	concentration of substance B
Å	angstrom (0.1 nm)
b	Langmuir isotherm constant (dm^3/mg)
Br	bromine
C	carbon
C_0	adsorbate initial concentration (mg/L)
$C_{12}H_{(10-x)}Br_xO$	general chemical formula for PBDE
C_{aq}	concentration of adsorbate in the aqueous phase
C_e	equilibrium concentration (mg/L)
CH_2Br_2	dibromomethane
CH_4	methane
CO_2	carbon dioxide
-COOH	carboxyl functional group
C_s	concentration of adsorbate in soil
d	exact differential
dQ^2/dr_{yy}	sensitivity of perturbation
F	F-test value
f_{OC}	fraction of organic carbon
H	hydrogen
H_2O	water
k	reaction rate constant
K_d	soil adsorption coefficient
K_F	Freundlich isotherm constant (mg/g)
K_L	Langmuir isotherm constant (L/mg)
K_{OC}	organic carbon-water partition coefficient
K_{OW}	octanol-water partition coefficient
n	adsorption intensity value

n	number of PLS analysis components
O	oxygen
pH	power of hydrogen
Q_0	maximum monolayer coverage capacities (mg/g)
q^2	cross-validated correlation coefficient
Q^2	variance of predictions
q_e	adsorbate in adsorbent equilibrium (mg/g)
r	reaction rate value
r^2	non-cross-validated correlation coefficient
r^2_{adj}	adjusted correlation coefficient
r^2_{pred}	correlation coefficient of testing set predictions
R_L	separation factor
t	time
x	partial order of reaction w.r.t. substance A
y	partial order of reaction w.r.t. substance B

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CHAPTER 1

INTRODUCTION

1.1 Background

Polybrominated Diphenyl Ethers (PBDEs) are a group of chemical compounds with a diphenyl ether structural foundation. Various quantities and positions of bromine atoms bond to sites on either aromatic ring forming 209 unique chemicals known as PBDE congeners (U.S. EPA, 2009). In the late 20th century, PBDEs were commonly used worldwide on commercial goods as a flame retardant to provide additional safety to users. The production of PBDEs for commercial use did not result in stock amounts of all 209 congeners. Instead, mixtures containing multiple common congeners at an average degree of bromination were produced, and were variants of either a penta-, octa-, or decaBDE mixture. During their peak times of usage, pentaBDE products were used mainly in the furniture and textile industries, octaBDE products were used for electronic and plastics, while decaBDE was used in a wide variety of applications involving polymers (ENVIRON, 2003a; ENVIRON, 2003b; Pohl et al., 2017). In recent years, however, adverse effects discovered about these chemicals have caused their phasing out in the manufacturing of products (Pohl et al., 2017). These compounds are considered to be emerging and persistent contaminants recognized by the Stockholm Convention brought forward by the United Nations council in 2009 (United Nations, 2009).

PBDEs have been detected in natural environments around the world as they have the ability to slowly detach from the products they are carried on and are then free to be transported via air and water (Lorber and Cleverly, 2010). Solid organic matter, such as soils and sediments, are a particular hotspot for PBDE contamination as these highly hydrophobic chemicals have an affinity towards the organics within these mediums (Ni et al., 2014). Once present in these natural environments, the bioavailability and exposure of PBDEs can have significant impacts on the well-

being of many organisms as they have been shown to be bioaccumulative and can biomagnify through an ecosystem (Liang et al., 2010; Currier et al., 2020). This route of exposure in combination with direct indoor contact has shown that the adverse effects on human beings include hormone and developmental disruptions as well as their potential for being carcinogenic (Pohl et al., 2017). In order to reduce exposure and mitigate the contamination, the understanding of behavioural characteristics of PBDEs in soil is a key strategy towards effective remediation.

Once introduced into soil, the best concept of understanding how they behave is by studying their adsorption tendencies to the soil particles through the principles of chemisorption and physisorption (Ni et al., 2014). Encompassing the mechanisms of ion-exchanges, charge transfers, interactions with metallic cations, hydrogen bond formations, and hydrophobic effects, the use of adsorption kinetics, equilibrium studies, and partition coefficients are reliable and widely adopted tools able to mathematically explain these mechanisms to the behaviour of PBDEs in soil. Understanding the distributional characteristics of PBDEs are relatively straighforward pieces of information to determine given that all required parameters are known. This task is mainly done with the use of isotherm curves, and partition coefficients for interactions between multiple mediums. Specifically speaking, the use of the soil adsorption coefficient (K_d) and fraction of organic matter present in a control volume of soil (f_{OC}) in combination with each other produces an organic carbon partition coefficient (K_{OC}) as the product of the ratio between the two former values (Kozerski et al., 2014). With an emphasis on the equilibrium state between organic matter and water, the K_{OC} value for a specific PBDE congener is an extremely useful tool made available to understand how these compounds interact and behave in a soil medium.

In addition to understanding the soil adsorption properties of PBDEs, focusing on their biodegradation process is key in approaching effective and efficient remediation. In general, the biodegradation process usually involves microorganisms, such as bacteria and fungi species, breaking down a substance for their survival. Often, a complex substance will be broken down into simpler and more useful substances that the microorganism can use for its needs. In order to perform biodegradation, these microorganisms produce extracellular enzymes known as exoenzymes that cause degradation as they are catalysts for required decomposition reactions (Gurung et al., 2013). From a chemical perspective, this means a substance, such as an organic contaminant, in the presence of an enzyme will have a reduced activation energy enabling the contaminant to decompose more easily (University of Arizona, 1996). This process has proven to be mutually beneficial for the survival of the secreting microorganisms as well as their surrounding environment since it promotes the cycling of organic matter and the return of the contaminating compounds back into a safe and natural state (Allison, 2012). The idea of using this principle to the advantage of remediation efforts is vital as many varieties of contaminants can be safely dealt with.

Through the use of in silico modelling, computational tools provide the ability to best understand and determine the specifics of how PBDE compounds in soil behave. This is best approached by modelling soil adsorption capacity and bioavailability with K_{OC} values as the measured metric while also analyzing the mechanisms present in the debromination process of multiple congeners occuring through enzymatic decomposition.

1.2 Statement of Problems

(1) Lack of sufficient understanding of the relationship between the structural properties of all 209 PBDE congeners to corresponding logK_{OC} values for evaluating the PBDE adsorption behavior in soil

To properly document and quantify the understanding of the behavior of PBDEs in soil, using mathematical tools to determine their equilibrium state as well as their distribution is necessary. Through the understanding of adsorption kinetics and isotherm models as well as partition coefficients for multiple media system, this can be accomplished. In particular, through using the organic carbon partition coefficient (K_{OC}), the attraction of any given substance to the organics found within soil particles can be determined. In the case of PBDEs, while there are many congeners that each pose threats, obtaining K_{OC} values for each would be very helpful in remediation efforts.

Currently, there are many publications that focus on the study of the adsorption characteristics that PBDEs display. Studies analyze either the kinetic behavior of the adsorption of PBDEs to natural materials or the desorption of PBDEs from natural materials to a third party medium to target effective remedial strategies (de la Cal et al., 2008; Liu W. et al., 2010; Bao et al., 2011; Liu et al., 2011; Liu et al., 2012; Ni et al., 2015; Deng and Tam, 2016; Jaafar et al., 2019; Sha'arani et al., 2019; Xu et al., 2019). In addition, other literatures look at the same topics with additional equilibrium studies which include adsorption isotherm information (Liu W. et al., 2011; Olshansky et al., 2011; Xin et al., 2012; Liu et al., 2017; Xu et al., 2019). While these publications are able to provide important discoveries on PBDE adsorption behaviors, there is still a need for information regarding the partition characteristics of PBDEs in soil. Previous studies on the partitioning of

PBDEs only deal with handfuls of commonly occurring congeners that have been made available through the use of commercial mixtures (Gustafsson et al., 1999; Braekevelt et al., 2003; Liang et al., 2010). There are no current available studies that display partitioning information for all 209 congeners of PBDE compounds. Determining the K_{OC} values for each of these compounds is a necessary step to develop proper remediation strategies that are able to account for any given congener that is present in the system. Creating such values with traditional laboratory experimental methods are too costly, time consuming, and laborious for purpose of solely determining K_{OC} values.

An alternative method for determining the K_{OC} values for all 209 congeners can be through the use of computational models such as Quantitative Structure Activity Relationship (QSAR) modelling. First developed by Hansch et al. in 1962, to understand the relation between the structures of growth receptor molecules in plants to their biological activity (Hansch et al., 1962), the general idea of a QSAR model was established. It later on was widely used to study the properties consequential to the structure of a compound and relate it back to a form of activity (Selassie and Rajeshwar, 2003; Cherkasov et al., 2014; Roy et al., 2015). In most cases, the application of QSAR is undertaken in biochemistry fields to understand biological activities, but in this case, the structures of PBDE congeners can be studied to understand aspects of their distributional behavior through a K_{OC} value. Many versions of QSAR models exist, however, the use of comparative molecular field analysis (CoMFA) is a good fitting model for this study since it is able to account for the 3D structures of the compounds, as well as measure the steric and electrostatic molecular fields (Cramer et al., 1988; Klebe et al., 1994; Suh et al., 2002). Current studies involving 3D-QSAR models to the application of PBDEs such as (Chu and Li, 2019; Drage et al., 2019; Zhang et al., 2019; Sheikh and Beg, 2020; Xiao et al., 2020) have focused their

attention to the biological activity between PBDE congeners and impacts to living organisms. Understanding how PBDEs interact with soil is a very important piece of information needed, but not tackled yet.

(2) Lack of information regarding the PBDEs bioavailability as well as the relationship between the adsorption and biodegradation of PBDEs in soil

The first step in the biodegradation process of PBDEs is debromination. Studies such as (Schmidt et al., 1992; Schmidt et al., 1993; Hundt et al., 1999; He et al., 2006; Kim et al., 2007; Zhou et al., 2007; Robrock et al., 2008; Robrock et al., 2009; Deng et al., 2011; Chou et al., 2013; Chou et al., 2016; Zhao et al., 2018) refer to the degradation pathways a brominated congener may experience before it is completely debrominated and reduced to simple/non-toxic materials through the use of biodegradation. While these studies portray an accurate use of microorganisms to assist in mitigation efforts, currently there is little information available on specific enzymes that exist within a soil medium that can effectively and efficiently focus on the debromination step of the biodegradation process of PBDEs.

Modern science and technology has been able to provide useful tools to guide the research and development of tracking the biodegradation of targeted contaminant compounds through enzymatic decomposition. One of the most useful methods is the study of molecular dynamics and the use of molecular docking. While molecular dynamics studies the physical movement of atoms and molecules (Allen, 2004), molecular docking is a tool used to describe how two or more molecular structures fit together and thus, how they interact with each other (Roy et al., 2015). In general, the use of molecular docking is not limited to the study of biodegradation and has a variety of applications. While there are some applications of using molecular docking to study

bioremediation (Liu et al., 2018; Srinivasan et al., 2019; Zhao and Li, 2019), many uses of this technology are applied in the medical field to better medicinal practices. For instance, studies such as (Lu et al., 2011; Wang et al., 2013; Mohan et al. 2015; Wang et al., 2015; Kumara M, 2017; Öztürk et al., 2017; Parthasarathy and Ajay-Kumar, 2019; El Hassab et al., 2020) advance the pharmaceutical field for drug discovery and design, while showcasing the mechanisms of receptors within living organisms. In the case of biodegradation, however, the use of molecular docking is a vital tool in understanding how effective and efficient the amino acid residues found in active sites on the surface of enzymes are at interacting with a ligand or contaminant molecule (Meng et al., 2011). The mechanisms present between any interactions that may occur will relay exactly how the ligands may decompose and thus can express and quantify biodegradability (Liu et al., 2018). While the potential for this tool to analyze and evaluate the interactions between specific PBDE congeners and enzymatic macromolecules found in soil environments can be useful, limit studies on this topic are currently available indicating more investigation is needed.

In addition to better understand the mitigation of PBDEs through enzymatic degradation, the adsorption behavior of these contaminants in soil are considered as well. As briefly mentioned previously, PBDE congeners are hydrophobic molecules that have an affinity towards the similarly hydrophobic organic material found in soil and sediment particles. If PBDE molecules are adsorbed to these external particles, they are presumed to become less bioavailable and thus less accessible for the debromination process. Adding this additional parameter for consideration can give insight on any relationship that may exist between the adsorption and biodegradable behavior of PBDEs. A study by (Huesemann et al., 2004) has shown that mass-transfer desorption rates have an influence on the biodegradability rates illustrating a clear relationship between these two parameters. In the case of PBDEs, there is available information describing their bioavailability in

soil through plant or animal uptake, however, there is currently little information regarding the relationship between the adsorption and biodegradation of PBDEs in soil (Xiang et al., 2019; Liang et al., 2010). Using useful tools and analyzing clear evidence for comparing the behaviors and establishing linkages is needed.

1.3 Objectives

This thesis focuses on investigating adsorption and biodegradation behaviors of PBDEs in soil. It entails the following research tasks:

(1) to develop an understanding of the relationship between the structural properties of all 209 PBDE congeners to a corresponding $\log K_{OC}$ value using 3D-QSAR modelling to establish stable and acceptable predictive K_{OC} abilities;

(2) to determine the effects of bromine atom positioning amongst PBDE compound structures and their effect on soil adsorption through $\log K_{OC}$ values;

(3) to identify potential enzymes for facilitating PBDE biodegradation through comparing selected enzymes on their interaction performance with respect to a place-holder PBDE congener;

(4) to conduct an investigation of the biodegradation of PBDE congeners through comparative analysis of their performance against the best suited enzyme with respect to multiple congeners with varying degrees of bromination; and

(5) to establish a relationship between the biodegradation and adsorption behavior of PBDEs in soil.

1.4 Thesis Structure

This document showcases a thesis consisting of five chapters. Chapter 1 creates the framework of the research scope, research objectives, as well as the written structure of the presented thesis. Chapter 2 consists of the literature review that explains relevant thesis topics in depth and details. Such topics include (1) a review of known information regarding PBDEs, (2) soil sorption and biodegradation mechanisms, and (3) introductory information regarding the computational tools used for optimization and modelling: 3D-QSAR and Molecular Docking. Chapter 3 presents the studies on the soil sorption characteristics of all 209 PBDE congeners with regards to a predictive computational model that can estimate $\log K_{OC}$ values. Chapter 4 investigates the debromination mechanisms present between three selected enzymes likely to be found in soil with eight commonly occurring PBDE congeners. Finally, Chapter 5 discusses conclusions of this research while also providing recommendations for future studies.

This thesis includes two independently conducted research topics. The first is to study the soil sorption characteristics and the soil adsorption capacity of all PBDE congeners. The second is to generate and understanding of the mechanisms present for the debromination of common PBDE congeners through enzymatic biodegradation in soil. These research topics will serve as important pieces of information to add to the understanding of PBDEs. With additional knowledge available on the understanding of PBDEs within a soil medium, future remediation techniques can be developed to efficiently and effectively control this contaminant while also decreasing exposure and thus removing any threats posed by the adverse effects these chemicals can cause.

CHAPTER 2

LITERATURE REVIEW

2.1 PBDEs

2.1.1 Commercial Production and Mixtures

As a type of brominated flame retardant (BFR), PBDEs do not occur naturally in the environment and are instead synthesized products made by humankind (Kodavanti et al., 2008). Since BFRs are used in a vast variety of commercial products and goods, the process of knowing how to properly synthesize PBDE compounds is necessary for large scale commercial production. The manufacturing process begins with diphenyl ether, which was previously mentioned as being the organic compound base of all PBDEs. Performing the bromination of diphenyl ether is done under certain reaction conditions that have not been disclosed by the manufacturers who have dealt with these compounds (Alaee et al., 2003). Using diphenyl ether in the presence of a Friedel-Craft catalyst, the organic molecule is often brominated with a dibromomethane (CH_2Br_2) solvent (Alaee et al., 2003). Even though it has been discussed that 209 congeners of PBDEs exist, the bromination of diphenyl ether consists of production preferences for certain congeners. This is due to a phenomenon known as steric hindrance in which the large shape of the phenyl rings may prevent or create difficulty for bromine bonding to occur at specific sites. The presence of the bridging oxygen atom can also have influencing properties (Alaee et al., 2003). The preference of certain congeners is highlighted in commercially sold PBDE mixtures.

When used on products, PBDEs are not available in one specific congener for use. Instead, commercial PBDEs are offered in three different mixtures that contain many congeners and are named after the average bromine content. These mixtures are pentaBDE, octaBDE, and decaBDE. Although it has been discussed that the prefixes of these names determine the homolog group, the naming of these mixtures is not as straightforward. Each of these three mixtures is named

according to which homologous congener is present in the highest quantity on average. Table 2.1 shows the percentage of which congener makes up the composition of each of the three commercial mixtures. Note that a majority percentage of the congeners that make up the pentaBDE mixture belong to the pentaBDE homolog group with traces of tetraBDE and hexaBDE in the mixture as well. This same logic is applied to both octaBDE and decaBDE mixtures.

In practice, these mixtures are used in different types of commercial products that need flame retardants to be recognized as safe for public use with respect to concerns of fire safety. PentaBDE mixtures were used mainly as flame retardants in the furniture and textile industries. Its primary use was in flexible polyurethane foam (FPUF) which is a product incorporated into goods such as bed mattresses and cushioning in automotive and airplane upholstered seats (ENVIRON, 2003a). Common trade names for commercial pentaBDE mixtures include but are not limited to: Bromkal 70-5DE, Pentabromprop, Tardex 50, DE 71, and Saytex 115 (Pohl et al., 2017). OctaBDE mixtures were used in the electronic and plastic industries as retardants for acrylonitrile butadiene styrene (ABS) terpolymers. These goods were mainly used for parts in computer casings and monitors (ENVIRON, 2003b). Common trade names for commercial octaBDE mixtures include but are not limited to: Bromkal 79-8DE, DE 79, Tardex 80, Adine 404, and Saytex 111 (Pohl et al., 2017). Finally, decaBDE mixtures were used in different polymer applications to reduce the chance of fire. The use of these polymers was almost exclusively used in the electronic industry ranging in products such as television cabinet backs to wires and cables. DecaBDE was also used in the textile industry in products like drapery and synthetic carpeting (European Union, 2002). Common trade names for commercial decaBDE mixtures include but are not limited to: Bromkal 81, Adine 505, Saytex 102, Berkflam B10E, Caliban F/R-P 39P, Flame Cut BR 100, Planelon DB 100, Hexcel PF1, Phoscon BR-250, and FR-300 BA (Pohl et al., 2017).

Table 2.1: Weight Percentage of PBDE Congeners in Commercial Mixtures (Pohl et al., 2017; U.S. EPA, 2010)

Congener	Compound Name	Commercial Mixture		
		PentaBDE	OctaBDE	DecaBDE
BDE 47	2,2',4,4'-tetrabromodiphenyl ether	25-37%		
BDE 99	2,2',4,4',5-pentabromodiphenyl ether	35-50%		
BDE 100	2,2',4,4',6-pentabromodiphenyl ether	6-10%		
BDE 153	2,2',4,4',5,5'-hexabromodiphenyl ether	3-5%	5-10%	
BDE 154	2,2',4,4',5,6'-hexabromodiphenyl ether	2-4%	1-5%	
BDE 183	2,2',3,4,4',5',6-heptabromodiphenyl ether		40%	
BDE 196	2,2',3,3',4,4',5',6-octabromodiphenyl ether		8%	
BDE 197	2,2',3,3',4,4',6,6'-octabromodiphenyl ether		21%	
BDE 203	2,2',3,4,4',5,5',6-octabromodiphenyl ether		5-35%	
BDE 206	2,2',3,3',4,4',5,5',6-nonabromodiphenyl ether			2.2%
BDE 207	2,2',3,3',4,4',5,6,6'-nonabromodiphenyl ether		7%	0.24%
BDE 208	2,2',3,3',4,5,5',6,6'-nonabromodiphenyl ether		10%	0.06%
BDE 209	2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether			97%

2.1.2 Chemical Properties

In order to properly understand the behavior of PBDE compounds, it will be useful to look at the chemical structure. Since PBDEs are a group of organohalide compounds, the general structure of each species consists of an organic hydrocarbon compound with bromine atoms bonded to carbon atoms at various positions. Specifically speaking, PBDEs consists of diphenyl ether as its base organic compound with a wide variety of individual species branching out from this base depending on the placement and quantity of bonded bromine atoms. The general structure is shown as an illustration in Figure 2.1

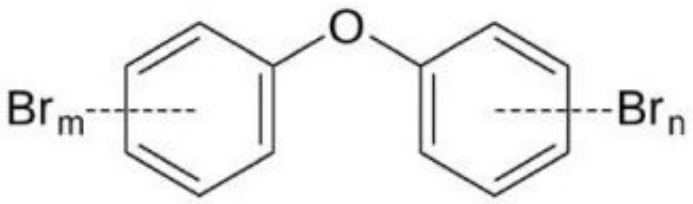


Figure 2.1: Chemical Structure of PBDE Compounds (Keet et al., 2010)

The organic compound, diphenyl ether, consists of two phenyl aromatic rings that are bonded to an oxygen atoms thus classifying this molecule as an ether. The attached bromine atoms that displace the hydrogen-carbon bonds can range from 1 to 10 in total. In writing, the nomenclature representing PBDEs is shown as $C_{12}H_{(10-x)}Br_xO$, where $x = 1 \sim 10$ while $x = m + n$. In consequence of the many different possible combinations and placements of bromine bonds, the multiple variations at different degrees of bromination are called congeners. PBDEs have 209 different congeners each of which can have a different behavior than another.

These chemical descriptions regarding PBDEs are not a foreign concept when dealing with their behavior. Another organohalide family that has already been well studied has a very similar chemical structure and in some ways can assist in the understanding of PBDEs. This group of

compounds are known as polychlorinated biphenyls (PCBs). In a similar fashion, the base hydrocarbon molecule that forms PCBs is biphenyl. Like diphenyl ether, biphenyl consists of two aromatic phenyl rings, however, these rings are directly bonded to each other through a single carbon-carbon bond rather than to an oxygen atom in between. There are also 10 positions that present opportunity for hydrogen atoms to be replaced with halogen atoms. In this case, chlorine atoms are the focus halogen in any quantity of position, also creating 209 congeners for PCBs. In fact, the nomenclature convention for naming PCBs popularized by (Ballschmiter and Zell, 1980) is the same system used to name the 209 PBDE congeners due to their structural similarities. Table A1 in Appendix A lists these 209 congeners associated with their formal scientific name for each PBDE compound.

Other important information to know regarding PBDE and PCB nomenclature is that of homologs and isomers. Seen in Table A1, the list of 209 congeners exists across 10 different group classifications. These groups are known as homologs which is the name given to a set of PBDE congeners that share the same number of bromine atoms. Since the "P" in PBDE represents the prefix word "poly", it is thus inferred that multiple numbered homologs exist. This is done through a prefix system for naming the number of bromine atoms followed by "BDE" which means brominated diphenyl ether. For instance, among the ten homologs in PBDE classification, there are mono-, di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-, and decaBDEs describing groups with 1 to 10 bromine atoms respectively. Each homolog group consists of 3, 12, 24, 42, 46, 42, 24, 12, 3, and 1 different isomers respectively from monoBDE to decaBDE (Pohl et al., 2017). This means that each homolog group has a certain number of ways in which the set amount of bromine atoms can bond. For instance, the monoBDE homologs have 3 distinct compounds, or isomers, all with a single bromine atom present. These distinctions are notable due to the difference in

properties the degree of bromination and molecule geometry homologs and isomers can have.

As both PCBs and PBDEs are halogenated organic compounds with two phenyl rings present, the similarities between these two groups can assist in classifying behavioral aspects of PBDEs through comparison. For instance, a report conducted by the Nordic Council of Ministers investigating food contamination and potential risks of PBDEs stated that the distribution and accumulation of PBDEs are similar to PCBs in an aquatic environment (Larsen et al., 1998). Moving forward with this knowledge, it is important to note that PBDEs and PCBs are different compounds and will behave in different ways under different circumstances, however for the purposes of this scope, realizing the similarities in their chemical structures is sufficient enough to illustrate the properties of PBDEs through resemblance. Figure 2.2 illustrates the structural similarities across different congeners of the same congener number. Although the structures are not identically analogous, the high degree of similarity is a strong indication that many traits between these two organohalide families are the same presented by logic.

In addition it may be important to note for proper understanding of these chemical congeners, that the bromine/chlorine – carbon bonds can happen at any position around either phenyl ring except for the position in which the phenyl rings are bonded to an oxygen atom in the case of PBDEs or to each other in the case of PCBs. This position is labelled at 1 on one of the rings and 1’ on the other. The other bonding sites are given the names ortho, meta, and para respectfully, and describe how far around the ring the bonding site is with respect to the 1/1’ position. Ortho sites would consist of halogen bonds closest to the 1/1’ site at positions 2, 2’, 6, and 6’, while meta sites are further away and consist of bonds at sites 3, 3’, 5 and 5’, and para sites would consist of bonds furthest away from the 1/1’ site with positions 4 and 4’.

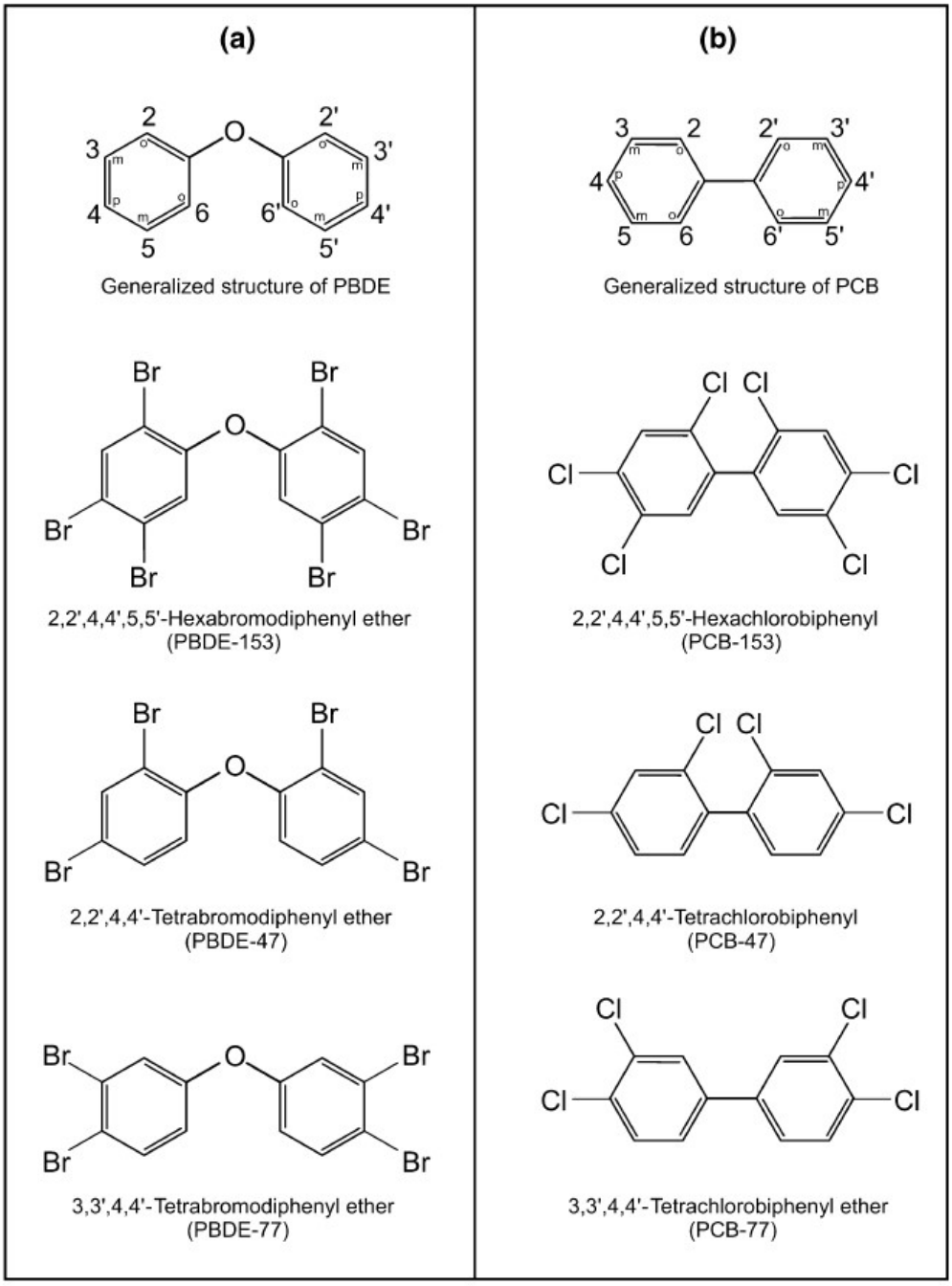


Figure 2.2: Chemical Structure Comparison between Select PBDE Congeners (a) and PCB Congeners (b) (Kodavanti et al., 2008)

2.1.3 Physical Properties

The physical properties of PBDE compounds are as diverse as the number of congeners there are. Since there is no one specific compound to define PBDEs by, a range of properties and trends can instead be discussed. Generally speaking, PBDE compounds are resistant to physical and chemical degradation (Kodavanti et al., 2008). They are a non-polar set of molecules that have very low solubility in water. They have a preference to bind to solid matter which may come in the form of aquatic sediments or soil in the natural environment (Pohl et al., 2017). The molecular geometry of these compounds can play a large part in their behavior and properties. For instance, since geometry can affect the polarity of a molecule, and polarity can affect many physical traits such as melting point, boiling point, and solubility (Ophardt, 2003a), knowing differences in molecular geometry amongst the many congeners is of interest. Three instances can occur which would affect a PBDE compounds shape. The first is if non-halogen bonding happens at any of the ortho positions, the molecule then takes a planar or near planar shape. Second and contrarily, if halogen bonding occurs at an ortho position, this will strongly influence the aromatic rings to orthogonal positions. DecaBDE is a very strong example of this phenomenon with a predicted dihedral angle of approximately 90 degrees and strong resistance of rotation around the linking bond. Finally, bonding at para or meta sites (non-ortho substitutions) will inflict a small dihedral angle but still present. In this case the molecule is considered to be near planar (Hardy, 2002). Since geometric symmetry is a good indicator of non-polar tendencies in molecules, PBDE compounds that are planar or near planar are going to be slightly less non-polar than those compounds that are non-planar. Molecular geometry is a consequence of where the bromine atoms are bonded to the diphenyl ether molecule, not its degree of bromination. Statistically speaking however, since high homolog groups of PBDEs contain more bromine bonds than that of lower brominated compounds,

it is much more likely that bromine bonding will occur in the ortho positions causing the molecule to be non-polar but consequently more polar. With that being said, it is fair to point out that through the likelihood of ortho bonding of bromine atoms, the degree of bromination indirectly influences polarity. Properties such as melting and boiling points are directly affected by polarity through stronger attractions, thus the more polar the PBDE compound, the higher the melting and boiling points will generally be. Additionally, since the general rule of solubility in chemistry is "likes dissolve likes", it may be appropriate to assume that higher brominated BDEs will have slightly higher water solubility and slightly lower organic solvent solubility. However, this is not the case. There are many factors that affect the different physical properties of chemicals. In this specific instance, the polarity of molecules is not the dominant factor affecting solubility. From a chemical factor perspective (assuming temperature and pressure to be held constant) polarity and molecule size influence the solubility of a molecule. The reason why decaBDE is the least soluble in water when it should be the most from a polarity stand point, is because the size of this molecule is the driving factor determining its solubility. Since the size of the compound increases with a higher degree of bromination, and size has an inverse relationship with solubility, the more bromine atoms present will decrease PBDE solubility. Since size is the dominant factor, this is also the case for its solubility in organic solvents. These trends are validated through numerical comparison with the data provided in Table A2 found in Appendix A presented by (Pohl et al., 2017). Table A2 also provides information on other physical characteristics of PBDEs such as appearance, physical state, density, and weight in addition to others.

2.1.4 Occurrence in Environments and the Associated Toxicity

With different BFR options in existence with two classifications, discussing which type PBDEs are is useful when understanding why these compounds are problematic in polluting the natural

environment. Another notable BFR is hexabromocyclododecane (HBCD), along with PBDEs are what are known as additive flame retardants. This means that when applied to commercial goods, these BFRs are blended in with the polymers of the product. This is in contrast to reactive flame retardants such as tetrabromobispenol-A (TBBPA) which are chemically bonded into the products (Hutzinger & Thoma, 1987). This distinction is important since additive BFRs are much more likely to leach out of their products and leak into their surrounding environment.

Once released into the environment, PBDEs can be particularly problematic. As mentioned previously, they are stubborn compounds that do not easily degrade upon physical or chemical intervention. They bind themselves to solids/sediments and are highly hydrophobic. In addition to leaching out of the products they were produced for, PBDEs have been proven to be challenging to deal with due to these characteristics as well as their tendencies to become present in extremely distant reaches from their initial source material such as the Arctic and Antarctic regions (Dickhut et al., 2012). Although resistant to degradation in general, PBDEs have been seen to degrade over long periods of time in an outdoor environment, mainly from photolysis for compounds present in air and water, however biodegradation is not significant (Pohl et al., 2017). Albeit not abundant, degradation does still occur which can transform PBDEs of a higher bromine content into lower homologs. Studies have shown over the past few decades that PBDE compounds, especially those of a lower bromine content, are highly susceptible to bioaccumulation and biomagnification (Pohl et al., 2017). While found in the air, water, and soil, these compounds are able to find their way into the food chain where their effects become stronger and more concentrated as they move to higher organisms in a respective chain.

As a chemical produced to minimize fire hazards in consumer goods and products, the predominant route of releasing PBDEs into the natural environment is through the disposing of these goods via

incineration and landfill. This is in addition to the relatively tiny amount of exposure accounted for from PBDE molecules that detach themselves from the polymers of products due to being an additive BFR. They can enter the environment through leachate in landfills and through airborne molecules from the incinerations of goods. With respect to leachate, the hydrophobicity and soil binding tendencies of PBDEs leave a small potential of contaminating the surrounding environment via leaching (European Union, 2002). However, under certain pH and temperature conditions it has been observed that PBDEs do exist in an aqueous form that can be cause for concern in landfills not properly optimized to deal with leachate. The highest concentration of aqueous phase PBDEs was found to be at a pH of 5 and at a temperature of 25^{o} Celsius in a mass transfer study evaluating the transport of PBDEs from electronic wastes (Danon-Schaffer et al., 2013; Pohl et al., 2017). Disposal of goods through incineration is another method that introduces PBDEs into the environment in the form of airborne molecules. This method may be the most toxic route of disposal however, since the combustion of PBDE compounds, especially those of a lower degree of bromination, are susceptible to the degrading into two even more toxic chemicals known as polybrominated dibenzo-p-dioxins (PBDDs) and polybrominated dibenzofurans (PBDFs). This route of disposal needs a high degree of caution to perform in order to prevent the introduction of worse pollutants into the environment (Zhang, Buekens, and Li, 2016).

Finally, understanding how PBDE compounds interact with the human body is of interest to this review as it can give some insight on why minimizing pollution and enforcing remeidal efforts is of great importance. A substance enters the body through three separate route which include ingestion, inhalation, and dermal absorption. In the case of PBDEs, exposure can come in many different forms but according to a study done by the U.S. EPA, it was found that a vast majority of exposure comes from the ingestion and inhalation of indoor dust particles. This study had also

mentioned that 80-90% of all exposures in the U.S. are through this route with the remainder of cases being from the ingestion of contaminanted food as a consequence of bioaccumulation and biomagnification (U.S. EPA, 2010; Pohl et al., 2017). Epidemiological studies regarding exposure to PBDEs suggest that relations to cryptorchidism, low birth weight, decreased sperm quality, increased likelihood of contracting diabetes, displaced thyroid hormone levels, and a reduction in IQ points exist for children who are exposed during development (Kodavanti et al., 2008). More studies are necessary to determine with certainty the toxic effects on the human body caused by PBDEs. However, in lieu of complete studies focused on PBDEs specifically, it is helpful to compare levels of PBDEs in humans to the same levels of exposure to PCBs at a concentration that is known to show adverse effects. It is estimated that PBDEs can cause developmental neurotoxicity in the same way it is know that PCBs do, due to the high degree of similarity in their chemical sturcutres (Kodavanti, et al., 2008). PBDEs have been found in human blood and due to their lipophilic nature, in breast milk as well. This is in addition to the ability of lower brominated BDEs and decaBDE to cross through the placenta into unborn babies causing concern for neurotoxic exposure and developmental issues (Pohl et al., 2017).

With polluting factors and toxicity in consideration, the regulations regarding PBDEs have changed significantly over the past few decades. With the production of PBDEs beginning in the 1970s and spanning many years of circulation in commerical goods up until relatively recently, many products in use today still contain PBDEs and the transport of these compounds into the environment is only increased by the use of unfit disposal practices as discussed previously. In the U.S., pentaBDE and octaBDE commerical mixtures were taken off the marketplace voluntarily by manufacturers at the end of 2004. In constrast, the production and implementation of decaBDE mixtures continued on until it was finally phased out for all uses by the end of 2013. Other parts

of the world also have placed regulations that restrict PBDEs from public circulation. The E.U., for instance, was to ban the marketing and use of penta- and octaBDE in 2004. DecaBDE was then subsequently severely restricted on the ground of being added to the Restriction of the use of certain Hazardous Substances E.U. Directive (Pohl et al., 2017).

Although most developed countries have been able to cease production efforts using PBDEs, production of goods needing flame retardants and the disposal methods for these goods in developing countries is still a cause for concern since they currently still rely on the use of PBDEs. Unfit incineration methods are also used in such places, resulting in the release of PBDEs, PBDDs, and PBDFs into the global natural environment that can effect everyone on an international scale (Zhang et al., 2016). To improve this situation, safe disposal methods of existing products carrying PBDEs and alternative safe flame retardants must be enforced worldwide. However, it may be noted that commerical pentaBDE and octaBDE were signed to Annex A (Elimination) under the Stockholm convention in 2009, while decaBDE followed in 2017 (UNEP, 2008; UNEP, 2019). This subsequently enforces obligations to stop using commerical PBDEs for any member countries.

2.2 Chemical and Biological Behaviors of PBDEs in Soil

2.2.1 Principles of Adsorption

In order to fully grasp and conceptualize why PBDEs can be such a persistent chemical to perform remediation techniques on and how they can be a potentially hazardous substance, the science behind chemical sorption and desorption must be discussed. To begin, the general meaning and understanding of the term "sorption" describes the consideration of the processes known as "absorption" and "adsorption" together as a single phenomenon. The definition of absorption is a mass-transfer process in which a substance dissolves itself into another, while adsorption is a mass-transfer process in which a substance is bonded to another; this is a surface occurrence only (Davis and Masten, 2004). In addition to sorption, a third process can also occur which represents its antithesis. This process is known as "desorption" and can be defined as the release of one substance from another, either from the surface or through the surface (International Labmate Ltd, 2014). In the context of this study, desorption is strictly applied as the opposite meaning of adsorption, while absorption will not be a focus. These ideas are illustrated clearly in Figure 2.3.

Additionally specific definitions of these process also exist and can assist in distinguishing specific field process from one another. Adsorption can be divided into two more process known as chemisorption and physisorption. Chemisorption describes the phenomenon in which an adsorbate is bonded to the surface of an adsorbent through chemical interactions, i.e., a covalent bond, while physisorption is where the adsorbate is attached to the surface of the adsorbent through an intermolecular force means such as van der Waals forces between the two substances (Allred, 2017). With that being said, it is understood that chemisorption is the stronger from of adsorption due to the stronger nature of the type of bonds that are in effect, thus for desorption to occur, more

energy is required to remove an adsorbate from its adsorbent if they are connected through a means of chemical bonds.

Adsorption kinetics and adsorption isotherms are the two main tools that are used to determine this information. Adsorption kinetics can be described as the measure of the adsorption uptake with respect to time at a constant pressure or concentration (Saha and Grappe, 2017), while an adsorption isotherm is a curve relating the equilibrium concentration of an adsorbate on the surface of an adsorbent, to the concentration of the adsorbate in the surrounding medium with which it is in contact with (Sahu and Singh, 2019). Simply put, the use of adsorption kinetics will study how quickly an adsorbate can adsorb or desorb to an adsorbent under constant pressure or concentration, while an adsorption isotherm is a numerical relationship that describes the equilibrium between the concentration of adsorbate on an adsorbent and the concentrations of adsorbate in the surrounding medium, which in most cases is a liquid. Moving forward with this information, understanding how adsorbates and adsorbents interact with each other is key in comprehending the behavior of either substance in a given situation.

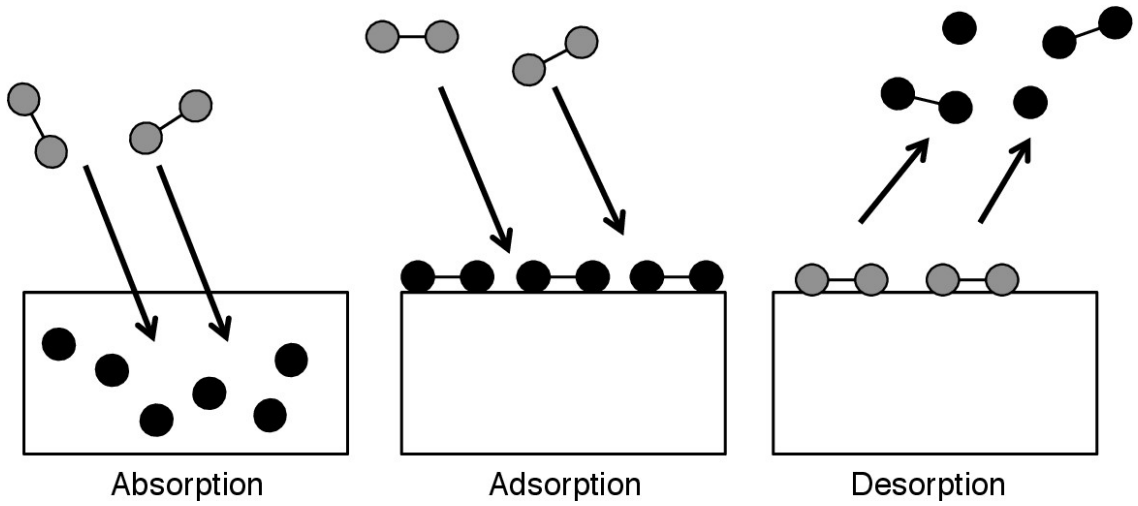


Figure 2.3: Illustration of the Differences between the Processes of Absorption, Adsorption, and Desorption (Allred, 2017)

The mathematics behind adsorption kinetics are represented through the use of reaction rate equations that can be modified and used in different reaction orders. In the most basic form, the mathematical expression used to represent the rate of a reaction is shown in equation 2.1

$$r = k[A]^x[B]^y \qquad (2.1)$$

Where k represents the reaction rate constant, [A] and [B] are the concentration values of substances A and B expressed in molarity, while x and y are the partial orders of the reaction in which their expresses the overall order of the reaction (Petrucci et al., 2012). The order of a reaction is the correlation between the concentration of the involved substances and the rate of the reaction. The most common displays of this principle can be represented in either a zero-, first-, or second-order reaction. In a reaction involving the decay of substance A into products, a zero-order reaction means there is no change in the reaction rate, while a first-order reaction will show this reaction rate doubled, and a second-order reaction will be quadrupled. In a plot of the concentration of substance A versus time, the concentration of A will change with time due to the chemical decaying of the species. No matter the reaction rate order, this plot can always be represented in the form of a line with modifications determined with integral calculus (Petrucci et al., 2012). Equations 2.2, 2.3, and 2.4 represent this relationship for zero, first, and second order reaction respectively.

$$\textit{Zero Order:} \quad -\frac{d[A]}{dt} = k \quad \rightarrow \quad [A] = [A]_0 - kt \qquad (2.2)$$

$$\textit{First Order:} \quad -\frac{d[A]}{dt} = k[A] \quad \rightarrow \quad [A] = [A]_0 e^{-kt} \qquad (2.3)$$

$$\textit{Second Order:} \quad -\frac{d[A]}{dt} = k[A]^2 \quad \rightarrow \quad \frac{1}{[A]} = \frac{1}{[A]_0} + kt \qquad (2.4)$$

Using these kinetic principles, the rate of adsorption and desorption can be modelled, calculated, and applied to a given situation in which this information is needed. Recent applications of adsorption kinetics in relation to studies dealing with PBDEs include relating the desorption rate of congeners from natural mediums (de la Cal et al., 2008; Liu et al., 2010; Liu et al., 2011; Liu et al., 2012; Ni et al., 2015) and sorption rates of PBDEs to other various mediums to enhance remediation techniques (Bao et al., 2011; Deng and Tam, 2016; Jaafar et al., 2019; Sha'arani et al., 2019; Xu et al., 2019). The main purpose of these studies is to understand the fate-transport actions of PBDEs as well as their bioavailability, thus touching upon hazardous exposures making this group of chemicals less threathening.

Adsorption isotherms are another mathematical tool able to assit in the understanding of how chemical substances interact with each other. In this case, the purpose is to gain an understanding of the concentration of an adsorbate to an adsorbent with resepect to an equilibrium within the surrounding medium. Specifically speaking, one of the most common and most relevant practices of this idea comes in the form of studying the retention or mobility of a substance from an aquatic setting to a solid-phase at a constant temperature and pH. This equilibrium is often depicted as a curve showing the solid-phase concentration of the substance against any residual, or remaining amounts of concentration (Foo and Hameed, 2010). Throughout the historical relevance of developing isotherm models, many have come into conclusion that each have their own advantages to a specific situation. Relevant and recent publications that undertake the task of studying PBDEs in this context often mainly rely on Freundlich and Langmuir isotherm models with other models occasionally being used in addition.

A Freundlich isotherm is generally widely applied to heterogeneous systems, espeically those involving organic compounds, and is mathematically represented with by either a nonlinear or linear equation. These are represented by equations 2.5 and 2.6 respectively.

$$q_e = K_F C_e^{1/n} \quad (2.5)$$

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (2.6)$$

Where q_e represents the concentration or amount of adsorbate at equilibrium, K_F is the adsorption capacity, 1/n is the adsorption intensity (n is a correction factor specific to the present material), and C_e is the equilibrium concentration of adsorbate on the adsorbent (Ayawei et al., 2017). The slope of the linear equation will range between 0 and 1, which is representative of adsorption intensity or surface heterogeneity becoming more intense as the slope approaches a value of zero (Foo and Hameed, 2010). Some of the shortcomings involved with this model are that these equations are purely empirical and it can only hold validity up to a threshold concentration in which this linear model become nonlinear and thus unreliable (Singh, 2016).

A Langmuir isotherm is another two-parameter model that was historically developed to describe the adsorption equilibrium relationship between the gas and solid phases (Ayawei et al., 2017). Unlike a Freundlich isotherm, this model is not empirically derived and is built from theoretical principles instead. However, this model assumes a monolayer adsorption idea in which only the top layer of the adsorbent is able to bond with the adsorbate (Foo and Hameed, 2010). Mathematically, this model can represented by a nonlinear and linear like the Freundlich model, but the linear equation can be expressed in four different ways depending on which axes are preferred to display the information needed. The nonlinear equation is expressed in equation 2.7. Where q_e and C_e are the same variables described in the Freundlich model, and the four linear

forms of the equation are shown in equations 2.8, 2.9, 2.10, and 2.11 respectively along with their associated plot axes (Foo and Hameed, 2010).

$$q_e = \frac{Q_0 b C_e}{1 + b C_e} \qquad (2.7)$$

$$\frac{C_e}{q_e} = \frac{1}{bQ_0} + \frac{C_e}{Q_0} \quad \rightarrow \quad \frac{C_e}{q_e} \; vs \; C_e \qquad (2.8)$$

$$\frac{1}{q_e} = \frac{1}{Q_0} + \frac{C_e}{bQ_0 C_e} \quad \rightarrow \quad \frac{1}{q_e} \; vs \; \frac{1}{C_e} \qquad (2.9)$$

$$q_e = Q_0 - \frac{q_e}{bC_e} \quad \rightarrow \quad q_e \; vs \; \frac{q_e}{bC_e} \qquad (2.10)$$

$$\frac{q_e}{C_e} = bQ_0 - bq_e \quad \rightarrow \quad \frac{q_e}{C_e} \; vs \; q_e \qquad (2.11)$$

In general, the Langmuir model is often assessed through the nonlinear equation by locating the numerical plateau in which equilibrium saturation has been reached and adsorption con no longer occur (Foo and Hameed, 2010). In addition, Webber and Chakkravorti had defined a separation factor, R_L, which reports on the resulting state of the Langmuir isotherm model in a dimensionless way using the expression defined by equation 2.12

$$R_L = \frac{1}{1 + K_L C_0} \qquad (2.12)$$

Where K_L is the Langmuir constant represented in an inverse concentration unit and C_0 is the initial concentration of the adsorbate represented in a manner able to counter the units of K_L. The value of R_L then represents the outcome of the adsorption system. The adsorption is unfavorable when $R_L > 1$, linear when $R_L = 1$, favorable when $0 < R_L < 1$, and irreversible when $R_L = 0$ (Ayawei, Ebelegi, and Wankasi, 2017).

Both of these isotherm models are used in recent studies that are relevant to this study. Applying isotherm principles to PBDE studies is very important in understanding how these compounds will behave in their natural surroundings and can express valuable information regarding compound concentrations in different situations. As previously mentioned with the examples of recent studies of PBDEs and their kinetic tendencies, most of these same studies also conduct isothermal calculations to understand equilibrium concentrations as well as their rates of adsorption or desorption. Some of these recent studies however have a more specific focus of determining the equilibrium relationship between the natural environment and PBDE concentrations or the equilibrium concentration tendencies of PBDEs and developing candidate mediums in remediation tactics (Liu W. et al., 2011; Olshansky et al., 2011; Xin et al., 2012; Liu et al., 2017; Xu et al., 2019). Understanding the basic principles of sorption and desorption is the corner stone knowledge needed by researchers to undertake the task of studying persistent organic pollutants in modern sicnece. Tools such as adsorption kinetics and adsorption isoterms allow for the study and understanding of how to best treat potentially hazardous and toxic chemical compounds such as PBDEs by learning how they behave under different settings in order to effectively and efficiently remove these chemicals from the natural environment.

2.2.2 Adsorption of PBDEs in Soil

Many common traits between persistent organic pollutants exist such as their adverse effects to living organisms, persistence within the natural environment, and their ability to bioaccumulate. However, their ability to unintentionally transport themselves far distances from their source material through wind and water is particularly troubling with respect to remediation efforts (U.S. EPA, 2002). PBDEs are no exception to this issue. As briefly mentioned in section 2.1, PBDEs are highly lipophilic compounds, having a strong affinity to sediments and soils. Using the principles

of sorption and desorption, along with the associated mathematical tools used to communicate, quantify, and give meaning to values; learning why PBDEs have an attraction to solid-phase materials is relatively straightforward task.

There are many chemical compounds of interest within the natural environment in the field of environmental remediation. Along with sorption and desorption kinetics and isotherms, extra mathematical knowledge is required in order to fully comprehend the presented values and subsequently understand the behavior of these chemicals in nature. This additional knowledge is mainly presented in the form of a ratio delegating the amount of a substance between the two mediums in which it exists. These are known as partition coefficients and are often used to describe the distribution of a substance throughout two mediums that do not typically react with one another (Bannan et al., 2016). One of the most common examples of this idea is that of the octanol-water partition coefficient (K_{OW}), which can express how soluble or lipophilic a substance is given that it is in an octanol-water two phase system. Since this ratio expresses its value with respect to the aqueous phase being the ratio denominator, a low K_{OW} value would mean that a substance is more hydrophilic than lipophilic, while a higher value would mean the opposite is true (Sangster, 1997). The use of K_{OW} in the research of chemical behavior can assist in explaining why certain compounds have the concentration distribution they have and can even assist in understanding the fate transport since highly soluble compounds may easily be moved once in contact with moving water. This trait is not unique to the octanol-water partition coefficient. Other similar methods are used to determine chemical distributions in specific scenarios that need to be studied to ensure the efficient removal of pollutants.

As stated, PBDEs are characteristically very lipophilic compounds and when given the choice, tend to not associate themselves in an aqueous phase if other lipophilic media is also within the

system. In the case of the present study, the most applicable scenario to analyze is that of PBDEs within a two-media system involving water and solid-phase material, such as soil, sand, or sediment. It should be recognized that PBDEs themselves do not have an attraction to the minerals that make up a large proportion of the components for most soils, sands and sediments. Instead, there is proof that PBDEs are attracted to these solid-phase materials due to their organic-matter portions. As lipophilic chemicals, it fundamentally stands that PBDEs would repel away from any interaction with water and instead try to interact with the lipophilic organic matter within these solid-phase materials. A study done by Yu et al. (2018), had analyzed the relation between the bioavailability of PBDEs in soil samples with their bioaccessibility through the use of different PBDE congeners in different soils with varying organic carbon contents, to find that congeners with a higher bromine content had lower bioavailability compared to those with fewer bromines. A negative relationship between their bioavailability and $\log K_{OW}$ value was also noticed, signifying that the more bromine atoms a congener had, the lower the bioavailability but also with higher K_{OW} values (Yu et al., 2018). In simple terms, this means that with more bromine atoms belonging to a congener molecule, the heavier and more lipophilic that compound becomes giving it a higher K_{OW} value. The risk associated with this can come in the form of damage to living organisms that interact with contaminated material over time, since these heavier congeners are not as likely to travel as far. Lower brominated congeners on the other hand have fewer bromine atoms making them lighter and more hydrophilic. The risk associated with these characteristics is that these compounds can then travel freely within whatever moving water they come into contact with, transporting these relatively hazardous compounds far distances. Thus determining the distribution characteristics of PBDEs in a water-solid phase system is a useful and relatively easy task when given all the needed information to calculate K_{OW} values.

Along with K_{OW}, other partition coefficient values have been developed over the years to try and gain more knowledge on how chemicals can distribute themselves throughout other specific mediums. Although very similar to the principle of K_{OW}, the use of the soil adsorption coefficient (K_d) and subsequently the organic carbon-water partition coefficient (K_{OC}) is a much more accurate representation of the distribution of chemicals in an aqueous-soil system. Although the use of K_{OW} is still very applicable and helpful in understanding the desried data, it does not consider the lipophilic solid-phase material for what it actually is. This is because it uses 1-octanol, a fatty alcohol compound, as a substitute for the organic matter in the solid-phase material (Kozerski et al., 2014). While this may provide a close estimation of the distribution behavior, it is well known that within two-phase systems, the distribution of the organic compound at question varies greatly with dependence on their diverse polarity (Nguyen et al., 2005). Thus, indicating that these sorption behaviors can not always be accurately replaced or properly reproduced with the use of 1-octanol as a replacement for organic carbon in soils.

The method of calculating K_d and K_{OC} remains relatively straigtforward and easy, as they still represent a ratio of concentrations between two mediums. Equation 2.13 shows the mathematical ratio for calculating K_d.

$$K_d = \frac{C_s}{C_{aq}} \qquad (2.13)$$

Where C_s is the concentration of the compound of question in the soil, and C_{aq} is the concentration of the compound in the aqueous phase. It should not be surprising that the ideas brought forward with this concept go hand-in-hand with the equilibrium studies allowed through the use of isotherms. Over a period of time, the introduction of a pollutant compound to the natural environment would cause the concentration of this chemical to reach equilibrium among the

surrounding mediums. It is the job of the adsorption isotherm to determine what this equilibrium concentration is, while the use of partition coefficients allow for the understanding of the distribution of the compound concentration. With that being said, a useful piece of information for the use of the K_d formula would be to determine the concentration of the pollutant at equilibrium. The use of a Freundlich isotherm is usually well equipped for this purpose and thus, equation 2.14 can be used to determine C_s at an equilibrium state.

$$C_s = K_F \times C_{aq}^n \qquad (2.14)$$

Where K_F and n remain the same parameters as they were in the Freundlich isotherm expression, and can be found using linear regression of $\log C_s$ on $\log C_{aq}$ (Kozerski et al., 2014). Although K_d can be a better estimation of the partitioning of a compound between water and soil, it does have some draw backs that should be considered. For instance, the value may vary greatly across a wide soil sample volume. The formulation for K_d does not include a parameter that considers the organic contents within the soil. Since the organic content can vary greatly across different samples, this can cause the meaning of K_d to become lost. Its value may also fluctuate greatly with respect to the soil medium it is dealing with.

Normalizing these K_d values to produce K_{OC} values is the best way to obtain distribution data that would make sense amongst different soil profiles. The simple fix for this adjustment is shown in equation 2.15.

$$K_{OC} = \frac{K_d}{foc} \qquad (2.15)$$

Where f_{OC} is the fraction of the soil mass strictly belonging to organic matter (Kozerski et al., 2014). Using K_{OC} values to determine the behavior of pollutant chemicals, including PBDEs, is an

excellent method that considers many factors. Although the use of K_{OW} and K_d values show promise in estimating distribution data as well, K_{OC} values are the best option available for the scenario of an aqueous-solid phase system.

With this information, understanding how PBDEs interact with solid-phase materials while in the presence of water, such as in ground water flow or shallow sediment scenario, is a task that is not impossible. Knowing this information would enhance the knowledge of how to best approach remediation efforts of PBDEs in a two phase system considerably. In addition, the assumption that a linear adsorption equilibrium relationship demonstrated through a K_{OC} value for analyzing PBDEs in a soil medium is valid. Studies such as Liu W. et al. (2011) and Liu et al. (2012) display similar protocols in which the use of linear distribution models have the capacity to accurately portray the sorption isotherms of some PBDE congeners in different natural soil samples.

Although the technology exists to determine the K_{OC} values for almost any chemical pollutant, there is a strong probability that the K_{OC} value for the exact chemical compound in question is not yet documented. This may be because the status of a compound many be very new and emerging and/or the experiments needed to produce these values may be too costly. PBDEs are not exception to this statement. While many studies have been produced in recent years studying the behavior of PBDEs, they are still a relatively new emerging contaminant and there remains a lot of missing information with regards to how they can effect entire ecosystems, human health, and how to properly undertake remediation techniques. As stated in section 2.1, PBDEs were largely made available in three different commercial mixtures that each had a concoction of different congeners in their makeup. For this reason some specific congeners of PBDEs are more commonly occurring than others. For instance, pentaBDE products mainly consist of BDE-47 and BDE-99, while octaBDE products mainly consist of BDE-183 and BDE-203 with considerable amounts of BDE-

153 and BDE-197 as well (U.S. EPA, 2010; Pohl et al., 2017). For this reason, current studies that focus on the sorption and desorption behaviors through kinetics, equilibrium isotherms, and chemical distributions such as the ones mentioned previously, have only ever focused their studies on a handful of common reoccuring congeners of PBDEs. This is a start towards gaining a perfect understanding of how PBDEs interact with the natural environment, but additional information is needed about all the possible congeners. As PBDEs sit within the environment, they can degrade over time into derivative congeners with very little know descriptive information. Determining any information possible about the more uncommon PBDE congeners is a requirement for dealing with these compounds on an overall scale so that no adverse surprises can arise.

2.2.4 Biodegradtion of PBDEs in Soil

In nature there is a large variety of living biota that all require energy to function for survival. This energy can be acquired in many different ways, with consuming and breaking down nutrients from external tissue being the most familiar as mammals. However, for other living biota, this process can be very diverse. In the context of environmental sciences, the energy sources of microorganisms is a particularly interesting topic. These microorganisms, such as bacteria and fungi species, source their energy from feeding on organic matter locally available in their surrounding environments (U.S EPA, 2012). In a lot of cases, these microbes are a vital part of the life cycle for many living creatures. Specifically speaking, bacteria and fungi obtain organic matter from the dead plants and animals in which they eat, causing decomposition. In an ecosystem, the decomposition of dead biota is extremely important as it recycles and frees important nutrients back into the environment, enabling the growth and support of new life (Ohkuma, 2003). The carbon, nitrogen and phosphorus cycles are prime examples of this phenomenon. Since carbon, nitrogen and phosphorus are specific elements that serve as essential nutrients in a soil

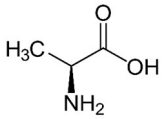
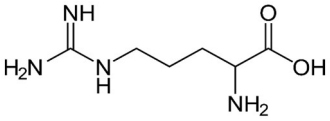
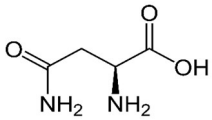
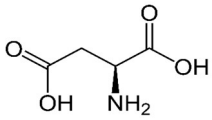
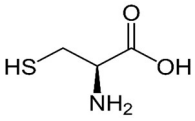
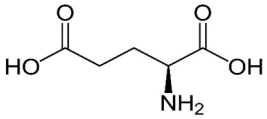
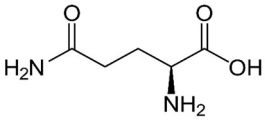
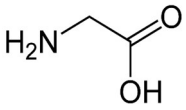
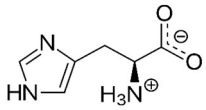
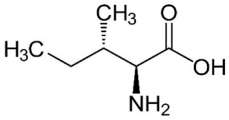
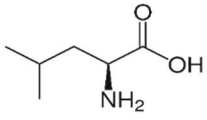
environment, they allow for optimal conditions for new plant life (Lines-Kelly, 1992) thus creating a solid foundation and support system for life at higher trophic levels.

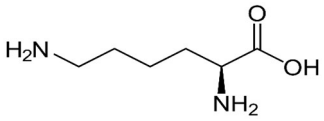
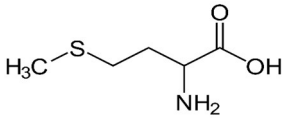
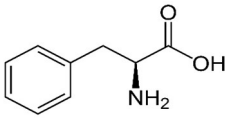
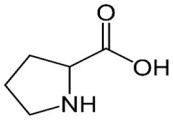
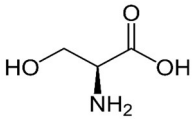
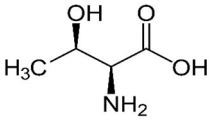
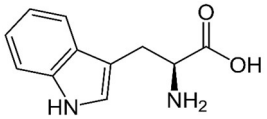
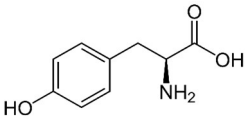
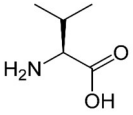
The microbes that act as decomposers in the biodegradation process are often categorized into two different groups known as aerobic and anaerobic microbes which indicate whether oxygen must be present for growth and survival. Like most living beings, these different types of microbes thrive in environments in which their needs can be optimally met. For instance, aerobic microbes are often found in terrestrial soils and sediments that are aerated and loosely packed through a means of plant roots, as well as where surface disturbances are common causing oxygen to circulate within these solid particles. Anaerobic microbes oppositely, are found in environments where oxygen is not as available such as in tightly packed soils or sediments, oxygen depleted marine environments, and even within the digestion tracts of animals (Gorbach, 1996; Hoorman, 2016). While both processes use carbon from organic matter to fuel themselves, they differ slightly in the way the carbon is metabolized thus producing different byproducts. The general chemical formula for the aerobic and anaerobic decomposition processes are similar since both produce carbon dioxide (CO_2) and water (H_2O) in addition to nutrients. However, an anaerobic process only requires organic carbon as a reactant while an aerobic process requires oxygen in addition. Anaerobic processes also produces methane (CH_4) as an additional product while aerobic processes do not (Zee and Maarten, 2011). With this knowledge considered, it is important to remember that these biogas products are produced and cycle naturally and generally do not cause excess concerns of greenhouse gas emissions as balance in the cycle occurs.

In either aerobic or anaerobic case, the microbes responsible for the decomposition of organic materials must have an efficient means of digesting them. Often the carbon source present can be too large and complicated for microbial digestion and in response, these microbes produce extra

cellular enzymes known as exoenzymes that have the specific task of reducing complex organic compounds into smaller and simpler forms more optimal for the microbes to use. These produced enzymes are specialized proteins that serve as a chemical catalyst to promote the decomposition of specific compounds (Kamalanathan et al., 2020). Specifically speaking, the structure of an enzyme is a large macromolecule consisting of long amino acid chains that fold and orient themselves in a particular way based on many external factors such as the polarity or charge of the amino acids present. In most cases, these macromolecules are folded and structured in a way to contain hydrophobic amino acids in the interior of the structure while orienting hydrophilic amino acids on the exterior in water containing environments and oppositely in lipophilic ones (Dyson et al., 2006; Voet et al., 2016; Foy et al., 2019). Generally speaking, amino acids are organic compounds that consist of an amino ($-NH_2$) and a carboxyl (-COOH) functional group as well as a unique side chain known as an R group that distinguishes one acid from another. There are 20 common and unique amino acids that make up the structures of proteins and enzymes. In addition to categorizing them by their polarity, they are often referred to as either essential or non-essential. This distinction groups these compounds through their ability to be produced internally by an animal organism (non-essential) or if they must be obtained through diet (essential). It may be noted that plant species are capable of producing all 20 varieties thus reinforcing their importance to sustain life for higher trophic organisms (Voet et al., 2016; Wax, 2019). Table 2.2 summarizes these amino acids in alphabetical order and accordance to the essentiality in human beings.

Table 2.2: General Information on the 20 Common Amino Acids (Ophardt, 2003b)

Amino Acids				
Name	**Code**	**Polarity**	**Essentiality**	**Common Structure**
Alanine	ALA	non-polar	non-essential	
Arginine	ARG	polar	non-essential	
Asparagine	ASN	polar	non-essential	
Aspartic Acid	ASP	polar	non-essential	
Cysteine	CYS	polar	non-essential	
Glutamic Acid	GLU	polar	non-essential	
Glutamine	GLN	polar	non-essential	
Glycine	GLY	non-polar	non-essential	
Histidine	HIS	polar	essential	
Isoleucine	ILE	non-polar	essential	
Leucine	LEU	non-polar	essential	

Lysine	LYS	polar	essential	
Methionine	MET	non-polar	essential	
Phenylalanine	PHE	non-polar	essential	
Proline	PRO	non-polar	non-essential	
Serine	SER	polar	non-essential	
Threonine	THR	polar	essential	
Tryptophan	TRP	non-polar	essential	
Tyrosine	TYR	polar	non-essential	
Valine	VAL	non-polar	essential	

The variety of amino acids present in a macromolecule decide on the structure and identity of the enzyme, thus each enzyme is a protein consisting of uniquely sequenced amino acid chains. In consequence of the macromolecule structure, each enzyme contains one or more locations called active sites, in which the structure is able to optimally accommodate a specific molecule known as a substrate to bind to the amino acids present at the active site (Srinivasan B, 2020). The typical nomenclature for identifying enzymes is organized around the specific substrate the enzyme is able to interact with. While there are many anomalies to this rule, it is common practice to add "-ase" as a suffix to the substrate name to label enzymes (Britannica, 2019).

The microbes that produce these specific enzymes do so to optimally digest the carbon compound food sources that are present and available thus, enzyme production is done to best suit microbial needs under the given environmental conditions. While interacting with present substrates, singular molecules, called ligands, are able to fit directly into the active site of the enzyme macromolecule creating an enzyme-substrate complex. Once the positioning is complete, the enzyme then enables the decomposition reaction of the ligand to occur by directly reducing the chemical activation energy required to perform this process (Schowen, 2003). With a lower amount of energy required to decompose the ligand, decompositions can happen at faster rates through a means of intermolecular interactions between the ligand molecules and present amino acid molecules. The most common interactions are composed of electrostatics, hydrogen bonds, and van der Waals forces (University of Arizona, 1996). Once the decomposition of the ligand is completed, the newly formed products are detached from the active site and the enzyme restores itself to enable another complex to be made, thus repeating the process. An illustration describing this phenomenon can be found in Figure 2.4.

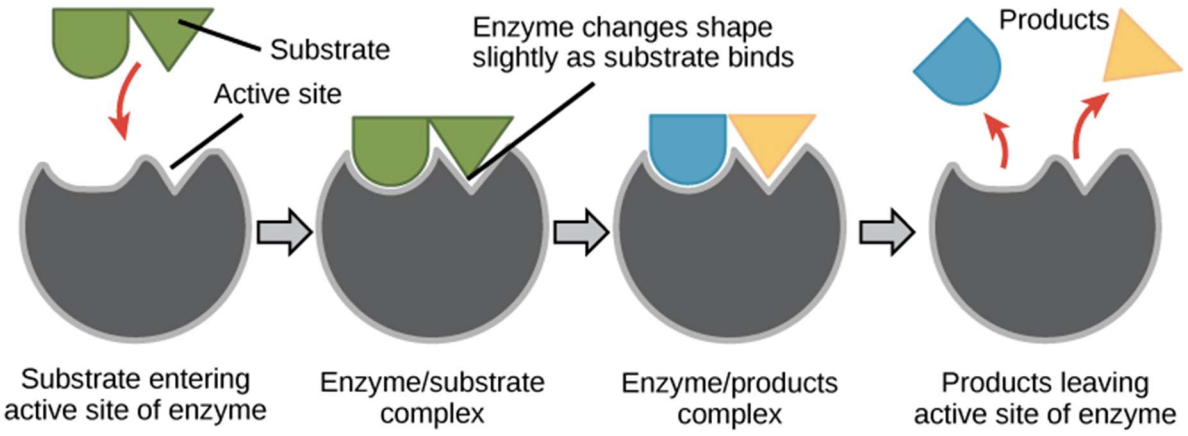


Figure 2.4: Enzymatic Decomposition Process (OpenStax College, 2013)

Biodegradation specifically, is this process of naturally found microorganisms feeding on organic matter to create energy for survival while also returning nutrients into the environment. However, this process is not exclusive to organic matter found naturally in the same biotic cycle. Organic matter used for food sources can also be acquired through xenobiotic materials (Ottow, 1989). In modern history, there have been many varieties of synthetically produced chemicals made for assisting society in one form or another. In some cases, these chemicals have been found to be dangerous with their adverse effects outweighing their usefulness and sometimes can find their way into the natural environment where they cause contamination and pollution issues. Interestingly, it is at this point where the intersection between biodegradation and bioremediation occurs. While biodegradation happens naturally, bioremediation is a manmade process in which biodegradation is harnessed and optimized to carry out the decomposition of harmful and foreign substances (Margesin and Schinner, 2001).

The first instance of using bioremediation to treat contamination in a commercial context was in 1972 when a pipeline spill occurred in Amber Pennsylvania consisting of relatively easy degraded petroleum chemicals (National Research Council, 1993). Since this point in time, bioremediation in soil specifically has advanced in practice. With additional research and technological advances in environmental engineering, this process can be aimed specifically at targeting chemical contaminants considered to be more difficult to remove such as halogenated hydrocarbons (PCE, TCE, and PCBs are common), polycyclic aromatic hydrocarbons (PAHs), and harmful pesticides like Aldrin and Endrin (Sharma and Reddy, 2004). While bioremediation has been shown to be a safe and effective practice of removing toxic contaminants, drawbacks to this method encompass the challenges faced when designing the optimal means of using biodegradation. For instance, microbes known to produce enzymes that are able to decompose the target contaminant must be

both present and optimized in quantity, or must be introduced. The temperature and pH of the soil medium at question must also be at ideal settings to support microbial life. Lastly, the saturation of the soil must be considered to determine if the correct microbial life can be supported and if not, air injection must be considered (National Research Council, 1993). It has been estimated that in-situ bioremediation for soil can cost in a range of $30 to $100 per cubic meter (Van Deuren et al., 2002). However, with advancing technologies, alternative methods of performing bioremediation can be considered to avoid site specific designs. Recent publications discussing the future of bioremediation such as (Kumar et al., 2011; Abboud 2019; Mutambu and Masaka, 2019) discuss the need for further advancement in researching different varieties of microbes that may be effective against different contaminating chemicals as well the need for more documented cases globally. While bioremediation remains a safe way to remove harmful chemicals through biodegradation, technologies and practices need cross disciplinary improvements to provide feasibility to in situ methods.

2.3 Computational Tools and Methods

2.3.1 Quantitative Structure-Activity Relationship Modelling

In 1962 a study titled “Correlation of Biological Activity of Phenoxyacetic Acids with Hammett Substituent Constants and Partition Coefficients” published by (Hansch et al., 1962) was the first glimpse at what would become the modern version of a Quantitative Structure-Activity Relationship (QSAR) model. The gist of this study was to comprehend the structure-activity relationships (SARs) of the growth regulators in plants. This was initially attempted through the use of Hammett relationships but an appropriate approach for their specific needs was not found. In lieu of this method, Hansch looked to other works to find a working methodology. This was eventually done through an investigation of the lipophilicity of relevant plant compounds to the biological potency (Hansch, 1969). The method in which this was measured was through octanol-water partition coefficients rather than more complex methodologies available at the time. Around the same time, another scholar by the name of Fujita, was measuring the value of these octanol-water coefficients through an experimental means (Fujita et al., 1959). In conjunction, Hansch and Fujita came to the realization that these partition coefficient values were an additive feature of the molecule due to the effects of substituents within, and that the same substituents in other molecules would often contribute the same amount of lipophilicity as seen through the measurement of the octanol-water partition coefficients (Cherkasov et al., 2014). While in some cases, the use of a method involving partitioning coefficients was not less complex than that of the methods using the relation between a Hammett equation and potency, it became more commonly come by in the field of research due to the values being easier to understand (Cherkasov et al., 2014). In the span of more than 50 years, the use of QSAR modelling has developed into a common method of analyzing the effects of molecule structures to their behaviors. These first studies had then created the

framework for which QSAR models are used for today.

With a useful tool at the disposal of researchers, QSAR has had many uses in different fields. One of the biggest examples of this is its usefulness in the research and development of drugs for the pharmaceutical industry. In the context of this study however, the focus of QSAR models will be aimed at chemical toxicology within an environmental engineering point of view. An overview prepared by the U.S. EPA of a Toxicity Estimation Software Tool (TEST) program provided through their website has a brief definition of what QSAR is in the correct context. It is defined as "a mathematical model used to predict measures of toxicity from the physical characteristics of the structure of chemicals (known as molecular descriptors)". Additional information goes on to say "Simple QSAR models calculate the toxicity of chemicals using a simple linear function of molecular descriptors: Toxicity = $ax_1 + bx_2 + c$, where x_1 and x_2 are the independent descriptor variables and a, b, and c are fitted parameters" (U.S. EPA, 2016). It may also be of interest to point out that different types of QSAR models exist for different types of analyses. The chemical or molecular descriptors representing the chemical structure can be portrayed at different levels and this will determine which level of QSAR model will be used. A single dimensional model (1D) will simply take into account the chemical formula, where 2D, 3D, and 4D QSAR models exists to look at the similarities in complete physical shape as well as time-dependent dynamics if required (Cherkasov et al., 2014). For the purposes of this study, 3D-QSAR models are used for the analysis of different PBDE congeners.

In a 3D-QSAR model, three dimensional information about the molecules being studied are required. This is because some aspects of SARs can only be fully realized in higher level dimensions other than single or two dimensional shapes. The way in which molecules are structured can have a significant impact on their biological activities, or in this case, their toxicity.

While a 2D-QSAR analysis is able to determine the structure-activity relationships of ligands in specific locations, more accurate readings of these relationships can be determined by analyzing the exact spatial locations of these ligand in a three dimensional space. Some of the ligand bonding processes to molecules have preferences that can only be read accurately with a 3D-QSAR method (Cherkasov et al., 2014). Over time, multiple variations of 3D-QSAR methodologies have emerged, while the original version known as a Comparative Molecular Field Analysis (CoMFA) is still one of the most popular and prominently used versions (Cramer et al., 1988). The formulations of 3D-QSARs that are used in this study are subjected to the methodologies of a CoMFA analysis.

A 1988 study by Cramer et al. (1988) was the first to use CoMFA as it is known today. In this study, the authors had acknowledged that work on this analytical tool had begun 12 years prior. The authors of this study had stated that two observations of molecular behavior were the basis of how this tool would operate. The first was that non-covalent interactions within a molecule seemed to be the interactions that would produce noticeable biological effects. The second was that a wide variety of molecular properties from these non-covalent interactions can be accounted for by steric and electrostatic forces. This had then laid the ground work for CoMFA and its use of calculating steric and electrostatic fields. This is done by representing molecules by these steric and electrostatic fields at the intersections of a three dimensional lattice grid and then calculated through Lennard-Jones and Coulomb fields (Klebe et al., 1994). If multiple molecules are at question, then a technique would be introduced to optimize mutual alignment through minimizing the root mean square (RMS) difference of the sum between steric and electrostatic interaction values. Depending on the number of molecules in a series, large amounts of data may be created and must be processed appropriately. Using partial least squares (PLS) as the data analysis method,

CoMFA is able to handle these large amounts of information with the accuracy needed for predictability (Cramer et al., 1988). It was later found that error in alignment sensitivity can commonly occur, which can lead to difficult interpretations of steric and electrostatic fields. This was addressed in 1994 by (Klebe et al., 1994) who improved on the current CoMFA model by developing a new one known as a Comparative Molecular Similarity Indices Analysis (CoMSIA). To build on the existing CoMFA method, Gaussian potentials are used to determine field values which allows for easier interpretation (Roy et al., 2015). Both methods of 3D-QSAR are reliable in producing predictive data and are adequate for the use of data analysis of the structure activity relationships presented in a series of molecules aligned properly.

2.3.2 Applications of QSAR Modelling

The field of chemistry describes the matter of everything that is all around us, whether it may be large or small, understanding chemicals and how to manipulate them to do what we need is an on-going investigation of modern problems that require modern tools to help bring solutions to. As a tool that was developed by (Hansch et al., 1962) to determine the structure activity relationship of regulating compounds for plants, QSAR has had its beginnings in the field of biochemistry and is arguably one of the most important tools used today for any field that requires the investigation of the biological effects the structural properties of molecules can have.

The use of QSAR is not limited to a specific branch of scientific study. Any relevance or interest in understanding how molecular structures can effect another dependent variable would value QSAR as an important application of study. Two specific branches of science that do rely on the use of QSAR for modern advancements are biochemistry and organic chemistry. As mentioned briefly, the creation of QSAR was to study the structure activity relationship of molecules related

to plant growth (Hansch, 1969). The use of the SARs for molecular investigation in a biochemistry setting has evolved far beyond the use of the tool for studying plant growth regulators. More advance topics and biotic organisms have also benefitted from QSAR in modern society. Over time, relationship equations have been created for specific tasks that have stemmed from the original approach of including the steric and electronic field contributions. These newer relationship models are often tweaked into non-linear equations to more accurately represent the SARs in cellular systems (Selassie and Rajeshwar, 2003). The use of QSAR in organic chemistry is no different with the exception for what the final purpose of the studied molecule is meant to accomplish. In addition to the design of new compounds, QSAR is able to use the appropriate molecular information to determine characteristics and behaviors of pollutant chemicals that would be important in chemical productions or environmental sciences. In particular, the use of QSAR in conjunction with molecular connectivity indices (MCIs) has assisted in the determination of different partitioning properties of various types of chemical pollutants (Sabljic, 2001).

As a tool that can be used for accurately predicting important chemical values, QSAR is able to assist in studies that involve the determination of media partitioning values of persistent organic pollutants based on their molecular structure. In this study, a 3D-QSAR approach is used to study the molecular structure activity relationships of all 209 PBDE congeners.

As an organohalide chemical, PBDEs have relatively recently become recognized and classified as POPs due to their environmental stubbornness and toxicity (Government of Canada, 2010). PBDEs have multiple routes of exposure to biotic organisms, but highly hydrophobic tendencies in combination with their affinity to bind with soils causes problems within ecological food chains since they have been shown to have tendencies of bioaccumulation and biomagnification (Government of Canada, 2010; Currier et al., 2020). There is no specific use for every PBDE

congener and as mentioned previously in section 2.1, PBDEs have only been commerically available three varying mixtures. Once exposed in the environment, higher brominated congeners can degrade to lower ones over time, and thus information on SARs of any given congeners may be valuble information required for appropriate environmental response at any given time.

As a compound with persistence in soil, understanding the behaviour of each congener within this medium is important for proper understanding. In relation to the hydrophobicity of a compound and its octanol-water partitioning coefficient, is the discussed K_{OC} value. Existing studies that are available in literature will touch upon a handful of PBDE congeners at best, but no current study has delivered the K_{OC} value for all 209 congeners. Using these existing values from literature in combination with the methods and techniques of QSAR, it is a goal and objective of this study to predict and determine each K_{OC} value with accuracy so that this information can be used in future response protocols.

In general, the development of a QSAR model requires input information about the molecular structure in order to study SARs to provide a desired output. Using available information provided from reputable online sources, access to such structural information is very accessible. In the case of three dimensional molecular structure information, the orientation of each atom in a molecule is enough to determine the steric and electrostatic field information of a given molecule. For a series of molecules being studied, the alignment of each to a common structure is necessary as a datum for each molecule to be compared to and calculated against. In the case of PBDE congeners, since diphenyl ether serves as the molecular base, this structure is how the congeners are aligned. Using these in combination with a PLS regression analysis of the inputted information, the model produces the desired CoMFA or CoMSIA values. Once these values are obtained and validated to a critical accuracy, they can be used in extrapolation to predict missing information on other

similarly structured molecules that are not in the series set, or to determine field values of modified molecules. An in depth discussion on how the model for this is specifically constructed can be found in Chapter 3 of this thesis.

2.3.5 Molecular Docking

In similarity to the uses of QSAR, molecular docking is another computational technique used to analyze chemical interactions based on molecular structures. In more detail, molecular docking is an in silico application that allows for the analysis of interactions that may occur between chemical compounds and protein macromolecules (Pagadala et al., 2017). This field of computational chemistry allows for inexpensive ways to learn about how biochemical compounds interact and behave when exposed to external chemicals without needing to perform laboratory experiments. In general, molecular docking uses various techniques and algorithms to determine how external compounds, called ligands, can optimally interact within the active site of a protein. Once stability is obtained between the two entities, the structure is known as a ligand-receptor complex. As mentioned previously in Section 2.2.4, the term protein defines long chained amino acids connecting and folding to create a large macromolecule. It is important to recall that enzymes are a type of protein that have the specific task of being catalysts to promote the decrease in chemical activation energy, thus enabling the decomposition of chemicals into smaller byproducts (University of Arizona, 1996). The use of molecular docking as a computational tool is able to focus on quantifying the specific details of this catalytic process as enzymes are representative of receptor molecules and any given chemical broken down through enzymatic decomposition are representative of ligand substrates.

Historically, the concept of modelling ligand-receptor complexes had begun with simple concepts that were developed in a time when computational power was not as disposable as it is in modern society. The processes involved in developing a working molecular docking model require the searching and understanding of predicting how ligands will conform and orient themselves to a receptor protein as well as then determining the binding affinity that occurs between them. The first approach at undertaking this model was were designed around developments proposed by (Fischer E, 1894) that suggests complexes are constructed by a means of a "lock and key" analogy, representative of treating both ligand and protein structures as ridged bodies trying to fit together. Over time with improving technologies, this method was questioned for its predictive accuracy due to the rigidity of both entities. In 1963, a study produced by (Koshland, 1963) suggested that to increase accuracy in molecular docking models, an "induced-fit" is considered. This theory would then go on to explain that the shape and geometry of an active site changes slightly to conform to that of the ligands it interacts with. This then suggests that there is relative flexibility between both ligands and receptors to induce as perfect of a fit as possible creating optimal affinities and stable interactions.

In regards to computational modelling of these ideas, many varieties of algorithms have been developed and produced into available software packages since the mid to late 20th century. While it has been known that allowing for flexibility in both ligand and receptor structures is ideal for determining the best docking results, computational power is now only recently beginning to become adequate enough for this task (Durrant & McMammon, 2010; Borhani and Shaw, 2012; Lexa and Carlson, 2012). Receptor flexibility has often been regarded as a challenging undertaking due to the large number of degrees of freedom that would be required to account for full flexibility. That is, considering six degrees of translational and rotational freedom in addition to conformation

degrees of freedom for both parts of a complex. For that purpose, optimizing computational techniques to provide flexibility to ligand structures while keeping receptor ridged has been a very common practice (Gagnon et al., 2016). During the last few decades, multiple approaches and strategies for modelling the two stages of molecular docking has been imperative in shaping how this tool is used.

2.3.6 Computational Approaches of Molecular Docking Models

As mentioned briefly, the process of molecular docking can be separated into two stages. The first being a sufficient searching algorithm to determine conformational poses that provide successful ligand orientations in protein or enzyme active sites. The second is then being able to rank these poses with a scoring function to determine which ones are best suited for binding affinities (Meng et al., 2011). With different options of approaching these tasks available in a computational context, brief discussion is provided to address commonly used methods. Out of the multiple searching algorithms available, the general principle in which they function can be broken down into four distinct categories. These include, geometry-based methods, fragment-based methods, stochastic searches, and methods that are able to further refine themselves after docking has taken place.

Geometry-based methods are dependent on the structural shape of both the ligand molecule and protein macromolecule. Using their geometric properties and chemical information, these methods are able to direct ligand shapes into an active site while considering both parties as pharmacophores, meaning that steric and electronic features are used to create optimal interactions with an intent to act on a biological response (Brint and Willett, 1987; Fischer et al., 1993). An example of this method that is used in computational software are Matching Algorithms (MA) that

have the advantage of fast computational times used to further the knowledge of compound activity on large scales (Norel et al., 1994).

Next, fragment-based searching algorithms approach the computational task by dividing the ligand into smaller fragments severed at rotatable bonds then are incrementally place into the active site of a receptor. Often, the fragment with the highest likelihood producing successful interactions is placed first with proceeding fragments placed around this anchor fragment one by one. With different orientations considered for the ligand as a whole, this algorithm is able to account for some flexibility while placing the structure into the active site (Meng et al., 2011). In addition, de novo designs can be considered along with fragment-based searches which relate specifically to designing ligand structures in incremental steps to ideally fit into and interact with the active site of a protein. Some examples of fragment-based methods include Incremental Construction (IC) (Miranker and Karplus, 1991), Multiple Copy Simultaneous Search (MCSS) (Eisen et al., 1994) and the program LUDI, which focuses on forming hydrogen bonds in active sites filled with hydrophobic pockets (Böhm, 1992).

The third method, stochastic searches, use randomly generated ligand orientations to fit into the conformational space in which interactions form based on the ligands degrees of freedom (Meng et al., 2011). One example of this algorithm is Monte Carlo (MC) simulation where a preset number of ligand conformations are inputted that are then randomly generated. These conformations are then cross-referenced to a set energy criterion that either eliminate the pose from consideration or advance the pose to additional rounds of testing (Hart and Read, 1992; Goodsell et al., 1993). Another example of a stochastic search method is Genetic Algorithms (GA) in which the degrees of freedom belonging to the ligand are treated as binary strings that represent genes within a chromosome structure, while these chromosomes are representative of a conformational pose.

Similarly to MC algorithms, these poses are then tested against a preset criterion that evaluates the success of the generated pose (Jones et al., 1997; Morris et al., 1998). While stochastic searching methods are efficient at searching through many varieties of pose configurations, they may also produce conformations that may not be achieved easily in realistic experimental settings.

Finally, there are approaches to searching algorithms that allow for further refinement of the conformational pose after it has been suggested. One specific method that explores this idea is Molecular Dynamics (MD) which is a very common tool used in the searching step of molecular docking due to its powerful simulation performance (Meng et al., 2011). In this method, the ligand molecule is considered by its individual atoms which then are placed individually to produce optimal conformations. This algorithm is often regarded as very useful since it can allow for some flexibility in both ligand and receptor structure creating poses that more closely resemble optimal induced fits. It should be mentioned however, that this method progresses in very small steps causing for large computational time and effort. To avoid pitfalls of produced unrealistic results in regards to energy barriers, MD is often used as an additional step to complement randomly generated conformations in order to further refine their docking success by moving atoms individually into more optimal positions (Brooks et al., 1983; Weiner et al., 1984; Cornall et al., 1995).

The second stage of the molecular docking process involves scoring functions that are created to rank the poses found during the searching stage according to their feasibility and docking success. In some instances, conformational poses found during the searching stage may not be optimally suited for predetermined needs and uses causing for correct and incorrect poses that must be sorted through (Meng et al., 2011). Additionally, each pose is scored on its success for creating stable interactions between the ligand and receptor. Traditionally, three classes of scoring functions are

used in molecular docking techniques with each having their own approach to the computational task. In each case, these functions are all physics-based in knowledge and operations. They include force-field based, knowledge based, and empirical based scoring functions (Kitchen et al., 2004). Within these scoring functions, the main purpose is based the thermodynamics of free energy. Negative values of free energy indicate that processes will proceed spontaneously, thus the principles of scoring functions aim to minimize the free energy in the docking process enabling for as spontaneous of a connection as possible.

Force-field based scoring functions will produce summations of the intermolecular forces present between that of the ligand molecule and amino acid molecules present at the receptors active site. Generally speaking, these forces often are either electrostatic or van der Waals. Electrostatics are often calculated using Coulombic formulations using electric point charge attractions between atoms, while van der Waals are accounted for using a Lennard-Jones potential function (Meng et al., 2011). In either case, computational times for this scoring function method can be lengthy thus distance restriction parameters are placed to ensure that only optimally close conformations are considered. Additional resources can be added to this method of scoring by also considering additional hydrogen bonds, entropy considerations and solvations between solute and solvent which are representative of the ligand and receptor respectively (Kollman, 1993; Carlson and Jorgensen, 1995; Åqvist, Luzhkov, and Brandsdal, 2002).

Secondly, knowledge based scoring functions use the generated list of conformations determined in the searching stage, and use statistics to determine how common intermolecular interactions are between that of the atoms in the ligand and the atoms in the active site of the receptor. The method is constructed around the idea that more frequent interaction occurrences are also more likely to be successful pairings. The scoring of the method specifically uses the attractive and repulsive

forces between these pairs while setting predetermined limits on the cutoff value for how intense a repulsion force must be before the pairing is removed from consideration (Verkhivker et al., 1995; Ishchenko and Shakhnovich, 2002; Feher, Deretey, and Roy, 2003).

Lastly, empirical scoring functions computationally approach the task of ranking generated conformations through dividing the overall binding energy that exists amongst the simulated poses. These divisions are often broken into down into ionic interactions, hydrogen bonds, hydrophobic effects existing in the site cavity, as well as binding entropy (Meng et al., 2011). In order to quantify these values into a form that allows for a proper summation score, they are multiplied by coefficients predetermined through regression analyses with a testing set of ligand-receptor complexes with already known binding energies. It should be noted that the means in which coefficients are determined will vary from program to program while the ultimate goal is to minimize the free energy as much as possible (Head et al., 1996; Böhm, 1998; Verkhivker et al., 2000).

While molecular docking has served as useful concept in modern society to understand the interactions between ligand molecules and proteins, the different means in which this can be done will be different and specifically customized to each specific pairing. With the different methods outlined to perform both stages of this process, the overarching concept of molecular docking is to find the best way in which a ligand fits into the active site of a receptor that also has the highest possible stability and binding energy while having the lowest possible free energy. It has been noted that obtaining a negative free energy value is ideal to indicate that no intervention is needed to produce successful pairings between a given ligand and protein structure (Pagadala et al., 2017).

2.3.7 Applications of Molecular Docking

This tool of computational chemistry was purposefully designed for biochemical fields to understand the biological outcomes between interactions of ligands to proteins. This tool is widely used in the pharmaceutical field to better understand how protein receptors in living organisms best receive medical treatments through the form of drug usage. Many drugs are specifically designed compounds that enable a specific biological reaction to take plus thus improving the health and well-being of the organism at question. Through the use of molecular docking, patterns can be studied which indicate and show researchers which aspects and traits of drug compounds best interact and perform in specific ways to target protein receptors. In addition to understanding the behavioral aspects of ligand features, molecular docking is also a tool that can be used to directly design drugs to fit into protein receptors which ultimately increase drug effectiveness. As mentioned briefly, searching algorithms such as MCSS and LUDI are specifically used for this purpose (Meng et al., 2011; Pagadala, Syed, and Tuszynski, 2017). Outside of the pharmaceutical field, molecular docking has become a promising technology used to study the biodegradation of pollutant compounds in the presence of microbial produced enzymes. With this tool, understanding how stubborn contaminants interact with environmental enzymes can be optimized to produce effective and efficient methods of bioremediation efforts (Suresh et al., 2008; Basharat, Bibi, and Yasmin, 2020). This principle is directly related to the research presented in this thesis as PBDEs are analyzed with soil enzymes to determine docking successfulness in biodegradation. This information is further discussed in detail in Chapter 4 of this document.

2.4 Summary

In this chapter, different topics have been addressed to add to the understanding of background knowledge in an effort to relay important information necessary to grasp the research and findings presented in the subsequent chapters. To summarize, the information presented in this chapter has been obtained from literature works and studies on keyword topics that serve as important supports in moving forward. Topics such as PBDEs, chemical and biological mechanisms, and relevant computational tools are touched upon.

In section 2.1, the chemical structure of PBDEs are discussed to gain insight on what exactly this group of chemicals are from a molecular perspective. This entails discussion on the halogenated hydrocarbon nature of all PBDEs with various bromine atoms binding to a diphenyl ether compound to create 209 unique chemicals known as PBDE congeners. While these congeners have their own individual properties and characteristics, physical and chemical property patters are noted with respect to varying degree of bromination. PBDEs had been synthetically developed in the mid to late 20^{th} century with the specific task of being a flame retardant that would be added to consumer goods to increase user safety. These flame retardants would be used in a commercial context through three distinct mixtures of PBDE congeners varying with an average degree of bromination. Over time it was determined that PBDEs can cause damaging adverse effects to the health and well-being of living organisms and human beings, but also to the natural environment as emerging contaminants.

Section 2.2 discusses the chemical and biological mechanisms in which PBDE behavior is described in the environment. Specifically speaking, since PBDEs are highly hydrophobic compounds that have been documented to have a particularly strong affinity for organic matter

content that often comes in the form of solid particles. For this reason, natural mediums consisting of solid organic matter are a particular hot spot for PBDE contamination. A strong example of this is soil contamination as the organic matter within soil can be a very large causing this medium to become a large sink for these chemicals. For this reason the discussion of adsorption kinetics and isotherms are of particular interest in order to mathematically deliberate and understand chemical equilibriums and behavioral characteristics of PBDEs in soil. Additionally the distribution of PBDEs in soil is addressed through the understanding of partition coefficients with the K_{OC} being a key interest. This section also touches upon the principles of biodegradation and how enzymatic activity between natural soil enzymes and external compounds play a big role in the safe and effective removal of organic based contaminants through bioremediation.

Lastly, section 2.3 address the computational methods used in research to determine quantitative models to provide insight and evidence on the characteristics of PBDEs in soil. With using QSAR, predicting PBDE partitioning actions for all 209 congeners is possible through statistically comparing the predetermined behavior of a small set of commonly studied PBDEs. The historical development of QSAR models is touched upon in addition to provide insight on how and why QSAR models are an essential tool in modern computational chemistry. Similarly, molecular docking models are introduced to gain an exact understanding of soil enzyme macromolecules interact and cause decomposition of given PBDE molecules as representative ligands. Different searching and scoring algorithms are discussed to provide context on how various molecular docking algorithms are useful in different ways.

Overall, while the purpose of this chapter is to present necessary information and ideas to properly understand the following studies, current literature regarding PBDEs in soil reaches a low threshold of understanding leaving research gaps and unstated information. Moving forward, as

QSAR and molecular docking are modern computational tools, there application is often only applied to biochemical fields of drug design and medicinal topics with a significantly less amount of information available in a bio-engineering context. Using these computational tools, additional information regarding the adsorption and enzymatic interaction behavior for PBDEs in soil is looked at in detail.

CHAPTER 3

PREDICTION OF SOIL ADSORPTION BEHAVIOUR OF PBDEs

3.1 Research Goal and Methodology

The aim of this chapter is to develop an understanding of the relationship between the structural properties of all 209 PBDE congeners to a corresponding $\log K_{OC}$ value using CoMFA techniques to establish stable models with acceptable predictive K_{OC} abilities. This is done through the use of collecting an adequate amount of existing information regarding partition coefficients of PBDEs from literature. These collected values will be used for construction and validation of a 3D-QSAR model. Once the model has been shown to prove they are capable of producing accurate K_{OC} values, interpolation measures will be used to predict the K_{OC} values for the remaining PBDE congeners.

3.1.1 Data Set for Construction of the 3D-QSAR Model

To properly create a working 3D-QSAR model that will accurately predict $\log K_{OC}$ values for PBDE congeners, existing values already known are taken from literature to create a basis. While some K_{OC} values are found in literature, it is not uncommon to find publications that represent their findings in the form of an octanol-water partition coefficient (K_{OW}) for PBDE congeners. Using the Estimation Program Interface (EPI) Suite™ software package provided for free by the United States Environmental Protection Agency, K_{OC} values are mathematically estimated through a linear function from given K_{OW} values (U.S. EPA, 2012). The relationship used is shown as equation 3.1.

$$\log K_{OC} = 0.5531(\log K_{OW}) + 0.9251 \qquad (3.1)$$

Table 3.1 displays the corresponding partition values found from literature, as well as the average value used to construct the preceding 3D-QSAR model. The model construction used 14 of these

$\log K_{OC}$ values at random as the training set, while the remaining 7 formed a testing set for model validation. These quantities thus show a training set to testing set ratio of 2:1 which has been used in a previous study by Manvar, et al., 2010. In this study, the authors had constructed a 3D-QSAR model consisting of variously structured 1,4-dihydropyridines by obtaining a training set quantities of 35 diverse molecules and a testing set of 19 diverse molecules thus providing a similar acceptable ratio of approximately 1.84:1 for the training set and testing set respectively.

3.1.2 Analysis of CoMFA Results

To conduct the 3D-QSAR analysis using a CoMFA model for the described dataset, the model was constructed and carried out in the SYBYL-X 2.0 (2012) software package on a provided workstation computer running Microsoft Windows 10 Enterprise, version 1803. The structural information of the PBDE congener compounds were obtained through SDF files found through online resources with the United States National Library of Medicine (United States National Library of Medicine, 2005). Once the SDF files for all 209 congeners were obtained, the files were then translated to a MOL2 type for which the analysis software could understand. Using the corresponding MOL2 files associated with the relevant input information, a training set and testing set were created through the use of databases within the software. Each of these sets were then subsequently aligned to a single target molecule to ensure the analysis would properly evaluate the necessary molecular properties in a way that would optimize the shape of each congener for comparison. The chosen target molecule of BDE-209 was selected due to its $\log K_{OC}$ value being the greatest (Li et al., 2020) and thus placed into both training and testing sets to influence as much continuity within the results as possible. This alignment was used to compare the portion of a PBDE molecule that is present across all 209 congeners which can be summarized by the C-C and C-O bonds which form the two phenyl rings bonded to an oxygen atom.

Table 3.1: Partition Coefficient Data Collected from Literature

Congener No.	Literature A[a]		Literature B[b]		Literature C[c]		Literature D[d]		Average	
	logKow	logKoc	logKow	logKoc	logKow	logKoc	logKow	logKoc	logKow	logKoc
BDE-15	5.82	4.14							5.82	4.14
BDE-17	5.63	4.04			5.74	4.10	5.70	4.08	5.69	4.07
BDE-28	6.24	4.38			5.94	4.21	5.94	4.21	6.04	4.27
BDE-47	6.80	4.69	6.81	4.69	6.81	4.69	6.39	4.46	6.70	4.63
BDE-66	7.00	4.80					6.73	4.65	6.87	4.72
BDE-77							6.73	4.65	6.73	4.65
BDE-85	7.27	4.95			7.37	5.00	7.12	4.86	7.25	4.94
BDE-99	7.38	5.01	7.32	4.97	7.32	4.97	7.10	4.85	7.28	4.95
BDE-100	7.09	4.85	7.24	4.93	7.24	4.93	7.24	4.93	7.20	4.91
BDE-126	7.86	5.27							7.86	5.27
BDE-138	8.17	5.44	7.91	5.30			7.91	5.30	8.00	5.35
BDE-153	7.86	5.27	7.90	5.29	7.90	5.29	7.9	5.29	7.89	5.29
BDE-154	7.62	5.14	7.82	5.25	7.82	5.25	7.82	5.25	7.77	5.22
BDE-166	8.11	5.41							8.11	5.41
BDE-183	8.61	5.69	8.27	5.50	8.27	5.50			8.38	5.56
BDE-190	8.61	5.69					9.44	6.15	9.03	5.92
BDE-196	9.29	6.06							9.29	6.06
BDE-204	9.26	6.05							9.26	6.05
BDE-207	9.65	6.26							9.65	6.26
BDE-208	9.65	6.26							9.65	6.26
BDE-209	9.87	6.38							9.87	6.38

[a] (Bao, You, and Zeng, 2011)

[b] (Liang, Zhu, Chen, and Zhu, 2010)

[c] (Braekevelt, Tittlemier, and Tomy, 2003)

[d] (Wu, Han, Li, and Wang, 2019)

The corresponding CoMFA analysis was calculated using a Tripos Standard CoMFA field class, with a steric type field value, distance dielectric, no smoothing, and the drop of electrostatics were within the steric cutoff for each row of information. In addition, the steric and electrostatic cutoff values were both set to 30.0 kcal/mol with a smooth transition. Using a Tripos Standard field class allows the software to evaluate the potentials at every lattice point in which the compound structural information is placed. After the confirmation of these settings, the respective CoMFA information column is displayed with corresponding values for each congener within the training set database.

3.1.3 Validation of the CoMFA Model

To ensure that the resulting $\log K_{OC}$ prediction values have accuracy and credibility, the constructed CoMFA model is subjected to a validation process available within the same software used for analysis. Seven congeners in addition to the target molecule of BDE-209 were used for the purpose of model validation through the testing set. The congeners set aside for the construction of the testing set are BDE-28, -47, -66, -99, -100, -138, and -204. The validation process within the software subjects the training set to a partial least squares (PLS) analysis in which a "Leave-One-Out" cross-validation is performed to determine the optimal number of components used for the PLS analysis (n), as well as the cross-validated correlation coefficient (q^2). After these values are obtained a second PLS analysis is run using the training set in which a "No Validation" run is completed using the n value determined in the previous step. This second run then displays the standard error of estimate (SEE), the non-cross-validated correlation coefficient (r^2), and the corresponding F-Test value (F). At this point, the testing set database is used to predict $\log K_{OC}$ values in which a third PLS analysis uses to determine the correlation coefficient of the testing set predictions (r^2_{pred}) and the standard error of prediction (SEP) value. Finally, a "Scrambling Stability

Test" is performed on the training set to ensure the model results are not reached through coincidence of the randomly selected input PBDE congeners. This test then produces a value illustrating the explained variance in the predictions (Q^2), the cross-validated standard error of prediction (cSDEP), and the slope of Q^2 with respect to the correlation of the original dependent variable against the perturbed dependent variables (dQ^2/dr_{yy}). Each of these statistical parameters determined by the software have specific threshold values that determine if the constructed CoMFA model is suitable for producing sufficient prediction results for the remaining PBDE congeners.

3.1.4 Selection of PBDE Substitution Positions to Decrease K_{OC} Values

Further understanding the interaction between PBDE characteristics and their individual $logK_{OC}$ values is important information that is required to improve knowledge of the fate and transport of these compounds by determining which parts of these compounds most significantly contribute towards a respective K_{OC} value. Within the 209 different PBDE congeners, many variations of bromine atom positions exist between the quantities of bromine atoms as well as their bonding site positions. While the quantity of bromine atoms present in the molecule effects the $logK_{OC}$ value, this trend can be seen clearly from one congener to another, while the effects on the $logK_{OC}$ values due to the bonding site positions is not so clearly recognized. To determine which bromine bonding sites have more of a significant effect on a compounds overall $logK_{OC}$ value, a resolution V 2^{10-3} fractional factorial design is used. This task is completed using the Experimental Design module in the Minitab software package.

The 10 factors involved in this design represent the 10 different substitution sites bromine atoms have the potential to bond with on a typical PBDE structure which can be illustrated in Figure 3.1. The levels involved for each factor are noted as either -1 or 1 to represent if a bromine atom is present. If a bromine atom is not present, the level is represented as -1 and signifies that a hydrogen atom has taken place at this particular bonding site. These factors are shown in Table 3.2. Subsequently, the software used is able to determine which sites are the most significant for $\log K_{OC}$ activity as well as any second-order interactions that may exist.

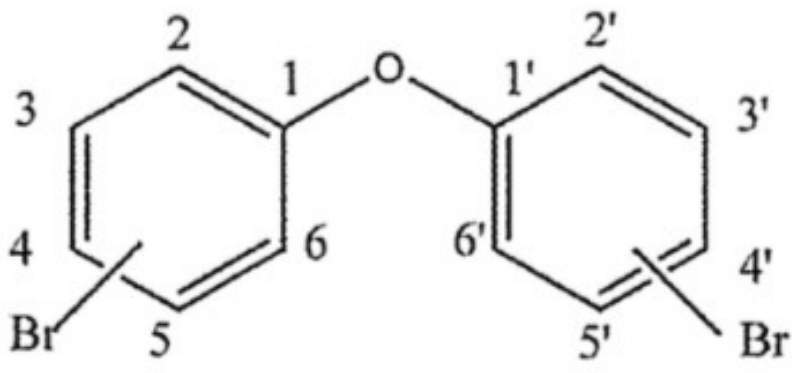


Figure 3.1: Generalized Molecular Structure for all PBDE Compounds (Chu & Li, 2019)

Table 3.2: Experimental Design Factors and Corresponding Substitution Positions

Factor	Substitution Position
A	ortho-2
B	meta-3
C	para-4
D	meta-5
E	ortho-6
F	ortho-2'
G	meta-3'
H	para-4'
J	meta-5'
K	ortho-6'

3.2 Results and Discussion

3.2.1 Evaluation of CoMFA Model Performance

Through the use of statistical values, the accuracy and predictability of the model can be justified and shown mathematically. Specifically speaking, the parameters in section 3.2.3 are addressed in Table 3.3 below. It has been shown that the constructed CoMFA model has an acceptable level of integrity with the q^2 being greater than a value of 0.5. The conventional r^2 value was also shown to be allowable which demonstrates that the model has to ability to make good predictions. This is usually the case with an r^2 value greater than 0.9 (Gu et al., 2016). It may be important to note that to determine which combination would give the most successful results a variety of testing set combinations were implemented. It was determined that using BDE-28, -66, -99, -138, and the target molecule of BDE-209 produced an r^2_{pred} value of 0.943. Since r^2_{pred} is greater than 0.6 and the SEE value is between 0.101 and 0.251, this indicates that the predictability of the model was fit for the estimation of the remaining PBDE congeners (Wang et al., 2017). Lastly, the corresponding correlation coefficients listed are close to a value of 1.00 indicating that the model is producing predictions that agree with the testing test used to compare the input information to the resulting predicted values.

In addition to these statistical parameters, Figure 3.2 illustrates the input $\log K_{OC}$ expressed as observed values, while the testing prediction values are expressed as predicted values. A 1:1 line is given through the center of the graphing area to represent how close the prediction values are to being the same as the observed values. The relevant trend line parameters for this given plot show that the corresponding slope to the data points is 0.993 with a standard error of 0.023 while the y-intercept is 0.036 with a standard error of 0.123.

This constructed CoMFA model has shown through numerical and graphical methods its competency towards the prediction of $\log K_{OC}$ values for the remaining PBDE congeners.

3.2.2 Predicted K_{OC} Values

The completed prediction model was successful at computing reasonable $\log K_{OC}$ values that could then subsequently be compared to the values used to construct the model as seen through the previously mentioned statistical parameters as well as the relative errors presented in Table 3.4. The relative error associated with each of the predicted values for these congeners all have values less than a single percentile, which further promotes the validity of the constructed CoMFA model. For instance, while the prediction results computed for BDE-28 are shown to have a relative error, with respect to the input value, of 0.138, this error is relatively low showing the high similarity between the observed and predicted values. While BDE-28 still produces agreeable results, the other data points only offer a better relative error value with BDE-154 showing a value to within the ten-thousandth of a decimal place.

Table 3.3: Statistical Parameters of the 3D-QSAR Model

Model	q^2	n	SEE	r^2	F	r^2_{pred}	SEP	Q^2	cSDEP	dQ^2/dr^2_{yy}
CoMFA	0.748	6	0.081	0.994	187.351	0.943	0.220	0.605	0.646	0.959

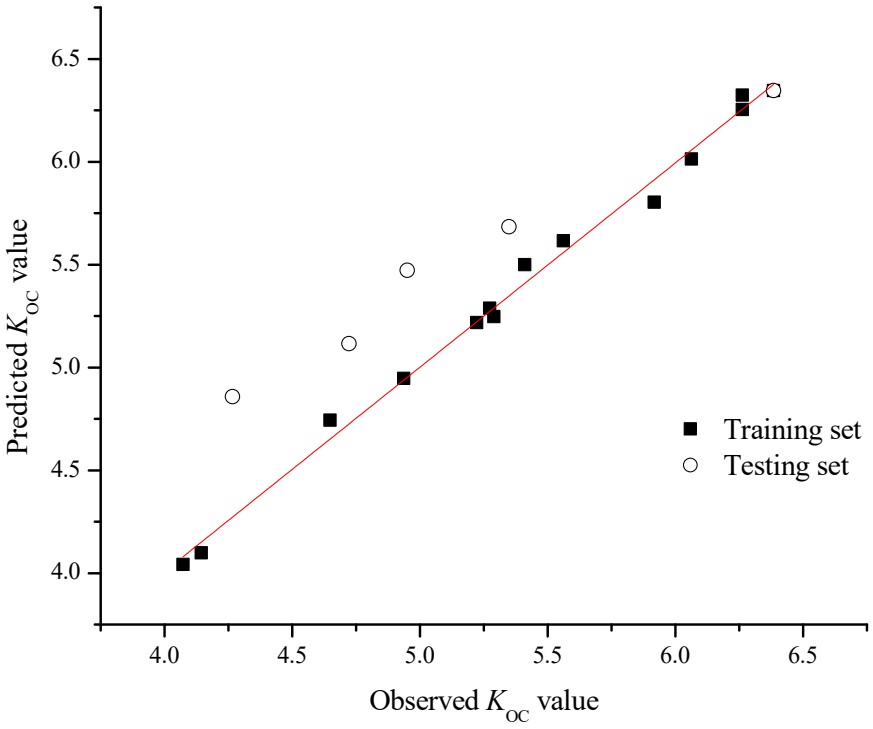


Figure 3.2: Model Predictability Plot

The final prediction results for all of the PBDE congeners can be found in Table 3.5. The common overall trend noticed within these values is that K_{OC} increases as the congener number increases. This means that a general rule can be applied stating that if a PBDE congener has a higher degree of bromination, its corresponding K_{OC} value will be higher as well. Thus, the more bromine atoms present in a given PBDE compound, the more immobile this compound will be within soil. This is more apparent as these compounds will have a stronger affinity towards the organic matter within a given soil type and location due to an increase in lipophilicity from additional halogen bonds. With additional bromine atoms present on the molecule structure, more lone pairs of electrons are able to interact with the organic matter in soil through induced dipole interactions. This trend of noticing a relationship between increased soil affinity and an increase in the degree of bromination has also been observed in studies such as (Nyholm et al., 2010; Venkatesan and Halden, 2014; O'Driscoll et al., 2016). It may also be noted that the increase in K_{OC} value does not consistently increase from one congener to the next. Although the nomenclature of PBDEs does extend the naming of these compounds from little bromine atoms present to a fully brominated compound, the systems does not name one congener to the next with respect to the quantity of bromine atoms only. Rather, this system considers quantity of bromines present first as well as the exact position these bromine atoms are bonded to second. With this into consideration it is important to realize that the K_{OC} value typically does increase through the congener list on an overall trend, but other factors are present to which the K_{OC} from one compound to another can fluctuate.

Table 3.4: 3D-QSAR Model Prediction Comparison

Congener	Chemical Name	Obs.	CoMFA	
			Pred.	Relative Error (%)
BDE-15	4,4'-dibromodiphenyl ether	4.14	4.098	0.010
BDE-17	2,2',4-tribromodiphenyl ether	4.07	4.041	0.007
BDE-28	2,4,4'-tribromodiphenyl ether	4.27	4.858	0.138
BDE-47	2,2'4,4'-tetrabromodiphenyl ether	4.63	4.319	0.067
BDE-66	2,3',4,4'-tetrabromodiphenyl ether	4.72	5.115	0.084
BDE-77	3,3',4,4'-tetrabromodiphenyl ether	4.65	4.743	0.020
BDE-85	2,2',3,4,4'-pentabromodiphenyl ether	4.94	4.946	0.001
BDE-99	2,2',4,4',5-pentabromodiphenyl ether	4.95	5.472	0.105
BDE-100	2,2',4,4',6-pentabromodiphenyl ether	4.91	4.900	0.002
BDE-126	3,3',4,4',5-pentabromodiphenyl ether	5.27	5.287	0.003
BDE-138	2,2',3,4,4',5'-hexabromodiphenyl ether	5.35	5.683	0.062
BDE-153	2,2',4,4',5,5'-hexabromodiphenyl ether	5.29	5.247	0.008
BDE-154	2,2',4,4',5,6'-hexabromodiphenyl ether	5.22	5.218	0.0004
BDE-166	2,3,4,4',5,6-hexabromodiphenyl ether	5.41	5.499	0.016
BDE-183	2,2',3,4,4',5',6-heptabromodiphenyl ether	5.56	5.615	0.010
BDE-190	2,3,3',4,4',5,6-heptabromodiphenyl ether	5.92	5.803	0.020
BDE-196	2,2',3,3',4,4',5,6'-octabromodiphenyl ether	6.06	6.013	0.008
BDE-204	2,2',3,4,4',5,6,6'-octabromodiphenyl ether	6.05	5.266	0.130
BDE-207	2,2',3,3',4,4',5,6,6'-nonabromodiphenyl ether	6.26	6.253	0.001
BDE-208	2,2',3,3',4,5,5',6,6'-nonabromodiphenyl ether	6.26	6.324	0.010
BDE-209	Decabromodiphenyl ether	6.38	6.345	0.005

Table 3.5: Predicted logK_{OC} Values

Congener	IUPAC Name	CoMFA
BDE-1	2-monobromodiphenyl ether	4.468
BDE-2	3-monobromodiphenyl ether	4.667
BDE-3	4-monobromodiphenyl ether	3.768
BDE-4	2,2'-dibromodiphenyl ether	3.911
BDE-5	2,3-dibromodiphenyl ether	5.126
BDE-6	2,3'-dibromodiphenyl ether	4.743
BDE-7	2,4-dibromodiphenyl ether	4.738
BDE-8	2,4'-dibromodiphenyl ether	4.590
BDE-9	2,5-dibromodiphenyl ether	4.879
BDE-10	2,6-dibromodiphenyl ether	4.633
BDE-11	3,3'-dibromodiphenyl ether	4.363
BDE-12	3,4-dibromodiphenyl ether	4.431
BDE-13	3,4'-dibromodiphenyl ether	4.740
BDE-14	3,5-dibromodiphenyl ether	4.617
BDE-15	4,4'-dibromodiphenyl ether	4.098
BDE-16	2,2',3-tribromodiphenyl ether	5.180
BDE-17	2,2',4-tribromodiphenyl ether	4.041
BDE-18	2,2',5-tribromodiphenyl ether	5.113
BDE-19	2,2',6-tribromodiphenyl ether	4.539
BDE-20	2,3,3'-tribromodiphenyl ether	4.803
BDE-21	2,3,4-tribromodiphenyl ether	5.272
BDE-22	2,3,4'-tribromodiphenyl ether	4.649
BDE-23	2,3,5-tribromodiphenyl ether	5.399
BDE-24	2,3,6-tribromodiphenyl ether	4.701
BDE-25	2,3',4-tribromodiphenyl ether	5.014
BDE-26	2,3',5-tribromodiphenyl ether	4.915
BDE-27	2,3',6-tribromodiphenyl ether	4.924
BDE-28	2,4,4'-tribromodiphenyl ether	4.858
BDE-29	2,4,5-tribromodiphenyl ether	5.046
BDE-30	2,4,6-tribromodiphenyl ether	4.776
BDE-31	2,4',5-tribromodiphenyl ether	5.133
BDE-32	2,4',6-tribromodiphenyl ether	4.863
BDE-33	2,3',4'-tribromodiphenyl ether	4.905
BDE-34	2,3',5'-tribromodiphenyl ether	5.102
BDE-35	3,3',4-tribromodiphenyl ether	4.508
BDE-36	3,3',5-tribromodiphenyl ether	4.093
BDE-37	3,4,4'-tribromodiphenyl ether	4.656
BDE-38	3,4,5-tribromodiphenyl ether	4.729
BDE-39	3,4',5-tribromodiphenyl ether	4.108
BDE-40	2,2',3,3'-tetrabromodiphenyl ether	5.351
BDE-41	2,2',3,4-tetrabromodiphenyl ether	5.364
BDE-42	2,2',3,4'-tetrabromodiphenyl ether	4.383
BDE-43	2,2',3,5-tetrabromodiphenyl ether	5.621
BDE-44	2,2',3,5'-tetrabromodiphenyl ether	4.908
BDE-45	2,2',3,6-tetrabromodiphenyl ether	4.605
BDE-46	2,2',3,6'-tetrabromodiphenyl ether	4.987
BDE-47	2,2'4,4'-tetrabromodiphenyl ether	4.319
BDE-48	2,2',4,5-tetrabromodiphenyl ether	5.242
BDE-49	2,2',4,5'-tetrabromodiphenyl ether	5.344
BDE-50	2,2',4,6-tetrabromodiphenyl ether	4.682
BDE-51	2,2',4,6'-tetrabromodiphenyl ether	4.762
BDE-52	2,2',5,5'-tetrabromodiphenyl ether	5.197
BDE-53	2,2',5,6'-tetrabromodiphenyl ether	4.837
BDE-54	2,2',6,6'-tetrabromodiphenyl ether	4.251
BDE-55	2,3,3',4-tetrabromodiphenyl ether	5.313
BDE-56	2,3,3',4'-tetrabromodiphenyl ether	4.906
BDE-57	2,3,3',5-tetrabromodiphenyl ether	5.436
BDE-58	2,3,3',5'-tetrabromodiphenyl ether	4.945
BDE-59	2,3,3',6-tetrabromodiphenyl ether	5.331
BDE-60	2,3,4,4'-tetrabromodiphenyl ether	5.051
BDE-61	2,3,4,5-tetrabromodiphenyl ether	5.189
BDE-62	2,3,4,6-tetrabromodiphenyl ether	5.184
BDE-63	2,3,4',5-tetrabromodiphenyl ether	5.130
BDE-64	2,3,4',6-tetrabromodiphenyl ether	5.276
BDE-65	2,3,5,6-tetrabromodiphenyl ether	5.110
BDE-66	2,3',4,4'-tetrabromodiphenyl ether	5.115
BDE-67	2,3',4,5-tetrabromodiphenyl ether	5.083
BDE-68	2,3',4,5'-tetrabromodiphenyl ether	5.311
BDE-69	2,3',4,6-tetrabromodiphenyl ether	5.065
BDE-70	2,3',4',5-tetrabromodiphenyl ether	5.335
BDE-71	2,3',4',6-tetrabromodiphenyl ether	5.169
BDE-72	2,3',5,5'-tetrabromodiphenyl ether	5.191
BDE-73	2,3',5',6-tetrabromodiphenyl ether	5.334
BDE-74	2,4,4',5-tetrabromodiphenyl ether	5.248
BDE-75	2,4,4',6-tetrabromodiphenyl ether	5.007
BDE-76	2,3',4',5'-tetrabromodiphenyl ether	5.197
BDE-77	3,3',4,4'-tetrabromodiphenyl ether	4.743
BDE-78	3,3',4,5-tetrabromodiphenyl ether	4.806
BDE-79	3,3',4,5'-tetrabromodiphenyl ether	4.140
BDE-80	3,3',5,5'-tetrabromodiphenyl ether	5.029
BDE-81	3,4,4',5-tetrabromodiphenyl ether	4.418
BDE-82	2,2',3,3',4-pentabromodiphenyl ether	5.535
BDE-83	2,2',3,3',5-pentabromodiphenyl ether	5.524
BDE-84	2,2',3,3',6-pentabromodiphenyl ether	5.047
BDE-85	2,2',3,4,4'-pentabromodiphenyl ether	4.946
BDE-86	2,2',3,4,5-pentabromodiphenyl ether	5.740
BDE-87	2,2',3,4,5'-pentabromodiphenyl ether	5.526
BDE-88	2,2',3,4,6-pentabromodiphenyl ether	5.074
BDE-89	2,2',3,4,6'-pentabromodiphenyl ether	5.180
BDE-90	2,2',3,4',5-pentabromodiphenyl ether	5.852
BDE-91	2,2',3,4',6-pentabromodiphenyl ether	5.160
BDE-92	2,2',3,5,5'-pentabromodiphenyl ether	5.557
BDE-93	2,2',3,5,6-pentabromodiphenyl ether	5.000
BDE-94	2,2',3,5,6'-pentabromodiphenyl ether	5.281
BDE-95	2,2',3,5',6-pentabromodiphenyl ether	5.235
BDE-96	2,2',3,6,6'-pentabromodiphenyl ether	5.289
BDE-97	2,2',3,4',5'-pentabromodiphenyl ether	5.419
BDE-98	2,2',3,4',6'-pentabromodiphenyl ether	5.122
BDE-99	2,2',4,4',5-pentabromodiphenyl ether	5.472
BDE-100	2,2',4,4',6-pentabromodiphenyl ether	4.900
BDE-101	2,2',4,5,5'-pentabromodiphenyl ether	5.055
BDE-102	2,2',4,5,6'-pentabromodiphenyl ether	5.079
BDE-103	2,2',4,5',6-pentabromodiphenyl ether	4.975

BDE-104	2,2',4,6,6'-pentabromodiphenyl ether	4.369	BDE-157	2,3,3',4,4',5'-hexabromodiphenyl ether	5.624
BDE-105	2,3,3',4,4'-pentabromodiphenyl ether	5.304	BDE-158	2,3,3',4,4',6-hexabromodiphenyl ether	5.721
BDE-106	2,3,3',4,5-pentabromodiphenyl ether	5.578	BDE-159	2,3,3',4,5,5'-hexabromodiphenyl ether	5.844
BDE-107	2,3,3',4',5-pentabromodiphenyl ether	5.385	BDE-160	2,3,3',4,5,6-hexabromodiphenyl ether	5.555
BDE-108	2,3,3',4,5'-pentabromodiphenyl ether	5.359	BDE-161	2,3,3',4,5',6-hexabromodiphenyl ether	5.881
BDE-109	2,3,3',4,6-pentabromodiphenyl ether	5.144	BDE-162	2,3,3',4',5,5'-hexabromodiphenyl ether	5.504
BDE-110	2,3,3',4',6-pentabromodiphenyl ether	5.235	BDE-163	2,3,3',4',5,6-hexabromodiphenyl ether	5.646
BDE-111	2,3,3',5,5'-pentabromodiphenyl ether	5.705	BDE-164	2,3,3',4',5',6-hexabromodiphenyl ether	5.979
BDE-112	2,3,3',5,6-pentabromodiphenyl ether	5.398	BDE-165	2,3,3',5,5',6-hexabromodiphenyl ether	5.808
BDE-113	2,3,3',5',6-pentabromodiphenyl ether	5.399	BDE-166	2,3,4,4',5,6-hexabromodiphenyl ether	5.499
BDE-114	2,3,4,4',5-pentabromodiphenyl ether	5.313	BDE-167	2,3',4,4',5,5'-hexabromodiphenyl ether	5.624
BDE-115	2,3,4,4',6-pentabromodiphenyl ether	5.085	BDE-168	2,3',4,4',5',6-hexabromodiphenyl ether	5.710
BDE-116	2,3,4,5,6-pentabromodiphenyl ether	5.267	BDE-169	3,3',4,4',5,5'-hexabromodiphenyl ether	4.480
BDE-117	2,3,4',5,6-pentabromodiphenyl ether	5.341	BDE-170	2,2',3,3',4,4',5-heptabromodiphenyl ether	5.896
BDE-118	2,3',4,4',5-pentabromodiphenyl ether	5.505	BDE-171	2,2',3,3',4,4',6-heptabromodiphenyl ether	5.705
BDE-119	2,3',4,4',6-pentabromodiphenyl ether	5.313	BDE-172	2,2',3,3',4,5,5'-heptabromodiphenyl ether	6.045
BDE-120	2,3',4,5,5'-pentabromodiphenyl ether	5.545	BDE-173	2,2',3,3',4,5,6-heptabromodiphenyl ether	5.587
BDE-121	2,3',4,5',6-pentabromodiphenyl ether	5.477	BDE-174	2,2',3,3',4,5,6'-heptabromodiphenyl ether	5.543
BDE-122	2,3,3',4',5'-pentabromodiphenyl ether	5.410	BDE-175	2,2',3,3',4,5',6-heptabromodiphenyl ether	5.487
BDE-123	2,3',4,4',5'-pentabromodiphenyl ether	5.408	BDE-176	2,2',3,3',4,6,6'-heptabromodiphenyl ether	5.340
BDE-124	2,3',4',5,5'-pentabromodiphenyl ether	5.452	BDE-177	2,2',3,3',4,5',6'-heptabromodiphenyl ether	5.633
BDE-125	2,3',4',5',6-pentabromodiphenyl ether	5.567	BDE-178	2,2',3,3',5,5',6-heptabromodiphenyl ether	5.734
BDE-126	3,3',4,4',5-pentabromodiphenyl ether	5.287	BDE-179	2,2',3,3',5,6,6'-heptabromodiphenyl ether	5.758
BDE-127	3,3',4,5,5'-pentabromodiphenyl ether	5.140	BDE-180	2,2',3,4,4',5,5'-heptabromodiphenyl ether	6.055
BDE-128	2,2',3,3',4,4'-hexabromodiphenyl ether	5.768	BDE-181	2,2',3,4,4',5,6-heptabromodiphenyl ether	5.373
BDE-129	2,2',3,3',4,5-hexabromodiphenyl ether	5.665	BDE-182	2,2',3,4,4',5,6'-heptabromodiphenyl ether	5.621
BDE-130	2,2',3,3',4,5'-hexabromodiphenyl ether	5.755	BDE-183	2,2',3,4,4',5',6-heptabromodiphenyl ether	5.615
BDE-131	2,2',3,3',4,6-hexabromodiphenyl ether	5.509	BDE-184	2,2',3,4,4',6,6'-heptabromodiphenyl ether	5.296
BDE-132	2,2',3,3',4,6'-hexabromodiphenyl ether	5.242	BDE-185	2,2',3,4,5,5',6-heptabromodiphenyl ether	5.448
BDE-133	2,2',3,3',5,5'-hexabromodiphenyl ether	5.898	BDE-186	2,2',3,4,5,6,6'-heptabromodiphenyl ether	5.154
BDE-134	2,2',3,3',5,6-hexabromodiphenyl ether	5.438	BDE-187	2,2',3,4',5,5',6-heptabromodiphenyl ether	5.538
BDE-135	2,2',3,3',5,6'-hexabromodiphenyl ether	5.674	BDE-188	2,2',3,4',5,6,6'-heptabromodiphenyl ether	5.713
BDE-136	2,2',3,3',6,6'-hexabromodiphenyl ether	5.184	BDE-189	2,3,3',4,4',5,5'-heptabromodiphenyl ether	5.686
BDE-137	2,2',3,4,4',5-hexabromodiphenyl ether	5.971	BDE-190	2,3,3',4,4',5,6-heptabromodiphenyl ether	5.803
BDE-138	2,2',3,4,4',5'-hexabromodiphenyl ether	5.683	BDE-191	2,3,3',4,4',5',6-heptabromodiphenyl ether	6.120
BDE-139	2,2',3,4,4',6-hexabromodiphenyl ether	4.974	BDE-192	2,3,3',4,5,5',6-heptabromodiphenyl ether	5.966
BDE-140	2,2',3,4,4',6'-hexabromodiphenyl ether	5.318	BDE-193	2,3,3',4',5,5',6-heptabromodiphenyl ether	6.042
BDE-141	2,2',3,4,5,5'-hexabromodiphenyl ether	5.703	BDE-194	2,2',3,3',4,4',5,5'-octabromodiphenyl ether	6.219
BDE-142	2,2',3,4,5,6-hexabromodiphenyl ether	5.151	BDE-195	2,2',3,3',4,4',5,6-octabromodiphenyl ether	5.783
BDE-143	2,2',3,4,5,6'-hexabromodiphenyl ether	5.487	BDE-196	2,2',3,3',4,4',5,6'-octabromodiphenyl ether	6.013
BDE-144	2,2',3,4,5',6-hexabromodiphenyl ether	5.370	BDE-197	2,2',3,3',4,4',6,6'-octabromodiphenyl ether	5.879
BDE-145	2,2',3,4,6,6'-hexabromodiphenyl ether	4.994	BDE-198	2,2',3,3',4,5,5',6-octabromodiphenyl ether	5.885
BDE-146	2,2',3,4',5,5'-hexabromodiphenyl ether	5.381	BDE-199	2,2',3,3',4,5,5',6'-octabromodiphenyl ether	5.936
BDE-147	2,2',3,4',5,6-hexabromodiphenyl ether	5.222	BDE-200	2,2',3,3',4,5,6,6'-octabromodiphenyl ether	6.012
BDE-148	2,2',3,4',5,6'-hexabromodiphenyl ether	5.417	BDE-201	2,2',3,3',4,5',6,6'-octabromodiphenyl ether	6.129
BDE-149	2,2',3,4',5',6-hexabromodiphenyl ether	5.477	BDE-202	2,2',3,3',5,5',6,6'-octabromodiphenyl ether	6.185
BDE-150	2,2',3,4',6,6'-hexabromodiphenyl ether	4.706	BDE-203	2,2',3,4,4',5,5',6-octabromodiphenyl ether	5.691
BDE-151	2,2',3,5,5',6-hexabromodiphenyl ether	5.296	BDE-204	2,2',3,4,4',5,6,6'-octabromodiphenyl ether	5.266
BDE-152	2,2',3,5,6,6'-hexabromodiphenyl ether	5.412	BDE-205	2,3,3',4,4',5,5',6-octabromodiphenyl ether	6.202
BDE-153	2,2',4,4',5,5'-hexabromodiphenyl ether	5.247	BDE-206	2,2',3,3',4,4',5,5',6-nonabromodiphenyl ether	6.086
BDE-154	2,2',4,4',5,6'-hexabromodiphenyl ether	5.218	BDE-207	2,2',3,3',4,4',5,6,6'-nonabromodiphenyl ether	6.253
BDE-155	2,2',4,4',6,6'-hexabromodiphenyl ether	4.670	BDE-208	2,2',3,3',4,5,5',6,6'-nonabromodiphenyl ether	6.324
BDE-156	2,3,3',4,4',5-hexabromodiphenyl ether	5.569	BDE-209	Decabromodiphenyl ether	6.345

3.2.3 CoMFA Contour Map Analysis

To add further insight and understanding of the PBDE congener compounds, a contour map analysis can be discussed to address specific regions of the general compound in order to determine which characteristics these regions produce for the overall adsorption behavior of any PBDE compound. The contour map produced by the SYBYL-X software can be found in Figure 3.3. The map image uses the target molecule, BDE-209, as a representation of the contours present for the PBDE CoMFA fields since this compound holds the highest K_{OC} value (Gu et al., 2019). While the analyses of a CoMFA model determine steric and electrostatic fields associated with the given input information, this model produced activities which only involved the steric field with no contributions from electrostatics. As seen in Figure 3.3, the PBDE compound is surrounded by yellow and green contours which represent unfavorable and favorable activities respectively. As positions 2, 4, 6, 2', and 6' are completely surrounded by yellow contours, this means the introduction of bulky groups to these positions may decrease the overall K_{OC} value of the compound as a general estimating guideline. While the remaining positions are either in close proximity to green contours or no contours, the introduction of bulky groups here would either increase K_{OC} or produce no change, which is not desired in this case. With the understanding that bromine atoms are the only bulky group available in the situation for PBDEs, the placement of these atoms within the compound can make large difference in K_{OC} and thus how well they adsorb to solid organic matter. It is desirable and ideal to work with PBDE compounds with bromine atoms bonded at sites surrounded by yellow contours for remediation efforts. While this is not always possible, it can be understood that PBDEs with bromine atoms bonded at other sites may be more difficult to deal with.

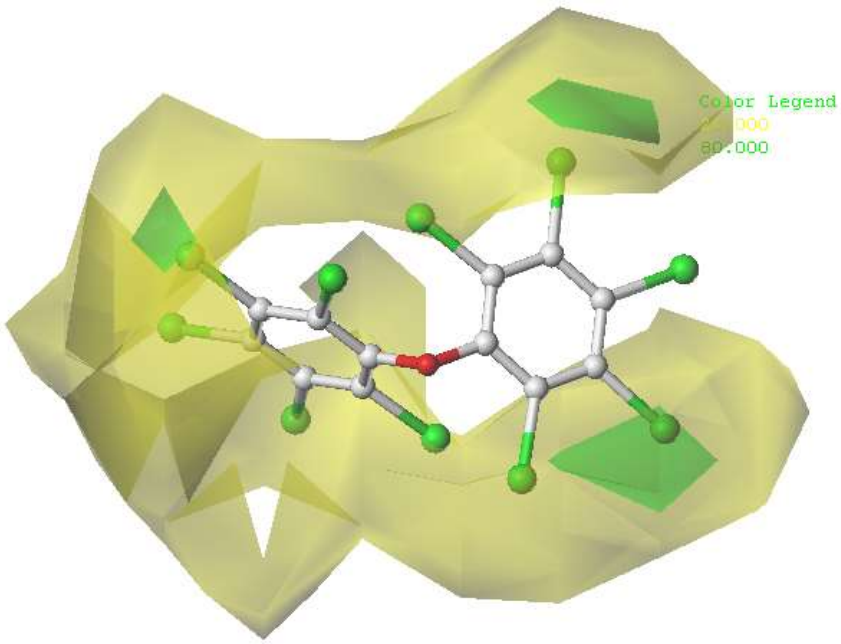


Figure 3.3: CoMFA Contour Map of Steric Field Contributions

3.2.4 Molecule Site Substitution Analysis

Using a contour map analysis to display which regions of the compound are more prone to changing the K_{OC} value is a very useful tool, although it can be considered a general estimation of the entire molecule. To further the understanding of the K_{OC} values from congener to congener, the bonding positions of the bromine atoms are still a key component. In order to fully understand which specific bonding sites may have significant effects on a PBDEs K_{OC} value, a resolution V fractional factorial design is used, as previously mentioned in section 3.2.4. Through the consideration of the bonding site information displayed in Table 3.2, the DOE style results can be found in Figure 3.4 below. As seen on the Normal Plot of the Standardized Effects, all of the main effects provide significance towards the increasing of the K_{OC} value. However, some of the second-order effects also show there is some significance when two bonding sites are specifically in use with bromine atoms together. Most notably, main factors B, D, G, and J are highly positive significant effects, meaning that when bromine is bonded at any four of the meta substitution sites, the K_{OC} value of the compound will be greater.

This phenomenon can be approximately seen visually through the contours in Figure 3.3. Notice that favorable green contours are located in proximity to the meta sites of the compound. Noting that there is very limited published information on this exact relationship, a similar study conducted by Gu et al. (2019) focused on environmentally friendly derivatives of polychlorinated naphthalenes (PCNs) while using a similar tool set to develop 3D-QSAR contour mapping.

This study had shown that the introduction of bulky groups to positions in resemblance to meta-bromine sites on PBDE congeners would also increase their target parameter with respect to their chosen PCN derivative molecule. In addition, eight of the thirteen second-order effects produce negative significance. For instance, as the most negatively significant interaction, factors A and F interact with each other in an interesting way. The bromine atom at site A (ortho-2) causes the K_{OC} to decrease if another bromine atom is present at site F (ortho-2'). It may be of interest to note that the most negatively significant second-order effects are all combinations of bromine atoms being present at ortho substitution sites.

The main effects plot as well as the second-order effect interactions plot can be found in Figure 3.5 and Figure 3.6 respectfully below and provides visual explanation for this phenomenon. While these statements are not concrete rules to determine the K_{OC} value of a congener, they are something to consider. As shown in Figure 3.4, the presence of a bromine atom at any of the bonding sites will increase the K_{OC} value along with some second-order interactions. However, as long as a congener contains bromine atoms at sites that represent second-order interactions to the left of the baseline, there will be a tendency for this congener to have a drop of K_{OC} in comparison to other closely named congeners. Thus, when approaching remediation efforts involving soil polluted with PBDE compounds, understanding that the congeners with bromine atoms at ortho substitution sites as well as a select few other substitution sites, will become the congeners removed from the system with the most ease.

In addition to this information discussed with respect to Figures 3.4, 3.5, and 3.6, the fractional factorial model is also able to produce a regression equation in uncoded units to describe effect significance numerically. The regression equation along with the fractional factorial design input data can be found within Appendix B.

It may be of interest to note that the statistical parameters for this model are also given through the analysis software. Since the provided statistics state that the R^2 is approximately 80% and since the R^2_{adj} and the R^2_{pred} are within close proximity to each other, this fractional factorial design is valid.

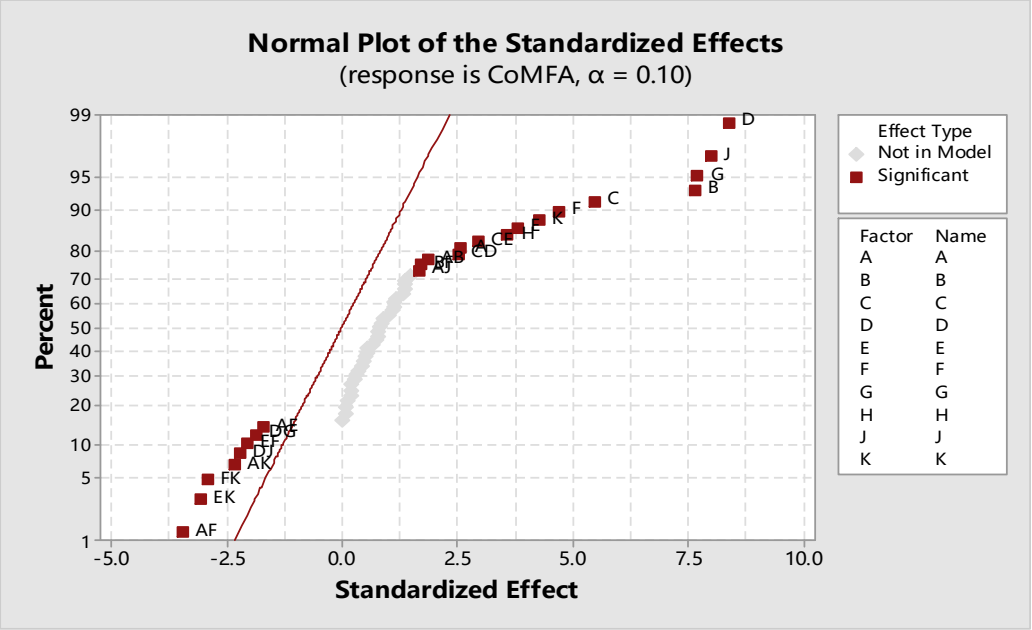


Figure 3.4: Resulting Effect Significance from the Fractional Factorial Design

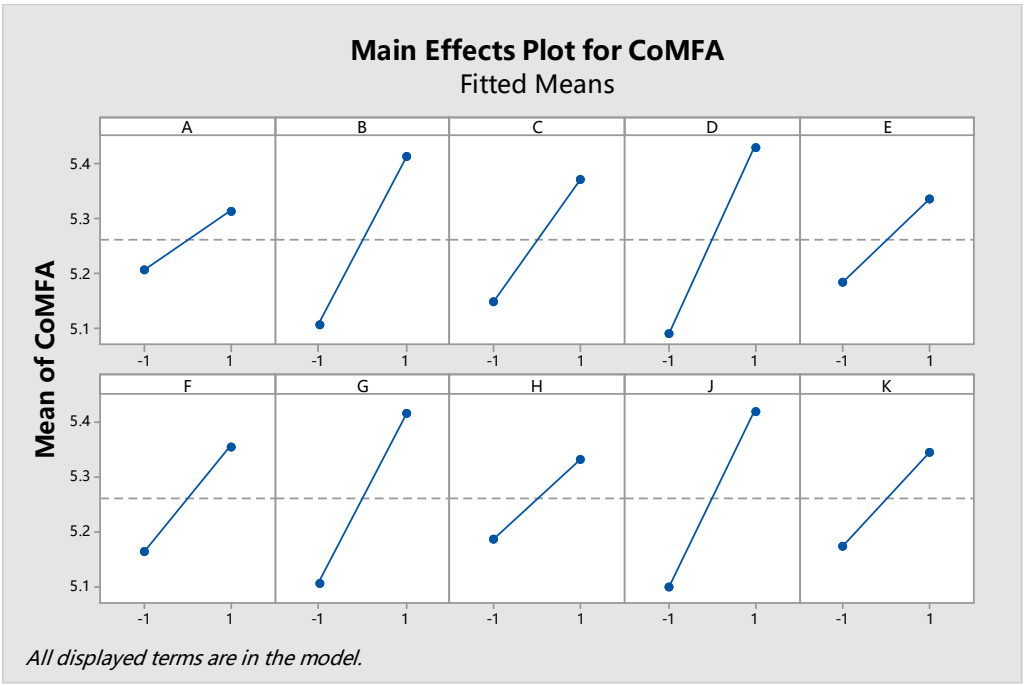


Figure 3.5: Main Effects Plot for CoMFA Koc Predictions. This plot represents the variance of each main effect between a hydrogen atom present (-1) and a bromine atom present (1)

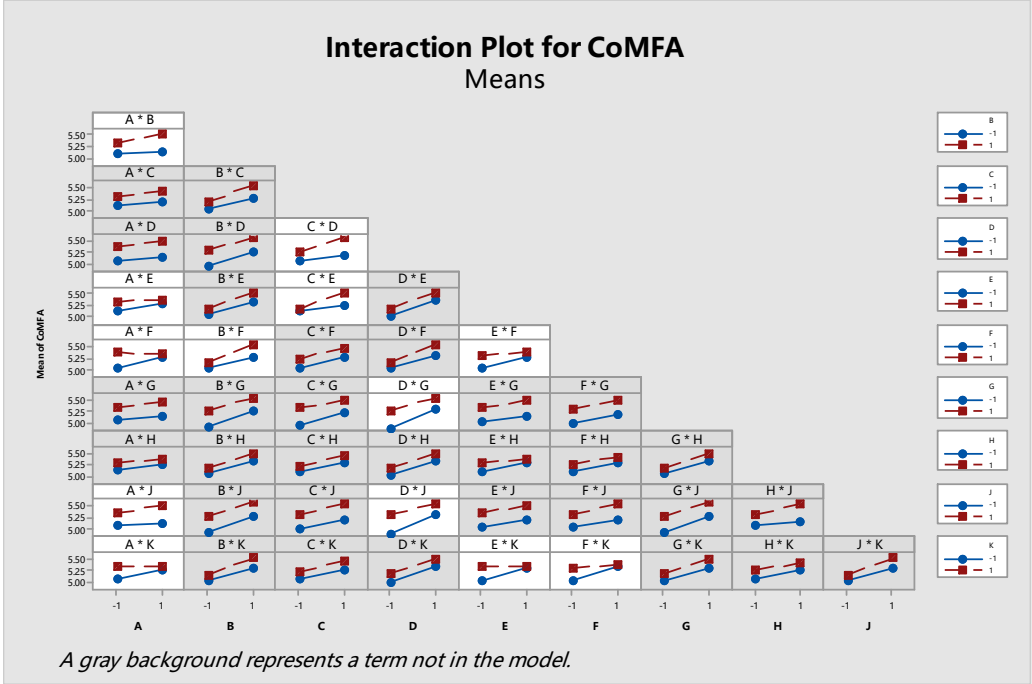


Figure 3.6: Interaction Plots of Main Effects. The significant interactions are highlighted in white cells, while -1 and 1 are associated with hydrogen and bromine bonds respectively

3.3 Summary

With the information presented here, it has been shown through the works of other studies that PBDEs do have a strong affinity towards the organic-matter located within natural solid-phase materials such as soils in contact with water. As an emerging contaminant, there is still a large portion of information regarding PBDEs that is still unknown. When addressing the fate and transport of these chemicals after their time used within consumer goods, relatively generalized statements about their behavior exist. It is known that PBDEs are hydrophobic organohalide compounds that pollute soil through their strong attraction to solid-phase materials and resistance to remediation. However, the in-depth analysis completed in this study provides additional context to understanding the exact adsorption behaviors of all 209 congeners through the use of an organic-carbon partition coefficient, or $K_{OC,}$ within a soil medium. By using 3D-QSAR techniques, a model had been created to determine the $\log K_{OC}$ values for each congener based on the limited information available regarding a handful of common PBDE congeners. The resulting information had shown that a PBDE congener will have a greater K_{OC} value with respect to a greater amount of bromine atoms present. The quantity of bromine atoms within the compound are not the only factor determining the intensity of the K_{OC} value. Additional inquiry regarding the effects of specific bromine bonding sites on the compound with respect to the significance of a compounds K_{OC} value was thus performed. It had been determined that a bromine atom at any of the bonding sites would increase K_{OC}, and some second-order interactions exist that state if two bromine atoms are present at two specific bonding sites, which may possibly decrease the K_{OC} value with relative respect to the degree of bromination.

Understanding the K_{OC} values for each PBDE congener is useful information in order to properly assess and implement environmental soil remediation techniques that may involve multiple PBDE congeners that are present. This is especially so when considering that most available information regarding PBDE behavior in a soil medium is limited to only the common congeners that make-up most of the commercial PBDE brominated flame retardant products. Through providing K_{OC} information about every PBDE congener, it is anticipated that soil remediation techniques and technologies can account for PBDE adsorption tendencies with a greater amount of simplicity, and thus create situations where PBDE contaminated soils are not regarded as complex problems.

CHAPTER 4

ANALYSIS OF DEBROMINATION CHARACTERISTICS OF PBDEs THROUGH ENZYMATIC MOLECULAR DOCKING

4.1 Research Goal and Methodology

The main purpose of this study is to determine and analyze information regarding the debromination process of PBDEs in a soil setting through three main objectives. The first is the identification of the potential enzymes for facilitating PBDE biodegradation through comparing selected enzymes on their interaction performance with respect to a place-holder PBDE congener. The second objective is the investigation of the biodegradation of PBDE congeners through comparing their performance against the best suited enzyme

4.1.1 Molecular Docking Procedure

The method of molecular docking used in this study was limited to the use of the CHARMM-based DOCKER (CDOCKER) which is an approach to molecular docking revolving around the principles of Chemistry at Harvard Macromolecular Mechanics (CHARMM) which is a computer program tasked with modelling macromolecular systems through the use of empirical energy functions (Brooks et al., 1983). Traditionally, the approach to docking a ligand to an active site on a protein would subject and limit the interactions to the rigidity of both the protein and the ligand geometry. Using the CDOCKER approach, the ligands are able to become flexible in the process, enabling the fit between an active site and ligand to become more realistic and representative of the snug fit that often occurs when a ligand interacts with the amino acid residues found within the active site. In addition to offering full ligand flexibility, CDOCKER also offers analysis with the flexibility of the CHARMM engine as well as a family of force fields incorporated into the method to maximize accuracy. Finally, CDOCKER is also extremely useful in its purpose as the computational times are considered to be suitable (Wu et al., 2003; Padariya et al., 2014; Mohan et al., 2015).

Generally speaking, the process in which CDOCKER performs is to produce randomized conformations of the given ligands at the active site using molecular dynamics based calculations. Each of the produced conformations is then handled using molecular dynamics at a high temperature based on a CHARMM variant. From this point, the conformation structures determined from the molecular dynamic calculations are located within the study area minimized. The ligands are then optimized into position within the active site using ridged body rotations which is then followed by simulated annealing. Lastly, a second annealing process is carried out to refine the conformations using a grid-based technique and a final full force field minimization takes place. (Padariya et al., 2014; Öztürk et al., 2017).

The molecular docking process for the selected enzymes and PBDE congeners suited to be ligands in this study was performed through the Discovery Studio 4.0 software. The process in which the docking was initiated reflects the existing research methods of (Gu et al., 2019; Holt et al., 2008; Yang et al., 2008; Gu et al., 2020). The resulting information from the docking analysis considers the value of the root mean square deviation (RMSD) of the ligand molecules once they have docking to the active site. The computational limitations are able to simulate the binding mode of the enzyme-ligand complex when the RMSD is in range of 2Å, or not less than 0.2 nm. This then illustrates and proves the rationale of the original software settings and thus the reliability of the results (Ewing et al., 2001; Gu et al., 2020).

4.1.2 Ligand Preparation

The PBDE congeners that are used as the ligands in this study represent the substrate material that will be given the opportunity to interact with the soil enzymes. Specifically speaking, while there are 209 unique PBDE congeners, only 8 are given focus.

These congeners include BDE-15, -28, -47, -99, -153, -183, -207, and -209. The selection process for choosing these congeners over any others has to do with their commonality and their high presence within commercial mixtures previously applied to consumer goods as well as for their representation of a wide variety regarding degrees of bromination. For instance, BDE-47 and BDE-99 account for 25 to 37% and 35 to 50% of the mass of commercial Penta-BDE mixtures respectfully, while BDE-153 and BDE-187 account for 5 to 10% and 40% of the mass of Octa-BDE respectfully and BDE-209 accounts for 97.5% of DecaBDE (Pohl et al., 2017; U.S. EPA, 2010). To further assist in the understanding of these specific congeners Table 4.1 and Figure 4.1 show additional information regarding these 8 selected congeners to be used as ligands. In order to successfully carry out the molecular docking procedures, information about the ligands that the software understand must be obtained. The 3D structural information of each of the selected congeners is retrieved from Pubchem and received as SDF files (United States National Library of Medicine, 2005). From this point the Discovery Studio software is capable of obtaining all the needed information from these files.

Table 4.1: Selected PBDE Congeners Used as Ligands

Congener	Chemical Name	Degree of Bromination
BDE-15	4,4'-dibromodiphenyl ether	2
BDE-28	2,4,4'-tribromodiphenyl ether	3
BDE-47	2,2',4,4'-tetrabromodiphenyl ether	4
BDE-99	2,2',4,4',5-pentabromodiphenyl ether	5
BDE-153	2,2',4,4',5,5'-hexabromodiphenyl ether	6
BDE-183	2,2',3,4,4',5',6-heptabromodiphenyl ether	7
BDE-207	2,2',3,3',4,4',5,6,6'-nonabromodiphenyl ether	9
BDE-209	Decabromodiphenyl ether	10

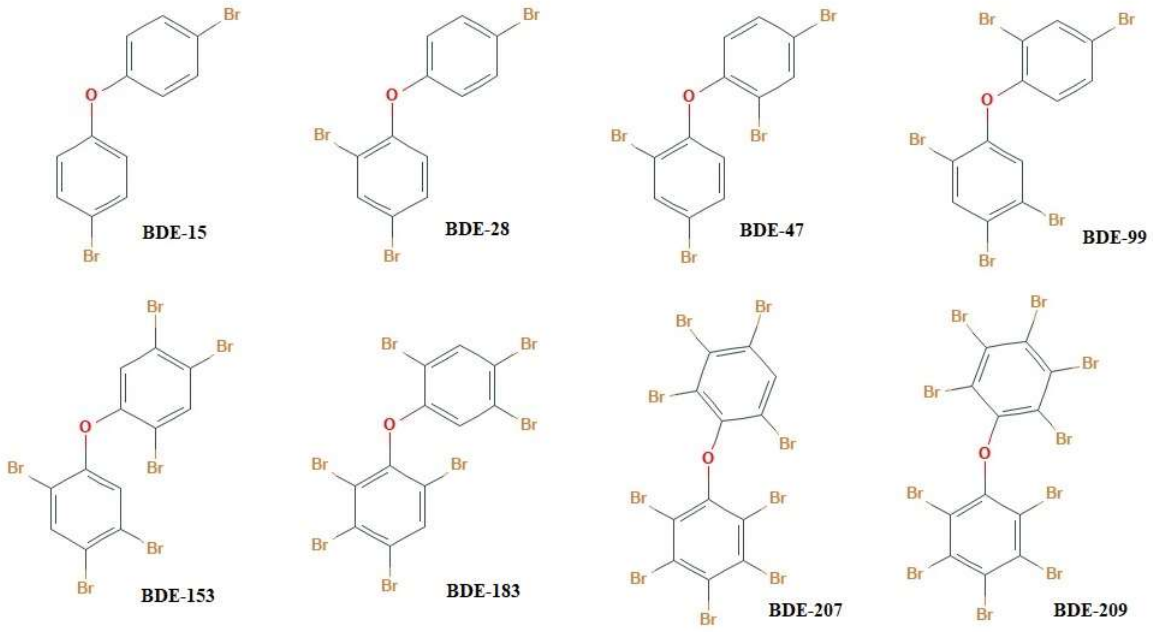


Figure 4.1: Chemical Structure of the Selected PBDE Congeners that Serve as Ligands

4.1.3 Enzyme Preparation

While understanding how enzymes are able to be used as a tool for progressing the biodegradation of pollutant chemicals is an important step, additional information is required to correctly choose enzymes that will potentially interact well with PBDEs successfully. As stated previously, the target goal of this study is to focus on the debromination of PBDEs located within a soil environment. Through extensive literature review, many enzymes were found to be studied for the decomposition of chemical pollutants in nature. However, three specific enzymes were noted and examined for the debromination of relevant brominated organic compounds in environments parallel to that of the focus media. Basic information regarding these enzymes was collected and considered form the Protein Data Bank (PDB).

The first is labelled as a hydrolytic haloalkane dehalogenase LinB (PDB ID: 1K5P) produced by the bacterial strain *Sphingomonas paucimobilis* UT26. This enzyme is created as a product of the mentioned bacteria; which exists within soil, water, and plant mediums (Berman et al., 2000; Streltsov et al., 2003; Ryan and Adley, 2010). The second target enzyme is a structure of the haloalkane dehalogenase mutant Dha15 (PDB ID: 3FWH) from the bacterial strain *Rhodococcus rhodochrous*; which can be found and isolated from contaminated soil mediums (Berman et al., 2000; Klvana et al., 2009; Larkin et al., 2010). Finally, the third target enzyme considered is a crystal structure of the Debrominase Bmp8 C82A in complex with 2, 3, 4-tribromopyrrole (PDB ID: 6OHJ) which is a biosynthetic protein from the bacterial strain *Marinomonas mediterranea MMB-1*. This bacteria is found and named after the seawater it has been isolated from (Berman et al., 2000; Chekan et al., 2019; Lucas-Elío et al., 2012).

The location and type of media these enzymes are found all consist or include a type of solid organic matter. In this case, 1K5P and 3FWH can be isolated from bacteria found in soil, while 6OHJ is found in seawater. While seawater itself is not a form of solid organic matter, PBDE molecules are known to persist in aquatic environments having an affinity towards suspended sediments. Figure 4.2a showcases illustrations of the overall macromolecule structure of these three selected enzymes while Figure 4.2b shows the detailed molecular structure displaying the atoms and bonds associated with the enzyme structure. Additional structure information about these enzymes as well as their sequencing can be found through the protein data bank.

In order for the computational experiments to run successfully, structural information about the enzyme macromolecules must be obtain in similar fashion to the ligands. To prepare the macromolecules, the structural information is downloaded from the Protein Data Bank and uploaded to Discovery Studio for interactive use. To find the most useful active site on each of the enzyme structures, the Discovery Studio software is able to rank these sites with the first listed as the best potential site for use. Although other sites are experimented with one by one, the first site listed was the selected site in the case for all three structures.

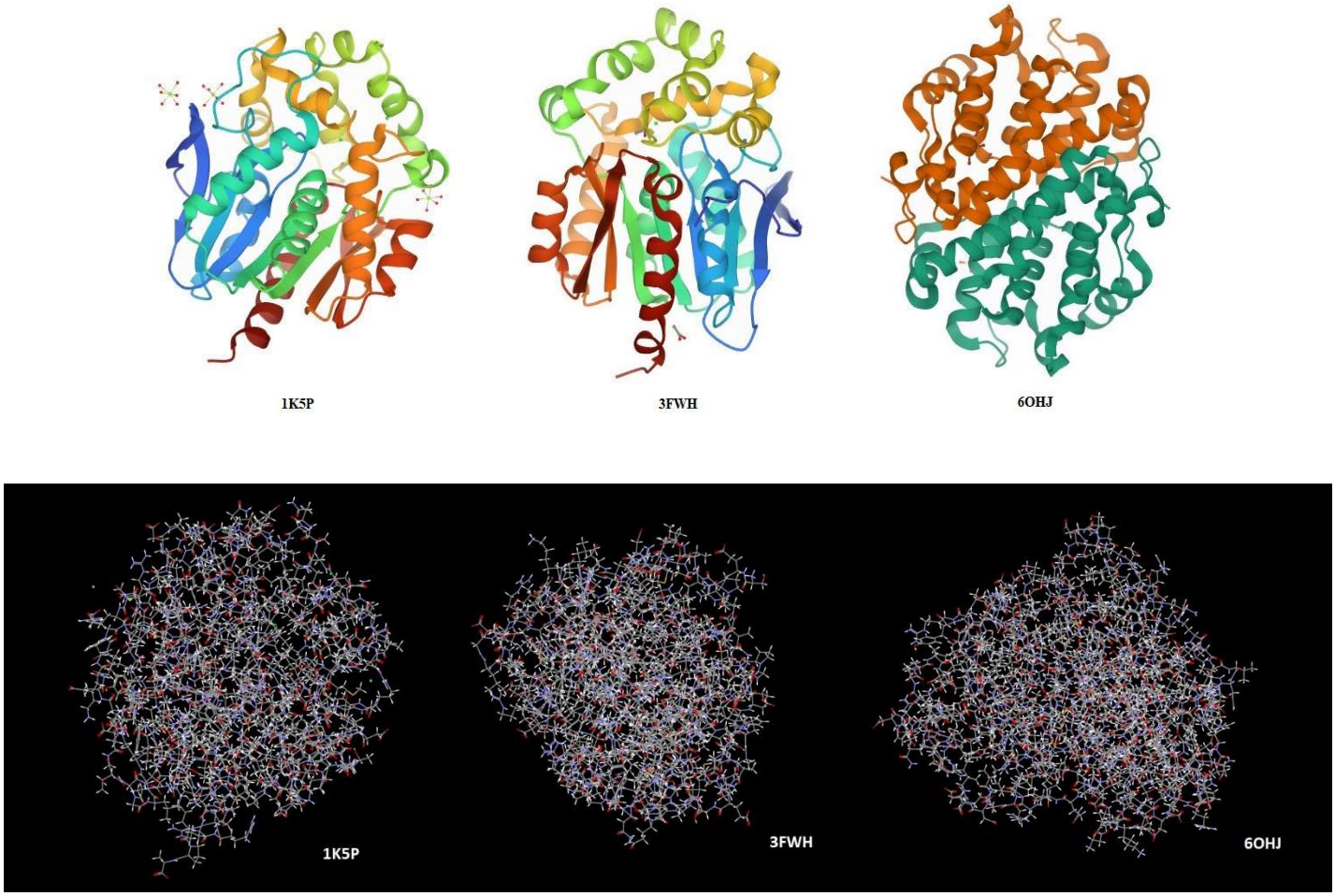


Figure 4.2: (a) Macromolecule and (b) Detailed Molecular Structures of Selected Enzymes

4.1.4 Results Analysis

The corresponding scoring method used to measure the success of an interaction is accounted for through two parameters, -CDOCKER Interaction Energy and –CDOCKER Energy. –CDOCKER Interaction Energy is simply the interaction energy present between the ligand and the amino acid residues in contact with each other. The interaction energy is expressed through the intermolecular forces that in turn describe the mechanism and affinity a ligand may have with the amino acid residues. In addition, -CDOCKER Energy is a summation energy value between the mentioned interaction energy as well as any strain energy that occurs within the ligand. Since the ligands are considered to be flexible in this docking method, any tensions or compressions that may cause the ligand to bend out of resting shape are accounted for (Rampogu and Rampogu-Lemuel, 2016; Parthasarathy and Ajay-Kumar, 2019). This is included in the energy scoring to account for the state in which a ligand fits into an active site during interactions. If a ligand experiences greater internal strain energy, it may be additionally difficult for the ligand to escape the active site. It is additionally important to note that while the parameters are named with a negative symbol preceding them, this indicates that the presented energy value is multiplied by a value of -1. This is done so to account for nomenclature in which bonding energies are understood. When bonds are broken, energy is applied to the system indicating positive energy. Oppositely, negative energy values signify that a bond forms. In the context of CDOCKER results, the creation of new bonds is desired, thus labelling negative energy values as positive. With that being said, the greater value of energy displayed in either parameter represents a greater success within the docking procedure. This would also be representative of a higher level of debromination and biodegradability.

4.2 Results and Discussion

4.2.1 Ranking of Enzymatic Debromination Performance

The interactions between the eight PBDE congeners constituted as ligands and the three enzyme structures are represented in Discovery Studio as unique conformations. That is, for each pairing of ligand and enzyme, the software generates as much as 10 positions in which the ligand may situate itself within the active site of the enzyme. In many cases however, it was determined that 10 individual and unique conformations were not always possible with every pairing, while some pairings had failed altogether. For pairings that have multiple successful interactions from different conformations, the software is able to rank these conformations in order from best to worst based on energy value probabilities for the pose at question. Therefore, the first conformation listed in the software is the chosen pose for each pairing and thus for further analysis as this pose will have the highest likelihood of success.

A sample ligand was chosen from the list of eight selected common PBDE congeners to evaluate the performance of the three selected enzymes. BDE-99 was chosen as the test ligand since it is a very commonly found and occurring congener. As previously mentioned, many studies conducting debromination analyses have a focus on the debromination pathways a fully brominated congener may take to becoming a completely debrominated diphenyl ether molecule. Many of these studies display that BDE-99 is a commonly achieved congener during this process. In addition, BDE-99 has a very high presence in many of the Penta-BDE mixtures that were once in use on commercial products and thus, this molecule is commonly occurring in nature. Lastly, within the list of selected congeners to be used as ligands, a wide variety of degree of bromination is displayed. Within this list, BDE-99 has the most average degree of bromination, allowing for it to be the best

representation of the selected congeners. In regards to the performance of the pairings between BDE-99 and the three selected enzymes, successful interactions were seen in each case.

As shown in Table 4.2, the 1K5P and 6OHJ enzymes produce resulting energy values that are negative, while 3FWH displays a positive results. While remembering that these values are multiplied by a value of -1, -CDOCKER Interaction Energy and –CDOCKER Energy results that are positive indicate a negative energy value with respect to the standard practice of energy in regards to intermolecular forces. While failures can occur in molecular docking, this represents the inability of the energy function algorithm to determine the predicted lowest energy a ligand conformation can obtain within the simulated complex (Verkhivker et al., 2000). Thus, while positive values indicate docking success from bonds forming, negative values are still representative of a successful docking, but this energy must be applied in order to create stable bonds.

The information shown in Figure 4.3 displays the exact mechanisms in which BDE-99 is interacting with these three enzymes. The 2D interaction diagrams show the structure of BDE-99 to clearly represent the ligand substrate used in these interactions while the remaining colored circles are representative of the amino acid residues found at the active site of the specific enzyme respectfully. The specific interaction mechanisms are represented through the colors of the circles as well as additional solid and dotted lines. Pink circles represent electrostatic interactions which include hydrogen bonding and polar interactions between the ligand and respective amino acid residue, while green circles represent van der Waal interactions. Orange lines represent π-bond interactions and blue dotted lines represent a direct hydrogen bond formed between the substrate and an amino acid side chain with the arrow pointing towards the electron donor. The number and type of interactions occurring, as illustrated in Figure 4.3, are summarized in Table 4.3. In

comparison with 1K5P and 6OHJ, which have 21 and 30 interactions respectively, 3FWH has the least amount at a total of 9 interactions.

Interestingly, while 3FWH has the least amount of interaction quantities, this enzyme has shown to be the most successful with respect to docking with BDE-99 to promote debromination. It is understood that with less amino acid residues available for interacting, there is additional spatial room for the interaction to occur naturally without additional strain on the flexibility of the ligand substrate. In addition, a decrease in steric hindrance would be observed as well as a decrease in any oppositional forces from other surrounding interactions. These ideas are also reflected in the energy results found in Table 4.2. While the –CDOCKER interaction energy between BDE-99 and 3FWH is a positive value, the –CDOCKER energy is also positive but with a smaller value.

Although both energy values are good indicators 3FWH is a suitable enzyme for the debromination of PBDE congeners, there is still straining energy present within the ligands and thus reducing the –CDOCKER energy value away from the –CDOCKER interaction energy value. The energy values of 1K5P and 6OHJ are also representative of a relation between the number of interactions and the success of the pairing. As seen in Table 4.2, 1K5P has the second worst energy results with modest negative values, while 6OHJ has the worst energy results with large negative values. While considering this in combination with 1K5P having more interactions than 3FWH and 6OHJ having more interactions than 1K5P, the number of interactions can be seen a relative and generic indicator of the success of the pairing. However, it must be noted that if no interactions occur, there is no possible way for the enzyme to catalyze debromination reactions and thus an optimal amount of interactions between the active site and ligand must exist.

Table 4.2: Molecular Docking Results between BDE-99 and the Three Selected Enzymes

Enzyme	-CODCKER Interaction Energy (kcal/mol)	-CODCKER Energy (kcal/mol)
1K5P	-20.1289	-74.3905
3FWH	22.8949	7.9310
6OHJ	-66.4377	-282.4060

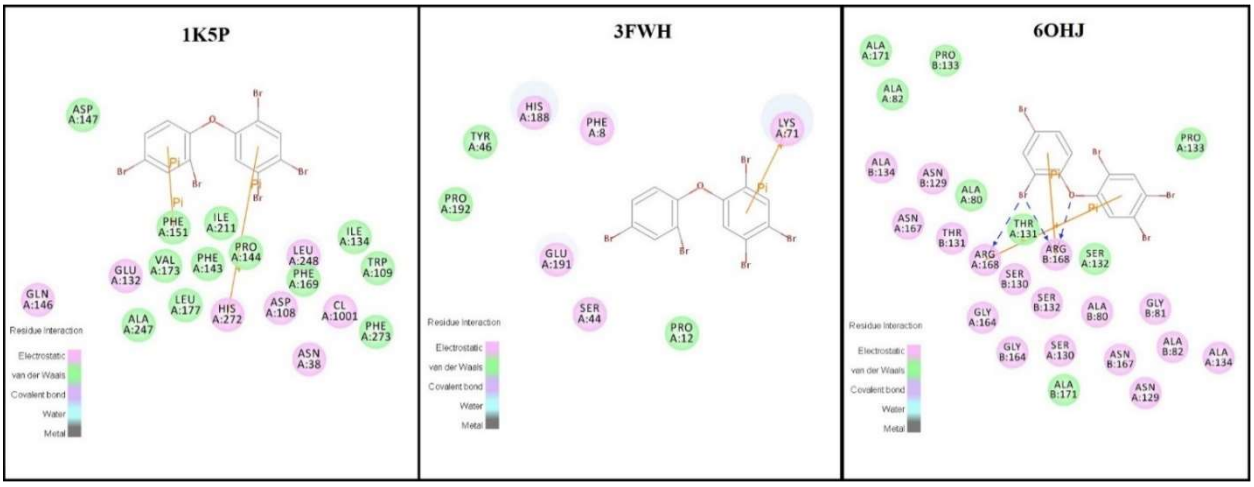


Figure 4.3: 2D Interaction Diagrams Showcasing the Interactions between BDE-99 and the Active Site Amino Acid Residues per Enzyme

Table 4.3: Number of Interactions between BDE-99 and the Selected Enzymes

Enzyme	Number of Interactions				
	Electrostatic	**van der Waals**	**Direct H-bond**	**π-bond**	**Total**
1K5P	7	12	0	2	21
3FWH	5	3	0	1	9
6OHJ	17	8	3	2	30

4.2.2 Debromination Performance of PBDE Congeners

Moving forward with 3FWH as the best candidate enzyme, the remaining congeners are paired and analyzed to determine if this enzyme is suitable for the debromination of a wide variety of PBDE molecules with varying degrees of bromination. The resulting information is found and shown in Table 4.4.

As noted in Table 4.4, it can be seen that successful interactions occur between every pairing with the exception of BDE-209. In general, both energy parameters indicate that as the degree of bromination increases, the success of the pairing performance decreases. It should be noted however, that this trend is not constant with regards to –CDOCKER Interaction Energy. This indicates that additional factors must be considered to fully understand how these PBDE congeners are interacting with the amino acid residues at the active site. For instance, BDE-15 should have the highest success with each succeeding congener progressively getting worse. Instead, discrepancies can be found in BDE-99 outperforming BDE-47 and BDE-207 outperforming BDE-183.

In addition to considering the degree of bromination, geometric factors are involved as well to account for the size of a bromine atom and the position in which they are bonded to either phenyl ring. Since bromine atoms are much larger in size compared to that of carbon, oxygen, and hydrogen, these larger atoms can act as a bumper against any external parties trying to access the smaller atoms at the molecules interior. This principle of steric hindrance can have significant effects on the amino acid residue interactions with respect to where the bromine atoms are bonded along the molecular structure of the congener. Furthermore, these ideas of steric hindrance and molecular geometry help explain why the –CDOCKER Energy values follow a constantly

decreasing trend. Due to the larger size of the higher brominated congener molecules, more contortions occur when fitting within the enzymes active site causing a larger amount of strain energy in comparison to the lower brominated congeners. With additional strain energy, these larger molecules lose success in the pairing interaction as the strain energy takes away from the interaction energy.

This is apparent with every selected congener to the point where the –CDOCKER Energy values for BDE-183 and BDE-207 become negative, meaning energy must be applied for the complex to stabilize. The failure of BDE-209 can also be explained through these concepts. As this particular molecule is completely brominated, these large atoms completely surround the interior of the structure making interactions difficult due to increased molecular stability as well as larger distance for intermolecular forces to persist.

As shown in Figure 4.4, there are a variety of amino acid residues present that make consistent appearances from one congener pairing to another in different trends. First, some residues are interacting with every selected congener through the same means. For instance, LYS A:71 and HIS A:188 are electrostatically interacting with each congener. This can also be noted for LYS A:71 individually as it bonds with each congener through π-bonds with the exception of BDE-207. There are no observable van der Waals interactions between a residue and every congener. Secondly, some residues are consistently interacting in the same way for only some of the selected congeners. This can be seen through SER A:44 as it electrostatically interacts with the lower brominated congeners until BDE-99. Another example of this is PRO A:12, PRO A:192 and TYR A:46. In this case, each of these residues are interacting through van der Waals forces. PRO A:12 interacts with each congener from BDE-15 to BDE-99, while PRO A:192 and TYR A:46 interact with each congener from BDE-15 to BDE-153.

Lastly, it can be shown that some residues change their type of interaction with respect to which congener it is paired with. GLU A:191 and PHE A:8 are observed to interact with BDE-15 through van der Waals forces, however, GLU A:191 begins to interact with BDE-28 and every succeeding congener through electrostatics while PHE A:8 makes the same interaction transition from BDE-47 onward. While these trends exist, there are also residue interactions that should be noted on an individual pairing basis. Some specific examples of these considerations include the van der Waals interactions between PHE A:10 with BDE-47, PRO A:9 and VAL A:184 with BDE-207, and the replacement of LYS A:71 with HIS A:188 as the π-bond with BDE-207.

To assist in the understanding of these results, the information presented in Table 4.5 lists the quantities of each type of interaction present for every congener pairing. While taking note of these values, it can be shown that in general, as the degree of bromination increases in a PBDE congener, the number of electrostatic interactions remain relatively constant while the number of van der Waals interactions significantly decrease.

The nature of the type of interaction is explanatory of this trend. Since each congener has its own unique chemical structure with varying quantities and positions of bromine atoms, the way in which each congener interacts with the presented residues is dependent on how it orients itself with respect to these residues. Electrostatic interactions are dependent on dipole-dipole intermolecular forces. In this case, the amino acid residues that consistently show electrostatic interactions must be in an optimal position to create dipole interactions with the generic structure of any PBDE congener; independent of its degree of bromination.

Oppositely, van der Waals interactions are due to induced dipole forces caused by the proximity of one molecule to another. This means that this type of interaction is highly dependent on the close distance between two or molecules to create the necessary forces of attraction. In the case of an increasing degree of bromination, additional large bromine atoms on the congener molecule represent a greater distance between the congener and amino acid residue, causing a drop in van der Waals interactions due to too large of a distance being created. In addition, while there are anomalies regarding this general relationship on a specific and individual pairing level, these differences are explained through the geometric uniqueness between each enzyme-ligand pairing. Studies such as (Loonen et al., 1999; Brenner et al., 2006; Markiewicz et al., 2017) were also able to confirm the effect of the degree of halogenation on the successfulness and quantities of amino acid residue interactions.

Through the use of molecular docking analysis results and discussion, it can be determined that while every PBDE congener may have a different experience while docking to the 3FWH enzyme, this type of dehalogenase is very capable of undertaking the debromination of a wide variety of PBDE congeners with respect to their degree of bromination. While lower brominated congeners have a higher success of creating a stable complex according to energy values, the more stable and higher brominated congeners share electrostatic interactions with the same amino acid residues. This infers that the main mechanism involved in the debromination of PBDEs with 3FWH is through electrostatics.

Table 4.4: Molecular Docking Results of all Target PBDE Congeners Paired with the 3FWH Enzyme

Congener	-CODCKER Interaction Energy (kcal/mol)	-CODCKER Energy (kcal/mol)
BDE-15	23.6658	12.1529
BDE-28	22.7402	10.4800
BDE-47	21.9953	10.0745
BDE-99	22.8948	7.9310
BDE-153	19.7924	0.7826
BDE-183	11.5860	-11.6714
BDE-207	13.9622	-35.3325
BDE-209	FAILED	

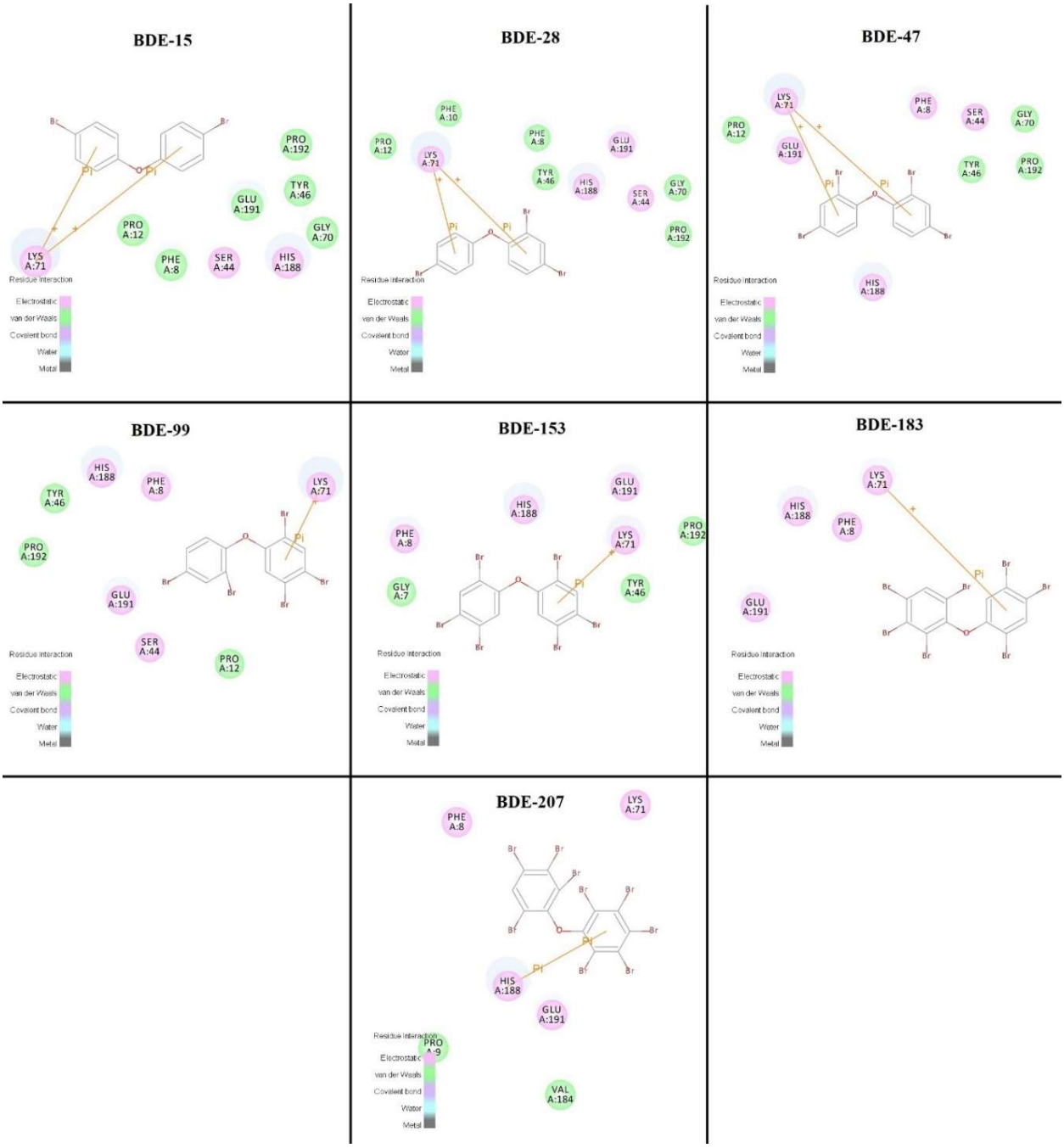


Figure 4.4: 2D Interaction Diagram between Selected PBDE Congeners and the 3FWH Enzyme

Table 4.5: Number of Interactions between the Selected PBDE Congeners and the 3FWH Enzyme

Congener	Number of Interactions			
	Electrostatic	**van der Waals**	**π-bond**	**Total**
BDE-15	3	6	2	11
BDE-28	4	6	2	12
BDE-47	5	4	2	11
BDE-99	5	3	1	9
BDE-153	4	3	1	8
BDE-183	4	0	1	5
BDE-207	4	2	1	7

4.2.3 Relationship between PBDE Biodegradability and Adsorption in Soil

It is important to understand that the debromination step of biodegradation will have a significant impact on bioavailability. Understanding the relation between the degree of bromination of PBDE congeners and how those congeners undergo debromination is important information to achieving more efficient and effective methods of dealing with their contamination safely. However, in a realistic setting, PBDE molecules that are found within a soil medium have additional corresponding factors to consider as well. While the biodegradation of PBDEs has been shown to proceed in the presence of the enzymatic secretions produced from bacteria that live in soil environments, the bioavailability of these molecules must also be considered to gain a clear grasp on how debromination and biodegradation pursues. Specifically speaking, since PBDE molecules are regarded as hydrophobic compounds, they have been shown to have a particular affinity towards the organic content found within soil particles. This feature of soil thus induces adsorptive properties of PBDEs onto these particles which in turn makes them less available to interaction with degradative enzymes. In this case, the bioavailability of the PBDE congeners is measured through their adsorption to soil particles. Subsequently, the measure of the adsorptive behavior of these congener molecules is done through the use of an organic-carbon partition coefficient (K_{OC}) value. The quantity of this metric is presented through the use of a ratio between the soil adsorption coefficient (K_d) and the percentage of organic carbon present in a control volume of soil (Kozerski, et al., 2014). It is important to distinguish that a low K_{OC} value indicates that the mobility of a substance within soil is high, while a high value indicates that the mobility is low.

With this information taken into consideration, comparative analysis can be done to highlight any overlapping trends that occur between the biodegradability of PBDEs and their adsorption behaviors with respect to their degree of bromination. Figure 4.5 illustrates the trend of

–CDOCKER Interaction Energy in a solid blue line and –CDOCKER Energy in a dashed blue line across the 7 selected congeners that were able to produce successful docking results. The quantity of energy is measured with kJ/mol and is shown on the left vertical axis. As mentioned previously, it can be seen that in general, as the degree of bromination increases, the docking energy decreases, meaning that higher brominated congeners have a greater difficulty of creating a stable enzyme-ligand complex and thus greater difficulty to biodegrade through debromination. The right vertical axis shows the K_{OC} ratio value, while the trend is illustrated with a red line. The K_{OC} values for the selected congeners were obtain from the study stated in Chapter 3 that used in silico modelling via 3D-QSAR techniques to accurately estimate the K_{OC} values of all 209 PBDE congeners. It can be shown that as the degree of bromination increases, the K_{OC} value also increases, meaning that with additional bromine atoms, the molecules become less mobile and more susceptible to adsorbing to the organic matter found in soil particles.

In context, the amount of bromine atoms present on any given PBDE congener molecule has a significant impact on its behavior. With that said, this parameter shows that it can independently effect the bioavailability through means of adsorption, as well as ease of obtaining interaction stability with present enzymes. Additionally, both cases include local peaks and depressions that occur on an individual congener basis due to the geometric properties of these molecules and location of bromine atom bond sites. The overall trends however state that higher brominated congeners are more difficult to move through the soil since they stick to particles and thus less bioavailable for enzymatic interactions. Studies such as (Huesemann et al., 2004; Congiu et al., 2015; Spasojević et al., 2015; Ren et al., 2018) also describe a similar relationship between the bioavailability of a substance and their readiness to biodegrade.

Moving forward, it can be stated that higher brominated congeners, especially BDE-209, have a larger amount of difficulty to treat with respect to contamination. These molecules are harder to remove from soil and are not as readily available to biodegrade as their lower brominated counterparts. While studies show treating higher brominated congeners like BDE-209 is possible, extra consideration is necessary for approaching problems involved with them. While the relationship between the degree of bromination with soil adsorption and biodegradability remains independent of on another, the amount of bromine atoms present creates significant behavioral characteristics that can affect the remediation of these compounds. By placing an emphasis on the debromination step of the overall biodegradation process may create more readily bioavailable compounds that can be dealt with more easily.

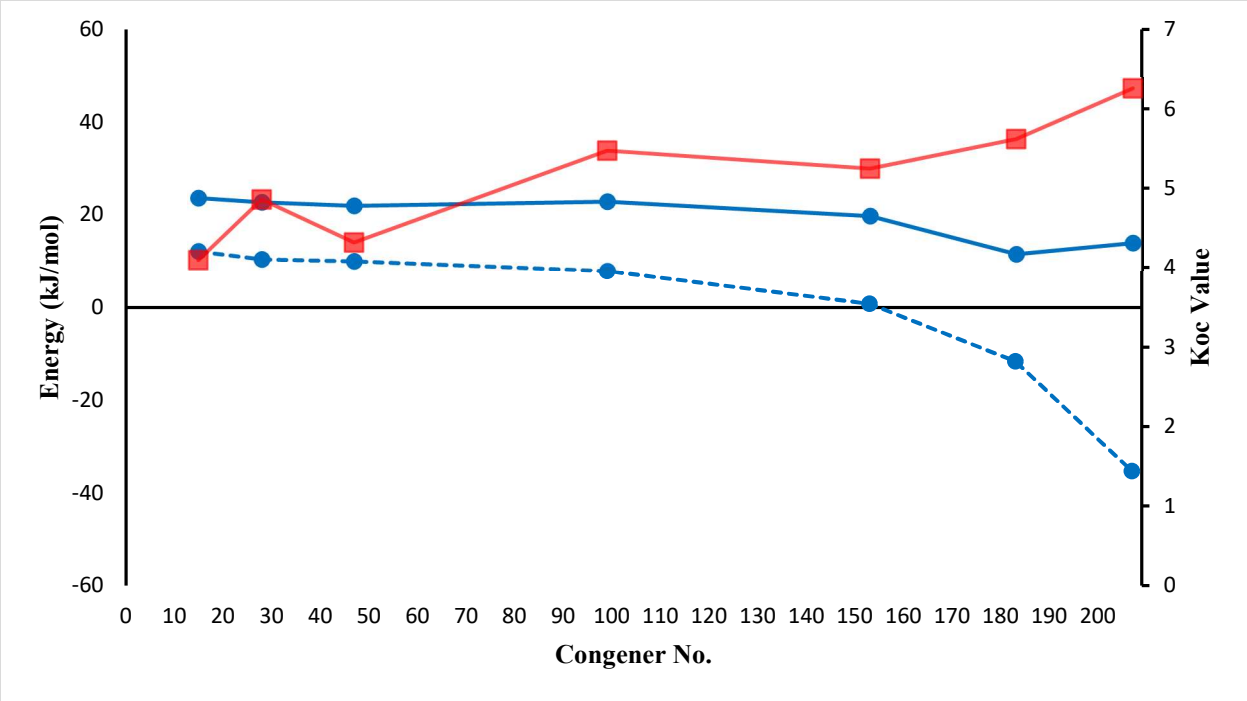


Figure 4.5: Relationship between the Degree of Bromination with Docking Energy and K_{OC} Values

4.4 Summary

Through the use of in silico molecular docking techniques, combined with information acquired from literature, the biodegradability behavior of PBDE compounds through enzymatic catalyzation is better understood. While many enzymes exist in nature with specific purposes, the three enzymes selected for this study were done so due to their likelihood of being found in soil environments that may also contain PBDE contamination. Since PBDEs are a relatively new and emerging source of contamination that can be found in multiple mediums, understanding their behavioral characteristics is imperative in implementing technologies and methods able to effectively and efficiently remediate them. Although there may be many solutions to this problem, the use of enzymes produced from existing natural microorganisms presented in the same soil medium is a safe, nontoxic, and fairly inexpensive process to obtain control of in order to optimize remediation techniques. While there may be many different kinds of enzymes that may help towards this goal, the 3FWH enzyme produced by *Rhodococcus rhodochrous* bacteria has shown consistency in the debromination of a wide variety of multi-brominated PBDE congeners. It has been shown that while individual exceptions exist on an individual congener pairing basis due to molecular geometry and steric hindrance, it can be stated that while the degree of bromination increases, the success of producing a stable complex decreases. Using this information in combination with the consideration of PBDEs adsorption to soil particles, remediation tactics can account for these behaviors and address them appropriately. With more effective and efficient means of removing PBDEs from nature, the adverse effects done onto ecosystems and living creatures can be mitigated.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

The use of PBDEs in the late 20th century on consumer goods was applied to improve the fire safety of the products, but has since become a problematic issue. Among the negative effects these compounds have been shown to have in relevance to the natural environment, their persistence within a soil medium is an important concern to address. As most information available about PBDEs is limited to interactions with living organisms, and the environmental impacts of a select few common congeners, the aim of the first task of this thesis is to provide adsorption information through the use of an organic-carbon partition coefficient (K_{OC}) for all 209 congeners. This goal is achieved through the development of a 3D-QSAR model to predict K_{OC} values for each congener based on input information collected from literature as well as the molecular structure and shape for each compound. To further add to this, a fractional factorial design is implemented to examine which exact bonding positions have the most significance on a congeners K_{OC} value in the presence of a bromine atom. It has been determined that a congeners K_{OC} value typically increases with an increase in bromination, as well as some tendencies for the K_{OC} to locally increase or decrease slightly depending on if bromine is present at certain bonding sites.

In addition to better understanding the behavioral tendencies of PBDEs in soil, an approach to their biodegradation through the use of enzymes produced from three different bacterial species commonly found in soil is explored as the second task of this thesis. Through literature review, three unique enzymes produced from three different bacterial species found in relevant mediums are analyzed and compared to determine which can best promote the debromination of a variety of commonly occurring PBDE congeners. Utilization of a CDOCKER molecular docking method has shown that the 3FWH enzyme produced by *Rhodococcus rhodochrous*, is the most appropriate

candidate for inducing a stable interaction as determined by the most optimal docking energy results. This enzyme is able to provide ample spatial room with consistently occurring amino acid residues that promote the electrostatic interaction between the active site and PBDE molecules with a varying degree of bromination. To further add to this study, the bioavailability of PBDEs are considered through a soil adsorption context based on the predicted K_{OC} values previously determined. While these two factors remain independent of one another, comparative analysis has shown that while the degree of bromination increases from one congener to another, the ability of the enzyme to create a stable complex decreases as well as a noticeable decrease in soil mobility. Thus, higher brominated congeners are shown to be less prone to degradation from the 3FWH enzyme due to a lower complex stability in addition to becoming less bioavailable as they are strongly adsorbed to the organic matter found in soil particles. By pursing the debromination of PBDE compounds in the soil, biodegradation will become less problematic as the remaining chemical from this process will be more bioavailable.

Overall, in the first study, it was shown that while PBDEs are recognized as a group of emerging contaminants with some information about them available, there are large gaps of knowledge pertaining to each congener in an environmental context. While some studies exist that discuss the environmental characteristics of PBDEs, these studies have a limited scope to only a handful of commonly occurring congeners. This thesis work has been contributive in that soil adsorption information regarding all 209 congeners is produced through the use of predictive in silico methods. It is the intent for this information to provide additional context for understanding the overall behavior of PBDEs in soil in hopes that approaching remediation efforts become easier. While the second study explores the biodegradation mechanisms present in enzymes likely to interact with PBDEs in a soil context. While molecular docking studies with PBDEs have been a

prevalent topic in research, this has mainly been in the conditions surrounding the health and interactions of living organisms with few studies available in an environmental degradation context. Through the topics explored in this thesis work, it is hoped that the understanding of the biodegradation mechanisms between the 3FWH enzyme and multiple varieties of PBDE congeners, provide additional insight on the debromination tendencies of these chemicals in a soil contamination context.

5.2 Research Contributions

(1) The focus of the first study was to obtain additional knowledge of how PBDEs distribute themselves in a soil setting that had previously not been documented and published. By creating a predictive 3D-QSAR model, organic carbon-water partition coefficient information on all 209 congeners has been reached to contribute to the limited information presently available regarding only a handful of commonly occurring congeners. Although other studies in the past have utilized QSAR techniques to study PBDEs, this thesis work presents and contributes unique soil adsorption information on all PBDEs.

(2) The aim of the second study was to quantify and perform molecular docking techniques to analyze the exact interactions and debromination mechanisms present between specific enzymes known to be found in a soil environment and a variety of PBDE congeners that represent a range of bromination. This is the first time that the molecular docking of PBDE compounds was tackled to understand the debromination process with the uses of specific soil enzymes.

5.3 Recommendations

(1) Although success was found in creating a 3D-QSAR model that is able to predict $\log K_{OC}$ values for each PBDE congener, it is recommended that future studies focus on confirming these quantities and trends through laboratory experimental analysis. In addition, further studies can focus on quantifying the fate and transport of congeners in a replicated soil setting.

(2) It had been determined that specific placement of bromine atoms at bonding sites around either phenyl ring can have a significance on a congeners K_{OC} value. Further studies may investigate and confirm these factors through additional modelling or practical laboratory experiments.

(3) While BDE-99 was used as a representative for a generic PBDE congener to determine the most appropriate soil enzyme to use, further studies may focus on the interactions between additional enzymes with additional congeners not considered in this study. This may provide additional information on more appropriately suited enzymes to promote the debromination and biodegradation of PBDEs in safe and nontoxic bioremediation methods.

(4) The degradative properties of the 3FWH soil enzyme was matched with eight commonly occurring enzymes with relative success and with an exception to a failure with BDE-209. Although this enzyme was able to successfully dock with most congeners, increased difficulty was observed as the degree of bromination in the congeners increased. Future studies may focus on designing additional means of promoting the debromination of highly brominated congeners in conjunction with the 3FWH enzyme.

(5) The degree of bromination in PBDE congeners has been noticeably prevalent in effecting both soil adsorption tendencies as well as biodegradation success. While these two factors remain independent of each other, it is recommended that future studies may perform additional analyses on the indirect relationship between the bioavailability and biodegradation of PBDEs to maximize effectiveness and efficiency when designing remediation strategies focused on these chemicals.

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APPENDICIES

APPENDIX A – Additional Information on PBDEs

Table A1: Chemical Identity of Polybrominated Diphenyl Ether (PBDE) Congeners

IUPAC Number[b]	Compound/substituents	CAS number[c]
	Biphenyl	92-52-4
	MonoBDE	101-55-3
1	2	
2	3	
3	4	
	DiBDE	2050-47-7
4	2,2'	
5	2,3	
6	2,3'	
7	2,4	
8	2,4'	
9	2,5	
10	2,6	
11	3,3'	
12	3,4	
13	3,4'	
14	3,5	
15	4,4'	
	TriBDE	49690-94-0
16	2,2',3	
17	2,2',4	
18	2,2',5	
19	2,2',6	
20	2,3,3'	
21	2,3,4	
22	2,3,4'	
23	2,3,5	
24	2,3,6	
25	2,3',4	
26	2,3',5	
27	2,3',6	
28	2,4,4'	
29	2,4,5	
30	2,4,6	
31	2,4',5	
32	2,4',6	
33	2',3,4	
34	2',3,5	
35	3,3',4	
36	3,3',5	
37	3,4,4'	
38	3,4,5	
39	3,4',5	

IUPAC Number[b]	Compound/substituents	CAS number[c]
	TetraBDE	40088-47-9
40	2,2',3,3'	
41	2,2',3,4	
42	2,2',3,4'	
43	2,2',3,5	
44	2,2',4,5'	
45	2,2',3,6	
46	2,2',3,6'	
47	2,2',4,4'	
48	2,2',4,5	
49	2,2',4,5'	
50	2,2',4,6	
51	2,2',4,6'	
52	2,2',5,5'	
53	2,2',5,6'	
54	2,2',6,6'	
55	2,3,3',4	
56	2,3,3',4'	
57	2,3,3',5	
58	2,3,3',5'	
59	2,3,3',6	
60	2,3,4,4'	
61	2,3,4,5	
62	2,3,4,6	
63	2,3,4',5	
64	2,3,4',6	
65	2,3,5,6	
66	2,3',4,4'	
67	2,3',4,5	
68	2,3',4,5'	
69	2,3',4,6	
70	2,3',4',5	
71	2,3',4',6	
72	2,3',5,5'	
73	2,3',5',6	
74	2,4,4',5	
75	2,4,4',6	
76	2',3,4,5	
77	3,3',4,4'	
78	3,3',4,5	
79	3,3',4,5'	
80	3,3',5,5'	
81	3,4,4',5	

IUPAC Number[b]	Compound/substituents	CAS number[c]
	PentaBDE	32534-81-9
82	2,2',3,3',4	
83	2,2',3,3',5	
84	2,2',3,3',6	
85	2,2',3,4,4'	
86	2,2',3,4,5	
87	2,2',3,4,5'	
88	2,2',3,4,6	
89	2,2',3,4,6'	
90	2,2',3,4',5	
91	2,2',3,4',6	
92	2,2',3,5,5'	
93	2,2',3,5,6	
94	2,2',3,5,6'	
95	2,2',3,5',6	
96	2,2',3,6,6'	
97	2,2',3',4,5	
98	2,2',3',4,6	
99	2,2',4,4',5	
100	2,2',4,4',6	
101	2,2',4,5,5'	
102	2,2',4,5,6'	
103	2,2',4,5',6	
104	2,2',4,6,6'	
105	2,3,3',4,4'	
106	2,3,3',4,5	
107	2,3,3',4',5	
108	2,3,3',4,5'	
109	2,3,3',4,6	
110	2,3,3',4',6	
111	2,3,3',5,5'	
112	2,3,3',5,6	
113	2,3,3',5',6	
114	2,3,4,4',5	
115	2,3,4,4',6	
116	2,3,4,5,6	
117	2,3,4',5,6	
118	2,3',4,4',5	
119	2,3',4,4',6	
120	2,3',4,5,5'	
121	2,3',4,5',6	
122	2',3,3',4,5	
123	2',3,4,4',5	
124	2',3,4,5,5'	
125	2',3,4,5,6'	
126	3,3',4,4',5	
127	3,3',4,5,5'	

IUPAC Number[b]	Compound/substituents	CAS number[c]
	HexaBDE	36483-60-0
128	2,2',3,3',4,4'	
129	2,2'3,3',4,5	
130	2,2',3,3',4,5'	
131	2,2',3,3',4,6	
132	2,2',3,3',4,6'	
133	2,2',3,3',5,5'	
134	2,2',3,3',5,6	
135	2,2',3,3',5,6'	
136	2,2',3,3',6,6'	
137	2,2',3,4,4',5	
138	2,2',3,4,4',5'	
139	2,2',3,4,4',6	
140	2,2',3,4,4',6'	
141	2,2',3,4,5,5'	
142	2,2',3,4,5,6	
143	2,2',3,4,5,6'	
144	2,2',3,4,5',6	
145	2,2',3,4,6,6'	
146	2,2',3,4',5,5'	
147	2,2',3,4',5,6	
148	2,2',3,4',5,6'	
149	2,2',3,4',5',6	
150	2,2',3,4',5,6'	
151	2,2',3,5,5',6	
152	2,2',3,5,6,6'	
153	2,2',4,4',5,5'	
154	2,2',4,4',5,6'	
155	2,2',4,4',6,6'	
156	2,3,3',4,4',5	
157	2,3,3',4,4',5'	
158	2,3,3',4,4',6	
159	2,3,3',4,5,5'	
160	2,3,3',4,5,6	
161	2,3,3',4,5',6	
162	2,3,3',4',5,5'	
163	2,3,3',4',5,6	
164	2,3,3',4',5',6	
165	2,3,3',5,5',6	
166	2,3,4,4',5,6	
167	2,3',4,4',5,5'	
168	2,3',4,4',5',6	
169	3,3',4,4',5,5'	

IUPAC Number[b]	Compound/substituents	CAS number[c]
	HeptaBDE	68928-80-3
170	2,2',3,3',4,4',5	
171	2,2',3,3',4,4',6	
172	2,2',3,3',4,5,5'	
173	2,2',3,3',4,5,6	
174	2,2',3,3',4,5,6'	
175	2,2',3,3',4,5',6	
176	2,2',3,3',4,6,6'	
177	2,2',3,3',4',5,6	
178	2,2',3,3',5,5',6	
179	2,2',3,3',5,6,6'	
180	2,2',3,4,4',5,5'	
181	2,2',3,4,4',5,6	
182	2,2',3,4,4',5,6'	
183	2,2',3,4,4',5',6	
184	2,2',3,4,4',6,6'	
185	2,2',3,4,5,5',6	
186	2,2',3,4,5,6,6'	
187	2,2',3,4',5,5',6	
188	2,2',3,4',5,6,6'	
189	2,3,3',4,4',5,5'	
190	2,3,3',4,4',5,6	
191	2,3,3',4,4',5',6	
192	2,3,3',4,5,5',6	
193	2,3,3',4',5,5',6	
	OctaBDE	32536-52-0
194	2,2',3,3',4,4',5,5'	
195	2,2',3,3',4,4',5,6	
196	2,2',3,3',4,4',5',6	
197	2,2',3,3',4,4',6,6'	
198	2,2',3,3',4,5,5',6	
199	2,2',3,3',4,5,6,6'	
200	2,2',3,3',4,5,6,6'	
201	2,2',3,3',4,5',6,6'	
202	2,2',3,3',5,5',6,6'	
203	2,2',3,4,4',5,5',6	
204	2,2',3,4,4',5,6,6'	
205	2,3,3',4,4',5,5',6	
	NonaBDE	63936-56-1
206	2,2',3,3',4,4',5,5',6	
207	2,2',3,3',4,4',5,6,6'	
208	2,2',3,3',4,5,5',6,6'	

IUPAC Number[b]	Compound/substituents	CAS number[c]
	DecaBDE	1163-19-5
209	2,2',3,3',4,4',5,5',6,6'	

[a]WHO 1994a
[b]Ballschmiter and Zell 1980
[c]No CAS numbers were identified for the individual PBDE congeners.

BDE = brominated diphenyl ether; CAS = Chemical Abstracts Service; IUPAC = International Union of Pure and Applied Chemistry

Source: (Pohl et al., 2017)

Table A2: Physical and Chemical Properties of Technical Polybrominated Diphenyl Ether (PBDE) Mixtures

Property	Pentabromodiphenyl ether	Octabromodiphenyl ether	Decabromodiphenyl ether
Molecular weight	Mixture	Mixture	959.22[a]
Color	Clear, amber to pale yellow[a]	Off-white[a]	Off-white[a]
Physical state	Highly viscous liquid	Powder	Powder[a]
Melting point	-7 to -3°C (commercial)[b]	85–89°C (commercial)[c]; 200°C (range, 167–257)[a]; 79–87°C[a]; 170–220°C[a]	290–306°C[a]
Boiling point	>300°C (decomposition starts above 200°C)[a,b]	Decomposes at >330°C (commercial)[c]	Decomposes at >320, >400, and 425°C[a]
Density (g/mL)	2.28 at 25°C[a]; 2.25–2.28[b]	2.76[a]; 2.8 (commercial)[c]	3.0[a]; 3.25[a]
Odor	No data	Faint[a]	Odorless[a]
Odor threshold:			
Water	No data	No data	Not applicable
Air	No data	No data	Not applicable
Solubility:			
Water	13.3 µg/L (commercial)[b,d]; 2.4 µg/L (pentabromodiphenyl ether component)[b]; 10.9 µg/L (tetrabromodiphenyl ether component)[b]	<1 ppb at 25°C (commercial)[c]; 1.98 µg/L (heptabromodiphenyl ether component)[c]	<0.1 µg/L[g]
Organic solvent(s)	10 g/kg methanol; miscible in toluene[d]	Acetone (20 g/L); benzene (200 g/L); methanol (2 g/L) all at 25°C[a]	No data
Partition coefficients:			
Log K_{ow}	6.64–6.97[d]; 6.57 (commercial)[b]	6.29 (commercial)[c]	6.265[e]
Log K_{oc}	4.89–5.10[e]	5.92–6.22[e]	6.80[e]
Vapor pressure	$2.2x10^{-7}$–$5.5x10^{-7}$ mm Hg at 25°C[d]; $3.5x10^{-7}$ mm Hg (commercial)[b]	$9.0x10^{-10}$–$1.7x10^{-9}$ mm Hg at 25°C[d]; $4.9x10^{-8}$ mm Hg at 21°C (commercial)[c]	$3.2x10^{-8}$ mm Hg[f]
Henry's Law constant (atm-m^3/mole)	$1.2x10^{-5}$[g]; $1.2x10^{-6}$[e]; $3.5x10^{-6}$[f]	$7.5x10^{-8}$[e]; $2.6x10^{-7}$[e]	$1.62x10^{-6}$[g]; $1.93x10^{-8}$[d]; $1.2x10^{-8}$[e]; $4.4x10^{-8}$[e]
Autoignition temperature	Decomposes above 200°C[b,d]	Decomposes above 330°C (commercial)[c]	Not applicable[a]
Flashpoint	No data	No data	None
Flammability limits	Not applicable (flame retardant)[b,d]	Not applicable (flame retardant)[c]	Non-flammable[a]

Property	Pentabromodiphenyl ether	Octabromodiphenyl ether	Decabromodiphenyl ether
Conversion factors	1 ppm=23.48 mg/m^3 at 20°C[d]	No data	No data
Explosive limits	None[b,f]	None[c]	No data

[a] WHO 1994a
[b] ENVIRON 2003a
[c] ENVIRON 2003b
[d] EU 2001
[e] Estimated values were calculated using EPIWIN v4.10 (EPA 2014e).
[f] Hardy 2002a
[g] Estimated value was calculated using vapor pressure and water solubility values in table.

Source: (Pohl et al., 2017)

APPENDIX B – Fractional Factorial Design Supplementary Information

- **Regression Equation in Uncoded Units (not all the effects are included, especially for the insignificant ones)**

CoMFA = 5.2599 + 0.0525 A + 0.1546 B + 0.1107 C + 0.1698 D + 0.0769 E + 0.0949 F
+ 0.1560 G + 0.0728 H + 0.1619 J + 0.0865 K + 0.0379 A*B - 0.0343 A*E
- 0.0695 A*F + 0.0342 A*J - 0.0470 A*K + 0.0346 B*F + 0.0513 C*D
+ 0.0598 C*E - 0.0376 D*G - 0.0444 D*J - 0.0416 E*F - 0.0619 E*K
- 0.0589 F*K

From the model, the negative effects indicated that if Br locate in these positions, the K_{OC} values will be decreased, such as AK, EF, DJ, DG, FK, EK, AE, AF.

- **Model evaluation**

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.228578	80.62%	76.34%	70.65%

The R^2 is 80%. The predicted R^2 and adjusted R^2 are close. The model is valid.

- **Analysis of Variance (ANOVA)**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	23	22.6081	0.98296	18.81	0.000
Linear	10	18.6878	1.86878	35.77	0.000
A	1	0.3524	0.35238	6.74	0.011
B	1	3.0610	3.06096	58.59	0.000
C	1	1.5687	1.56866	30.02	0.000
D	1	3.6904	3.69037	70.63	0.000
E	1	0.7568	0.75676	14.48	0.000
F	1	1.1533	1.15330	22.07	0.000
G	1	3.1156	3.11563	59.63	0.000
H	1	0.6783	0.67832	12.98	0.000
J	1	3.3541	3.35405	64.19	0.000
K	1	0.9574	0.95738	18.32	0.000
2-Way Interactions	13	3.9203	0.30156	5.77	0.000
A*B	1	0.1838	0.18377	3.52	0.064
A*E	1	0.1508	0.15084	2.89	0.092
A*F	1	0.6186	0.61855	11.84	0.001
A*J	1	0.1493	0.14933	2.86	0.094
A*K	1	0.2826	0.28256	5.41	0.022
B*F	1	0.1532	0.15318	2.93	0.090
C*D	1	0.3362	0.33620	6.43	0.013
C*E	1	0.4579	0.45792	8.76	0.004
D*G	1	0.1809	0.18090	3.46	0.066
D*J	1	0.2522	0.25223	4.83	0.030
E*F	1	0.2211	0.22111	4.23	0.042
E*K	1	0.4900	0.49005	9.38	0.003
F*K	1	0.4437	0.44368	8.49	0.004
Error	104	5.4338	0.05225		
Total	127	28.0419			