ABSTRACT

Title of Document: ASSESSING VARIABILITY IN BIOAVAILABILITY OF POPS IN SOIL TO NATIVE EARTHWORMS USING TRADITIONAL BIOLOGICAL AND THIN-FILM SOLID-PHASE EXTRACTION

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A variety of man-made chemicals, from pesticides to flame retardants, have been identified as persistent organic pollutants (POPs). To examine the true effect of POPs on the environment the bioavailability must be determined. In this experiment two families of POPs, DDT and its constituents and PBDEs, were examined using a traditional and an alternate bioavailability method. Polymer thin-film solid-phase extraction (TF-SPE) uses a polymer, EVA, to mimic earthworm bioavailability. The TF-SPE method is faster and easier than the biological method. Soil and native earthworms were obtained from a historically DDT contaminated orchard, and two commercial farm fields in which PBDEs were introduced through multiple biosolids applications. This

study establishes a correlation between the TF-SPE method and native earthworm accumulation for the two types of contaminants. TF-SPE has the potential to be an easy and effective method of assessing variability in bioavailability due to field management techniques or remediation efforts.

ASSESSING VARIABILITY IN BIOAVAILABILITY OF POPS IN SOIL TO NATIVE EARTHWORMS USING TRADITIONAL BIOLOGICAL AND THIN-FILM SOLID-PHASE EXTRACTION

By

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Dedication

To my parents for being the best role models I could ask for, and to my husband Kyle, for going through the highs and lows of graduate school with me.

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Chapter 1: Introduction

1.1 Literature Review

1.1.1 Introduction to POPs

Persistent organic pollutants (POPs) are chemicals that remain in the environment for long periods of time, bioaccumulate in organisms, and have deleterious effects on human and animal health and the environment (US EPA, 2013). The international community has recognized the potential harm from POPs to human health and the environment. A global treaty, the Stockholm Convention on Persistent Organic Pollutants, was developed in 2001 to address this issue. The Convention requires countries that are participating parties to eliminate or reduce the release of POPs into the environment. Currently there are 179 countries that are parties to the Convention and 152 countries participating as signatories, including the United States. Since its implementation in 2004, the Convention has been reviewed and updated twice to include new information and to add pollutants to the initial 12 POPs (Table 1.1)

The 12 initial POPs under the Stockholm Convention	
Chemical	Use
Aldrin	Pesticide
Chlordane	insecticide, especially termite control
DDT	insecticide, malaria prevention
Dieldrin	insecticide, especially termite control
Endrin	insecticide, rodenticide
Heptachlor	Insecticide
Hexachlorobenzene (HCB)	fungicide, by-product
Mirex	inseciticide, fire retardant
Toxaphene	Insecticide
Polychlorinated biphenyls (PCB)	heat exchange fluids, paint additive,plastics
Polychlorinated dibenzo-p-dioxins (PCDD)	by-product during incomplete combustion of certain wastes
Polychlorinated dibenzofurans (PCDF)	similar to PCDD, and by-product PCB production

Table 1.1 – The initial 12 POPs listed under the Stockholm Treaty

POPs have been highly regulated in the United States and throughout the world, and an extensive effort has gone into their measurement in various phases of the environment. While many POPs of concern are no longer in production, soils and sediments act as reservoirs for these compounds (Gaw et. al., 2012). This is due to their physical and chemical properties, including low water solubility and long half-lives. This is highlighted in Figure 1.1 which shows the dissipation of several POPs in contaminated field sites over time (Alexander, 2000). The concentrations of contaminants decreased slowly at first due to

volatilization, by plant uptake and removal during harvest, in water runoff and through chemical transformations (photodegradation and biodegradation) (Semple et. al., 2003)(ATSDR, 2002a). Approximately 35% of the total dissipation in the soil occurs during the first 5 years. After that time, the rate of POP dissipation slowed and became close to non-existent. The contaminants become entrapped within the soil and become less accessible to microorganisms, essentially eliminating their movement and degradation (Alexander, 2000). After 15 years there was still over 60% of the initial DDT and over 30% of the initial dieldrin concentration remaining.

Because soil provides a sink for POPs, the risk associated with the contaminants can linger. The main concern is the accumulation of POPs in the fatty tissue of soil dwelling organisms which introduces them into the terrestrial food chain. The same properties that keep POPs in the soil, mainly their lipophilic nature, are the reason for this accumulation.

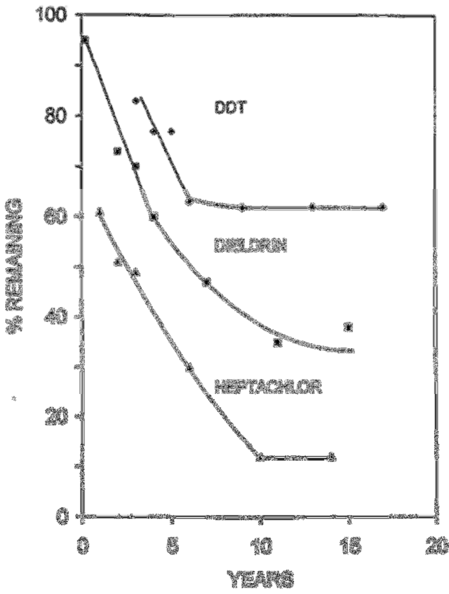


Figure 1.1 - Changes in concentrations of three POPs in long term monitoring of several field sites (Alexander, 2000)

1.1.2 POPs Studied

The three families of contaminants examined in this research are dichlorodiphenyltrichloroethane (DDT), Dieldrin, and Polybromodiphenyl ethers (PBDEs) and are listed as POPs identified by the Stockholm Convention (SC, 2013). The first two, DDT and Dieldrin, were part of the initial 12 POPs identified, and are organochlorine pesticides. Both of these chemicals were historically applied as insecticides, mainly on crops. The third family of POPs investigated in this research, PBDEs, was added to the Stockholm Convention in 2009. PBDEs are a family of brominated flame retardants, and have been used on a wide variety of products, from furniture to electronics. PBDEs were manufactured under three commercial products, Pentabromodiphenyl ether (PentaBDE), Octabromodiphenyl ether (OctaBDE) and Decabromodiphenyl ether (DecaBDE). Basic chemical structure and properties of the POPs studied in this research are presented in Table 1.2. A key characteristic of these POPS is their halogenation which contributes to their potential toxicity and resistance to degradation. Also, differences in size and structure affect the POP’s behavior and therefore bioavailability to organisms and the environment.

Chemical	Structure	Solubility (mg/L)	log Kow	logKoc	Vapor Pressure (mmHg at 25°C)	Molecular Weight (g/mol)
p,p'-DDT		0.025	6.91	5.18	1.6×10^{-7} (20°C)	354.49
p,p'-DDE		0.12	6.51	4.7	6.0×10^{-6}	318.03
p,p-DDD		0.09	6.02	5.18	1.35×10^{-6}	320.05
o,p-DDT		0.085	6.79	5.35	1.1×10^{-7} (20°C)	354.49
o,p-DDE		0.14	6	5.19	6.2×10^{-6}	318.03
Dieldrin		0.11	6.2	6.7	5.89×10^{-6}	380.91
pentaBDE (mainly BDE-99)		13.3	6.57	4.9-5.1	3.5×10^{-7}	564.69
octaBDE (mainly BDE-183, pictured, and BDE-209)		<1ppb	6.29	5.9-6.2	1.7×10^{-9}	801.31
decaBDE (mainly BDE-209)		<0.1	6.265	6.8	3.2×10^{-8}	959.22

Table 1.2 - Chemical structure and properties of the POPs studied

DDT is one of the most prominent POPs. It was the cause of increased public concern over possible hazards of pesticide use in the mid-20th century. This led to the U.S. government taking regulatory actions to restrict and then prohibit the use of DDT (Young, 1975). DDT primarily degrades to DDE (dichlorodiphenyldichloroethylene), through dehydrochlorination but also degrades to DDD (dichlorodiphenyldichloroethane), through reductive dechlorination (Schwarzenbach et. al. 2003). There are two configurations of DDT and DDE encountered in the environment, the para, para configuration (p,p'- or 4,4'), and the ortho, para configuration (o,p'- or 2,4'), which are shown in Table 1.1. The DDT compounds degrade to their corresponding configurations of DDE. There are several observed effects of DDT exposure; the most concerning is the lethality to birds and fish and reproductive effects to birds including eggshell thinning caused by DDE exposure. In humans and animals, nervous system effects have been observed, including tremors and convulsions. DDT and it's metabolites can also cause hormone-altering actions, the most concerning of which is DDE which has been shown to alter the development of reproductive organs in rats (ATSDR, 2002b.)

Dieldrin is another organochlorine pesticide with historical use and is also the by-product of a similarly applied pesticide, Aldrin (ATSDR, 2002a.). It has been banned in the U.S. since 1987, but was widely used from 1950-1974 on crops, and from 1972-1987 for termite control. Aldrin can transform to dieldrin photolytically and through biodegradation. Dieldrin degrades very slowly, and the primary process of loss is volatilization. However, volatilization is also slow, with

a half-life of 868 days. This is because dieldrin has a low vapor pressure and is strongly sorbed to the soil. In humans, dieldrin has been shown to affect the central nervous system, causing convulsions and even death in acute poisoning. Animal toxicity studies have shown similar results, with main effects on the central nervous system (ATSDR, 2002a.).

The final family of POPs investigated in this research is PBDEs. Of the three commercial mixtures of PBDEs, the Stockholm Convention only includes the first two commercial products; however the third is being voluntarily phased out in the United States and other participating countries by the manufacturers. PBDEs were used in mattresses, furniture, carpet padding, textiles as well as electronics and other plastics as flame retardants. The chemicals are not chemically bound to the products they are used on, and therefore easily leach and then enter the environment (de Wit, 2002). The commercial BDE products are made up of a mixture of individual BDE constituents, which are known as congeners. There are 209 possible compounds with the PBDE structure, however only a subset of the possible congeners are observed (ATSDR, 2004). Because the commercial products are mixtures, the names are only indicative of the main component of the product. For example, commercial OctaBDE is a mixture of hexa-, hepta- octa- and nonaBDEs and trace amounts of decaBDE. Generally, the main components of each commercial mixture are: DecaBDE: 97% BDE-209, PentaBDE: 43% BDE-99 and 8% BDE-100, OctaBDE: 45% heptaBDE (mainly BDE-183) and 14% BDE-153(ATSDR, 2004). The percentage of each congener in the mixtures can vary between manufacturers.

PBDEs can be debrominated photolytically, and the photodegradation increases with increasing bromination, therefore decaBDE degrades more quickly than pentaBDE and octaBDE. PBDEs do not show an appreciable amount of biodegradation. DecaBDE also shows lower toxicity compared to the lower brominated PBDEs, octaBDE and pentaBDE. In humans these lower brominated PBDEs target the liver, thyroid and neurobehavioral development and may cause cancer (McDonald, 2002; ATSDR, 2004).

1.1.3 Bioavailability as a Measure of Risk

Currently environmental risks of POPs are determined by measuring the total concentrations of the chemicals in different mediums. For example, in the U.S. the Environmental Protection Agency (EPA) has implemented ecological soil screening levels (Eco-SSLs) to assess and regulate potential ecological risks from POPs. Eco-SSLs are concentrations of contaminants in soil that are considered low enough to be protective of ecological receptors of the contaminants. The levels for DDT determined in the Eco-SSLs are 0.093 mg/kg for avian receptors and 0.021 mg/kg for mammalian receptors. For dieldrin the levels are 0.022 mg/kg for avian receptors and 0.005 for mammalian receptors (OSWER, 2007;OSWER, 2005). In Canada, the soil quality guidelines for DDT are 0.7 mg/kg for agricultural and residential land (CCME, 1999). However, it has been shown that total concentration of contaminants in soil is not related to biological effects (Harmsen, 2007). There is a time-dependent sequestration of POPs in soil in which the availability of contaminants to organisms decreases without a parallel decrease in total concentration (Morrison, 2000). Therefore

total concentrations can often over-estimate the risk from these contaminants, as they are not directly assessing the exposure to living organisms (Alexander, 2000). This means that soil may contain relatively high levels of DDT and dieldrin, but still be low enough to be protective of the ecological receptors of concern.

As a replacement for total concentration, bioavailability has been suggested to provide a better measurement for regulation and risk assessment purposes (Harmsen, 2007). The bioavailable portion of POPs is the fraction of the chemical that is accessible, therefore can be taken up or transformed by living organisms (Semple et. al, 2003; Alexander, 2000). This gives a more accurate measure of the level of risk in a medium, in this research soil.

Generally, bioaccumulation is used as a measure of the bioavailable portion of POPs in soil and sediments. The amount of contaminant that is bioavailable can be taken up by an organism, where it collects and accumulates in the fatty tissue. Bioaccumulation evaluates this accumulation in the organism (Stumm and Morgan, 1996). This buildup in one type of organism, for example earthworms, leads to biomagnification. Biomagnification is the progressive accumulation of compounds in the food chain (Stumm and Morgan, 1996). As POPs travel up the food chain, the concentration in the organism gets increasingly higher as the contaminant continues to accumulate. Biomagnification has been seen in previous studies on DDT, for example, one study showed that soil with 10ppm concentration of DDT contained earthworms with a DDT concentration of 141 ppm, and robins in the same area were found to

have a concentration of 444ppm (Mongillo and Zierdt-Warshaw, 2000). Biomagnification highlights the significant concern regarding POPs. The bioaccumulation in the low end of the food chain, such as earthworms, may not cause any deleterious effects, but the fauna further up the food chain can potentially experience damaging effects.

The bioaccumulation of POPs in organisms is often linearly related to their octanol/water partitioning coefficient (K_{ow}) (Stumm and Morgan, 1996; vanLoon and Duffy, 2005). This relationship allows for the Bioconcentration Factor (BCF) to be modeled for an organism, in the form of (Stumm and Morgan, 1996):

$$\log \mathrm{BCF} = y \log \mathrm{K_{OW}} - x$$

This model highlights the importance of a contaminants K_{OW} value and it's use when determining the BCF. One example is a study done examining organochlorines in Lake Baikal, where the logBCF and log K_{OW} relationship was determined for several fish and seal species. However, there was a weaker than expected correlation, as the r^2 for the fits ranged from 0.45 to 0.62 (Kucklick et. al., 1994). Another study comparing BCF of two species of fish to the K_{OW} for a range of chlorinated organic compounds found weak correlations as well, ranging from an r^2 of 0.46 to 0.73 (Swackhamer and Hites, 1988). These weak correlation results show that the exclusive use of the octanol/water partitioning coefficient to approximate lipids for bioaccumulation estimates may not be appropriate (Swackhamer and Hites, 1988).

BCFs, Bioaccumulation Factors (BAF) and a third parameter, Biota-Sediment Bioaccumulation Factor (BSAF) are widely used when researching bioavailability (Schwarzenbach et. al. 2003) BCFs are often used interchangeably with BAFs, however traditionally BCF has been used in aquatic environments, and BAF is used to describe all mediums (Stumm and Morgan 1996; Schwarzenbach, 2003). The distinction between the BAF and BSAF occurs when dealing with aquatic systems. In aquatic systems, the BAF compares an organism's POP concentrations to the aqueous POP concentrations, where the BSAF compares the organism's POP concentrations to the sediment POP concentrations. Therefore if the organism of interest lives exclusively in the sediment, BAF=BSAF (Schwarzenbach et. al., 2003). In this research, BAF was used, and is defined as the ratio of mass of contaminant in the organism, the earthworms (by weight and lipid normalized), and the mass of contaminant in the medium, soil:

$$\mathrm{BAF} = \frac{\mathrm{g_{POP}}/\mathrm{kg_{worm}}}{\mathrm{g_{POP}}/\mathrm{kg_{soil}}}$$

The mass of POPs in the earthworms are lipid normalized because the POPs are lipophilic and accumulate in the lipids. This normalization allows comparison between samples, for example different species and/or sizes of earthworms, which contain different amounts of fat (Swackhamer and Hites, 1988).

Several factors can affect bioavailability, especially in a heterogeneous medium such as soil and sediment. These factors include soil characteristics as

well as contaminant aging in the soil and biological factors such as species or size of the organism of interest (Meloche et. al., 2009; Semple et. al. 2003). This demonstrates how bioavailability is specific for soil and organisms. Soil characteristics such as pH, organic carbon (OC) content and particle pore size may make contaminants more or less available (Harmsen, 2007). Also, as previously mentioned the bioavailable portion decreases over time as the soil ages. This is illustrated in Figure 1.2. There are four different routes that POPs can take once they have been introduced into the soil. Some of the total concentration is lost, while the remaining concentration can partition into three different sections of the soil. Some of the contaminant stays available or labile. Some of the contaminant can partition into the inorganic portions of soil, and become irreversibly bound. Finally, contaminants can partition into organic matter, or into nano-pores of the soil particles, and become recalcitrant. Different variables can affect the bioavailability of POPs in soil by increasing or decreasing the recalcitrant portion of contaminants. The recalcitrant portion is not available to organisms, but can be extracted as part of the soil total concentration. By taking advantage of the variables that reduce bioavailability, *in situ* techniques can be applied to assist in remediation of highly POP contaminated soil.

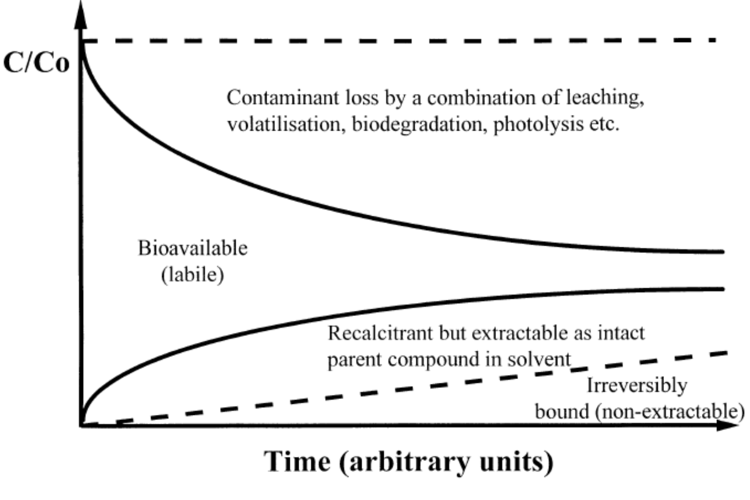


Figure 1.2 - Theoretical diagram showing the four sections POPs partition into once introduced to soil (Jones and de Voogt, 1999)

1.1.4 Methods of Bioavailability Measurement

Traditional methods of analyzing bioavailability of POPs involve extraction of biological samples collected from contaminated sites to determine the total concentration of contaminant in the biological sample (Harmsen, 2007). During biological extraction samples are usually ground or blended and then extracted using exhaustive solvent extraction, for example using Soxhlet extraction with a hexane/acetone mixture or dichloromethane (DCM) (Swackhamer and Hites, 1988; Kucklick et. al., 1994). The organism extraction is similar to the medium extraction, in which the total concentration of POPs contained in the sample will be extracted into the organic solvent (Kucklock et. al., 1994; Gomez-Eyles et al., 2011). These types of methods have several disadvantages, including the need to collect enough biological samples to get data, complexity of sample

processing, expense and the large amount of time and labor involved (Zabiegala et. al. 2010).

Because of these disadvantages, several extraction methods have been developed and used to measure bioavailability as an alternative to biological extraction methods. One method includes mild solvent extraction which determines the ‘readily extractable fraction’ of contaminant by using mild organic solvents and extraction conditions (Semple et. al., 2003; Gomez-Eyles, 2011). For example, a process used to estimate the bioavailability of polycyclic aromatic hydrocarbons (PAHs) involved vortexing soil with butanol for 50 seconds (Gomez-Eyles, 2011). However, these milder solvent extractions still have not reliably predicted the bioavailability when compared to biological extractions (Semple et. al., 2003; Gomez-Eyles, 2011).

Other suggested alternative bioavailability measurement methods, for use in soil and sediments, involve measuring the pore water concentration directly or the use of an adsorbent to extract the contaminant by coming to equilibrium with the pore water phase (Harmsen, 2007). By measuring pore water concentration, which is the freely dissolved concentration, the chemical activities of the contaminants in the multiphasic environmental system of soil and sediment can be assessed (Gschwend et. al., 2011). However, measuring the pore water concentration is very challenging. Collecting sufficient amount of pore water to be analyzed is difficult, and the presence of colloids in the pore water will misleadingly increase the concentration present in the sample (Gschwend et. al., 2011). Therefore, the use of an adsorbent could provide a better method of

analysis. Polymeric samplers can be used to assess the pore water concentrations of contaminants, and the performance and sampling techniques of various polymers have been widely investigated (Gschwend et. al., 2011; Ouyang and Pawliszyn, 2007).

Polymer passive sampling (PPS) devices have been used to measure equilibrium concentrations of pollutants for many years (Mills et. al., 2011). More recently, polymer passive samplers are being used to evaluate contaminant bioavailability, and not just to monitor their concentration and fate. In PPS, the contaminants gather in the polymer much as they do in a living organism. However, the PPS devices are not perfect models of biological organisms, but can be used to model the bioaccumulation or biomagnification or be correlated to biological organism uptake (Zabiegala et. al., 2010; Gaw et. al. 2012). The main benefits of PPS devices are their low tech characteristics such as the elimination of power requirements, ease of use and ease of analysis, and their low cost when compared to active sampling techniques (Zabiegala et. al., 2010; Ouyang and Pawliszyn, 2007). This is particularly important when examining bioavailability because there is often the need for a large number of samples to be taken over large periods of time (Zabiegala et. al., 2010). PPS relies on the difference in chemical potentials of POPs across separate phases resulting in a net flow from one medium to another (George et. al., 2011). PPS calibration and use is based on this free flow of contaminants from the environmental sample matrix to the polymer receiving phase (Ouyang and Pawliszyn, 2007). Following this principle, there are two types of passive sampler

operation methods, non-equilibrium and equilibrium samplers (Mills et.al., 2011; Zabiegala et. al., 2010). Contaminant uptake in PPS is often described by a first-order one-compartment model or two-compartment model. The two types of samplers are based on the behavior of different portions of this thermodynamic equilibrium curve (Mills et. al., 2011).

The non-equilibrium PPSs, also referred to as linear uptake passive samplers, do not reach equilibrium with the environmental medium within the sampling period. This type of PPS is applied most often to aquatic medium (Mayer et. al., 2003). The PPS are sampled at the beginning of the thermodynamic equilibrium curve, when the mass transfer of contaminant from environmental medium to sampler is still linear. This means the samplers give time-weighted average (TWA) concentrations of contaminants. The main requirement when selecting a linear uptake PPS device is that the sampler must act as a zero-sink, which means the sampler takes up all of the contaminants that are transported to it so that none is lost before extraction (Zabiegala et. al., 2009). The main drawback of these types of passive samplers is the need for the sampling rate to be determined in the laboratory, so that the samplers are calibrated before use in the field. Often, performance reference compounds (PRCs) are spiked into the samplers before deployment to increase the reliability of the TWA concentration data. However, this adds an additional pre-deployment for sampler use (Mills et. al., 2011). Also, the calibration done in the laboratory is typically done using distilled water which may not reflect real environmental conditions (Mills et. al., 2011).

The types of PPS devices that have been commonly used as linear uptake devices include: solid-phase microextraction (SPME), often used for air/water sampling, semipermeable membrane device (SPMD), passive *in situ* concentration/extraction sampler (PISCES), membrane-enclosed sorptive coating sampler (MESCO), ceramic dosimeter, and Chemcatcher are all used for water sampling (Ouyang and Pawliszyn, 2007; Zabiegala et. al. 2010). The most common types of polymer used with these techniques are low density polyethylene (LDPE) and polydimethylsiloxane (PDMS).

Equilibrium PPS devices differ from the linear uptake samplers because they are deployed for a longer time, enough for thermodynamic equilibrium between the environmental medium and the polymer (Ouyang and Pawliszyn, 2007; Zabiegala et. al. 2010). This means that there is a stable concentration reached after a certain response time, which is the flattened region of the thermodynamic equilibrium curve when the concentration is approaching equilibrium concentration. The equilibrium time varies with the type of PPS, ranging from seconds to months (Ouyang and Pawliszyn, 2007). The basic requirements for equilibrium PPS devices are: 1.the extraction medium reaches equilibrium with the environmental medium by reaching stable concentrations in the polymer; 2. this equilibrium time must be reasonably short; and 3. the PPS should not remove a significant portion of, or deplete, the POP concentration in the sample (Mayer et. al., 2003; Wilcockson and Gobas, 2001).It is also important to choose a polymer that will not be contaminated by lipids or other organic matter during extraction (Wilcockson and Gobas, 2001).

When choosing a method and type of PPS device, it is important to select properties that maximize performance. To minimize equilibrium times, some PPS have turbulence applied to the sampling matrix, increasing the rate of uptake (Mayer et. al., 2003; Gschwend et. al., 2011). Another factor affecting equilibrium times is the surface-to-volume-ratio (A/V). Generally a high A/V ratio indicates a fast sampling device (Mayer et. al., 2003). To avoid depletion of contaminants in the sample medium, it is ideal to keep the amount extracted into the sampler below 5% of the total contaminant concentration in the sample (Mayer et. al., 2003). Finally, to confirm equilibrium has been reached, three methods are commonly applied, as described in Mayer *et. al.* 2003. The first is to measure concentration in the sampler versus time, until the concentration levels out. The second is to simultaneously extract two passive samplers, one of which is spiked with contaminant(s) at a concentration above the equilibrium concentration. The convergence between the spiked and clean samplers occurs at equilibrium. The final is to use multiple coating thicknesses for the same polymer (Reichenberg et al., 2008). The thin coating will take up the contaminants at a faster rate, so once the two samplers reach a similar/equal concentration, equilibrium has been achieved.

Types of equilibrium PPS devices that have been researched include SPME, SPMD, empore disks, and diffusive multi-layer samplers (Zabiegala et. al., 2010). However, SPMDs and empore disks are not ideal for very hydrophobic organic substances, such as those investigated in this research. This is because they tend to have long equilibrium times and often extract a significant fraction of

the chemicals from the sample (Wilcockson, J.B., and Gobas2001). Equilibrium PPSs that have proven more applicable include polymer sheets/strips, polymer beads and thin coatings of polymer applied to other mediums, most often glass (Gschwend et. al., 2011). The polymer sheets and glass fibers coated in polymers, SPME fibers, can be directly inserted into the sample matrix, eliminating the need for bulk sample collection (Ouyang and Pawliszyn, 2007). Often polymer beads and strips of polymer are tumbled with soil or sediment for extended times making use of turbulence to minimize equilibrium time (Gschwend et. al., 2011). Thin films of polymer have been used to coat the inside of glass vials, which can be loaded with contaminated sample (Wilcockson and Gobas, 2001; Reichenberg et. al., 2008). This technique is known as thin-film solid-phase extraction (TF-SPE) and takes advantage of high A/V ratios to lower time to equilibrium.

Calibration of the equilibrium PPSs is based on the equilibrium partitioning coefficient of the analytes between the polymer phase and the sample, Kps. The bioavailable concentrations can be estimated by the equation:

$$C_S = C_P / K_{PS}$$

Often, these partition coefficients are determined in the laboratory prior to PPS use, and when concentration in the polymer (C_P) is determined, it is used to deduce concentration in the sample (C_S) (Mayer, et. al., 2003; George et. al., 2011; Gschwend et. el., 2011).

While pore water concentration is the amount of contaminant that is “freely available”, it is not a direct measurement of bioavailability. The bioavailable concentration is not only the amount in the pore water, but may include the amount of contaminant that desorbs during the time an organism is in contact with the soil and/or the amount of contaminant that can be taken up through alternate routes such as digestion (Harmsen, 2007; Shang et. al., 2013). The only direct way determining if the organism or food chain is protected, is the direct biological measurement of that organism (Harmsen, 2007). However, a potential surrogate method can be validated against biological measurement (Gaw et. al., 2012). This is accomplished by correlating an equilibrium passive sampler to biological data.

1.1.5 Thin- Film Solid-Phase Extraction

TF-SPE is a unique polymer passive sampling technique, and has many benefits. As mentioned, there are a large number of variables that can affect the bioavailability of POPs. Soil properties, such as pH and OC content can affect the concentration available for uptake, and the type of organism can take up the contaminant to varying degrees. Even different species of earthworms show different amounts of bioaccumulation. Also, there are multi- species (flora and fauna) interactions that affect uptake (Kelsey and White, 2005). This means that to accurately assess risk, a correlation between a polymer TF-SPE to individual organisms should be established. This correlation then allows for large number of samples to be collected across a contaminated site and be easily and quickly assessed.

One characteristic of TF-SPE that makes it a good technique to use when establishing a correlation is the large A/V ratio. This large A/V relationship allows the contaminants in the sample media, in this research soil, to quickly achieve an equilibrium distribution with the thin-film solid-phase, in this research ethylene vinyl acetate (EVA) (Meloche et. al., 2009). EVA has been shown to make an appropriate polymer for TF-SPE when examining hydrophobic substances such as polychlorinated biphenyls (PCBs), organochlorine pesticides and current use pesticides (Gaw et. al., 2010; Meloche et. al., 2009). It's also been shown that the EVA TF-SPE method is sensitive enough for real-world contaminated site concentrations (Meloche et. al., 2009).

The use of PPSs has mostly been applied to aquatic environments; the mediums examined being water and sediment. Studying contamination in soil changes the type of PPS that can be easily used. It is more difficult to insert polymer sheets into soil compared to sediment. The coated vial is a more ideal fit, it is easy to load with soil, and the soil does not need any extra processing compared to the total concentration samples. Also the method for coated vial TF-SPE is simple and doesn't require the same extensive extraction and subsequent clean up procedures needed in some methods (Wilcockson and Gobas, 2001).

When evaluating the TF-SPE performance, the application of a mathematical model to time dependent C_P data is used. As previously mentioned when discussing equilibrium confirmation, time-dependent polymer concentration data proves that equilibrium between sample and polymer has been reached. Beyond that, the true equilibrium concentration of the polymer can be calculated

using the mathematical fit. Often, instead of determining the pore water concentration from the polymer concentration, this true equilibrium polymer concentration is correlated to biological extraction concentration data.

1.1.6 Bioavailability Reduction Strategies

Because bioavailability expresses the true risk of POPs, research into the reduction of the bioavailable portion of POPs in soil is important as it could provide simple and inexpensive *in situ* remediation techniques. The abundance of POP contaminated sites means that the reduction of risk is potentially widely applicable. Ideally, TF-SPE can be used to evaluate the effectiveness of potential reduction strategies faster and easier than biological extraction.

It has been well documented that soil aging reduces the bioavailability of POPs (Jones and de Voogt, 1999; Reid et. al., 2000; Alexander, 2000). As previously mentioned, this is displayed in Figure 1.2. There is a reduction in total concentration of contaminants from degradation, volatilization and other activities, but also an increase in irreversibly bound and recalcitrant portions of POPs (Jones and de Voogt, 1999). This increase is caused by various soil-compound interactions with the organic and inorganic constituents of soil matrix. The POPs can be located in either exterior or interior sites within the soil matrix, or become absorbed into soil components (Reid et. al., 2000). This is illustrated in Figure 1.3.The exterior sites, which exist freely in the pore water, are easily accessible and so contain the bioavailable fraction of contaminants. The interior sites, such as nano-pores, allow little to no desorption of contaminants, and therefore the contaminants have a very low bioavailability when sorbed onto

these sites and are recalcitrant (Reid et. al., 2000). When contaminants enter the solid phase of organic and inorganic matter and become absorbed, they become inaccessible. This sorption and diffusion into the soil particles is known as sequestration (Harmsen, 2007; Smple et. al., 2003). The contaminants that enter the inorganic particles become irreversibly bound, whereas the contaminants that are sequestered in the organic matter are part of recalcitrant portion.

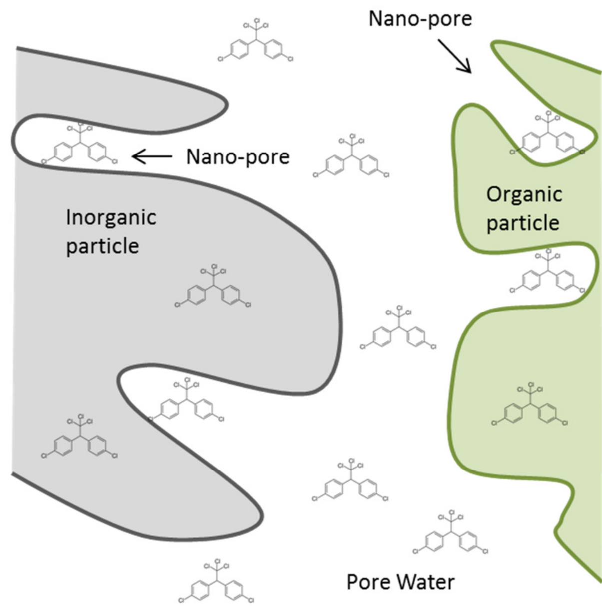


Figure 1.3 – Theoretical illustration of contaminant partitioning in soil. One portion stays in the pore water, one portion is sequestered in nano-pores a partitioning into organic matter, and the final portion becomes irreversibly bound by partitioning into inorganic particles.

This extensive characterization of POP interactions, sorption and sequestration with soil matrix allows bioremediation techniques to be proposed and investigated. By encouraging the aging characteristic of recalcitrance, the bioavailability can be lowered (Jones and de Voogt, 1999). One technique that makes use of this is the addition of amendments high in organic matter to contaminated soil to improve soil conditions (Lunney et. al., 2010). This

improvement is accomplished by an increase in the amount of contaminant that is recalcitrant, therefore removing it from availability. The type of amendment used to add organic matter to soil can affect the amount of bioavailability reduction. Part of the recalcitrant contaminant concentration is the compounds trapped in nano-pores of soil particles. Therefore, amendments with organic matter containing more nano-pores have a higher potential for bioavailability reduction.

Another remediation technique that has been researched is the use of phytoremediation. The phytoremediation technique involves plants removing POPs from the environment through two routes. One route is degradation in the rhizosphere and the other route involves contaminants crossing the plant root barrier, where it can then be degraded, transpired or stored in plant tissue (White et. al., 2007). Phytoremediation is an inexpensive technique, and several plants have proven effective at reducing POP concentrations including *Cucurbita pepo* (includes zucchini and pumpkin) (Kelsey and White, 2005). However, the amount of POPs removed by this method is not very high due to the high recalcitrance of POPs in soil. Amendments to increase the accumulation of POPs by plants have been investigated, but this adds additional time and cost to this remediation technique (White et. al., 2007).

1.2 Scope of Work and Objectives

It has been well established that measuring the bioavailability of POPs in soil is useful when estimating risk to environmental and human health, as opposed to POP total concentration measurements. In POP contaminated soil,

the more of the pollutant that is sorbed and recalcitrant, the lower the risk posed by the contaminant. Traditional biological assays to assess bioavailability are often expensive, time consuming and require a large amount of work for collection and processing. When researching bioavailabilty of POPs in soil samples, such as assessing the viability of remediation actions or the variability of bioavailability in soil, an alternate method could be advantageous.

It has been illustrated that the TF-SPE method using EVA polymer has effectively assessed the bioavailability of soil-bound POPs to earthworms as the polymer coating used has similar sorptive capacity to earthworm lipids. During a controlled pot study, it was shown that there was a strong correlation between the soil-polymer equilibrium concentrations and *L. terrestris* BAFs for DDX, DDE and dieldrin (Andrade et al., 2013). Continuing research into this method by investigating on site contaminated soil conditions can prove its potential as a bioavailability screening tool.

The main goal of this research is to examine bioavailability of POPs from contaminated soil to native earthworms. Part of this study is assessing whether the established EVA TF-SPE methodology applies when compared to native earthworm biological assays. With TF-SPE application, there is the potential for easier analysis of factors affecting bioavailability such as field management practices or remediation efforts. To accomplish our goals, the objectives of the research were:

1. Develop and validate an accelerated extraction methodology to quantify soil and earthworm POP concentrations.

2. Assess the effectiveness of previously developed abiotic methodology in mimicking bioavailability to native earthworm populations from PBDE contaminated soil.
3. Utilize the EVA TF-SPE method to assess bioavailability across a DDT and dieldrin contaminated site.

Chapter 2: Materials and Methods

2.1 Site Descriptions

2.1.1 DDT and Dieldrin Contaminated Site

An abandoned orchard site on the USDA Beltsville Agricultural Research Center (BARC) campus in Beltsville, MD contains DDT and dieldrin contamination due to historical pesticide use, up until the 1970s. The soil concentrations of the POPs were determined to be above the United States-Environmental Protection Agency (US-EPA) regulated levels when sampled in the early 1990s. The orchard, along with a neighboring storage barn and pesticide mixing area, were incorporated into a US-EPA Superfund site at BARC, which includes pesticides and other contaminants throughout BARC property. In 2010, a contractor sampled soil, earthworms and small mammals from the contaminated fields for the USDA (Figure 2.1). None of the wildlife exceeded the criteria for harm and the soil levels were found to have decreased, but were still high. The area that had encompassed the storage barn and pesticide mixing area, known as BARC 04, was highly contaminated and soil had to be removed (Figure 2.1). However, in an effort to minimize the area of soil that had to be removed, an alternative remediation effort for the rest of the site, labeled BARC 19, has been proposed.

A broader research project to assess the effectiveness of organic amendments and phytoremediation for *in situ* remediation of DDT and dieldrin is currently being carried out by an USDA/USGS/UMD collaboration effort. The aim

of the project is to perform *in situ* bioremediation, primarily by adding organic amendments to reduce the bioavailability of the POPs to earthworms, and therefore the terrestrial food chain. A second potential bioavailability reduction strategy investigated was phytoremediation using orchard grass (*Dactylis glomerata* L.). An initial experiment, a pot study in the controlled environment of a growth chamber using seeded earthworms, was carried out in 2011. During this pot study, 6 amendments were incorporated into contaminated soil collected from the BARC 19 area (Figure 2.1). The amendments were: dairy manure compost, aged four months (4-mo. Compost); dairy manure compost, aged two years (2-yr. Compost); Orgro®, a biosolids compost (Biosolids Compost); pine biochar pyrolyzed at 500℃ (Biochar); and lime stabilized Biosolids. The 2-yr compost was applied at two different rates, the same rate as the other amendments, 112 dry t/ha, and an increased rate of 224 dry t/ha. This higher rate of the 2-yr compost was the 6th amendment. Eight 4L pots of each amended soil were prepared: 4 without plants and 4 planted with orchard grass. After being left for 45 days to stabilize, each pot received 12 *Lumbricus terrestris* (Linnaeus) earthworms and then was allowed to incubate for an additional 45 days. The methods discussed in this research were used to determine total soil and earthworm concentrations after the incubation period. Results are discussed in detail in the results and discussion section, however, the main findings of the pot study were the noticeable bioavailability reduction using manure compost as an amendment. The 4 mo. Compost showed the largest reduction in bioavailabilityfor most compounds, and the 2 yr. compost showed significant

reduction as well. Along with the promising results, these two amendments were relatively inexpensive and easily accessible, therefore they were chosen to use in the next phase of the study.

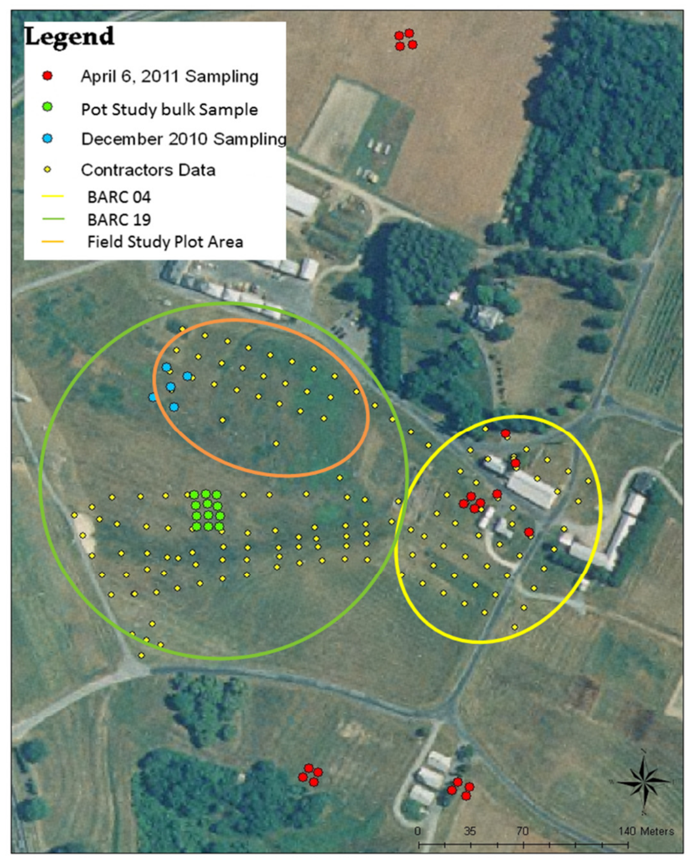


Figure 2.1 – DDT and dieldrin contaminated site on the USDA-BARC campus.

The project has progressed to a second phase, a pilot study in the BARC contaminated field using the two chosen forms of manure compost. Seven field plots, 7.3m x 12.2m, were randomly positioned over the contaminated site (Figure 2.1). Each plot was split into 4 sub-plots, 3m x 7.3m, each of which received one of the designated treatments: compost A tilled; compost B tilled; no amendment tilled; and no amendment and no till. The two types of manure compost were compost A, that had been aged for 2 months and compost B,

which had been aged for 2 years. These composts were obtained at BARC. The application rate was approximately 250 dry t/ ha. The compost was applied using a skid loader, and it was determined that this application rate would require approximately 2 skid loads, if the loader bucket was shaken so that the level was just below even. Before compost application, there was an initial, time zero sampling for this field study, which is discussed as part of this research.

2.1.2 Biosolids Applied Farms

Biosolids are applied as soil amendments in agricultural fields, and this application is regulated in the US by 40 CFR Part 503. However, the regulation does not currently cover organic pollutants that may be introduced into the soil through this application. It is well known that there are organic pollutants found in biosolids, in particular, PBDEs have been found in biosolids samples throughout the world, and appear to be at the highest levels in the US (Andrade et. al., 2010). Previous research in our group has been carried out to determine the soil concentration of PBDE congeners on a number of farms which have received biosolids applications throughout Virginia (VA), USA (Andrade et. al., 2010). Andrade *et. al.* 2010 reported a large amount of PBDE concentration data collected at 27 farm fields which had received biosolids application.

The fields were sampled in 2006 and again in 2009, with multiple samples taken throughout each field. Because of the historical concentration data available, two of the previously sampled farms were selected for bioavailability analysis, one in Orange County, VA and one in Fauquier County, VA. Criteria for selection included high contamination levels and varied field

management practices. The high PBDE concentrations were desired as they presented higher risk and a high concentration would assure breach of the detection limit during TF-SPE analysis. Because fields that had received multiple biosolids applications had concentrations that were significantly higher than those with just one application (Andrade et. al., 2010), only multiple applications fields were considered. The two selected farms had different field management practices. The first farm field was used for grazing, and the second was used for crop production, most recently corn. These farms had been labeled as MA2 and MD1 in Andrade et. al. 2010. The high concentration, as well as the variability in concentration for field MA2 is illustrated in Figure 2.2.

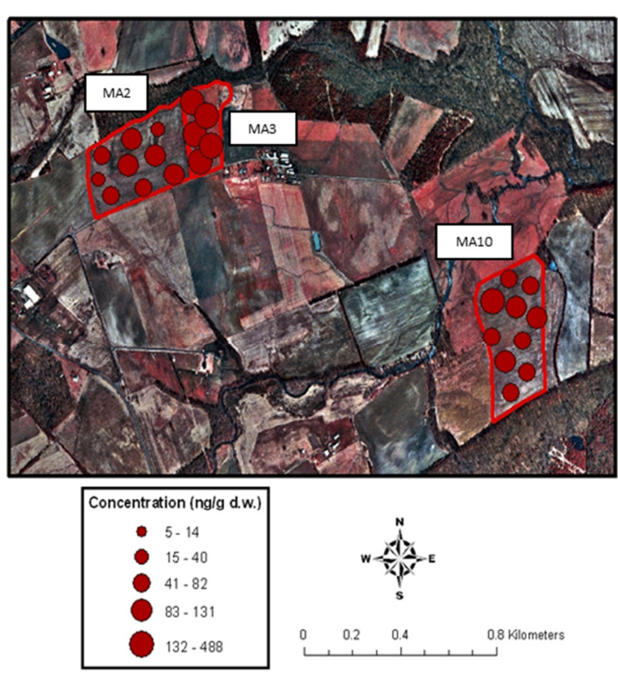


Figure 2.2 - Total PBDE concentrations observed for farm MA fields in 2006

Andrade 2012 discussed the effects of field management practices, which seemed to indicate that tilling and planting results in lower total concentration of

PBDEs in the soil, most likely due to distribution of the chemicals further down in the soil profile. Also, there is high variability across contaminated sites, illustrated in figure 2.2, most likely due to non-uniform application of biosolids and soil heterogeneity (Andrade, 2012). Another observation from Andrade 2012 was the understanding that after biosolids application, soil PBDE concentrations increase, however, after 3-4 years of no subsequent biosolids applications the concentrations tend to decrease. The investigation into bioavailability carried out during this research benefitted from these observations and the large amount of historical data on the sampling sites. The characteristics of the two sites that were chosen for this research are summed in Table 2.1.

Site Location	Field use at time of sampling	Time span of biosolids applications	Total amount of dry sludge applied (ton/acre)	Soil carbon content (%) from 2009	Predominant Soil Type
Orange County, VA	Pasture	1997-2009	34	3.09	Fluvanna loam
Fauquier County, VA	Corn Production, harvested	1995-2012	20	1.58	Catlett loam

Table 2.1 – PBDE contaminated site characteristics

2.2 Sampling Protocol

2.2.1 Soil and Earthworm collection

The larger research project being carried out at the DDX and dieldrin contaminated field required a time zero sampling before addition of amendments to get an initial measure of bioavailability. The sampling area of each sub-plot was 2m x 6m, which was inside the application area of 3m x 7.3m. Sections of soil were collected to a 15cm depth randomly throughout each sub-plot using a shovel, and the soil was broken apart by hand (Figure 2.3a). Any earthworms

found were placed in glass jars for future processing (Figure2.3b). During sampling, the aim was to collect 30g of earthworms, ensuring enough sample for replicate analysis. Two grams of earthworm dry weight per replicate was the ideal amount needed for extraction, with 1g per replicate the minimum weight required. Knowing that the average percent moisture of the pot study earthworms was approximately 75%, 30 g was determined to be sufficient. One 19 L container of soil from each sub-plot was collected, ensuring that soil from each hole was included in the sample.

At each of the two farms where biosolids were applied, one 1.5m^2 area was dug to 15 cm depth. The soil was placed on top of a tarp, and was broken apart by hand (Figure 2.3a). Any earthworms were located, removed, and collected in glass jars (Figure 2.3b). Two 19 L buckets of soil from throughout the collection area were taken. All PBDE processing and analysis was done in a laboratory space with light filters to block wavelengths below 620nm to minimize photodegredation of PBDEs (Andrade et. al., 2010).

Figure 2.3 – a) Soil being broken apart by hand to locate earthworms. b) Earthworms collected on site

2.2.2 Earthworm Processing

The earthworm samples were kept alive for 1-2 days after sampling in glass jars filled with soil from their respective collection sites. The first processing step was to identify the predominant species of earthworms. The DDX and dieldrin contaminated field plots contained two main species of earthworms, *Aporrectodea turgida* (Eisen) (Figure 2.4a) and an unidentified *Lumbricus* species. The two VA farm sites contained both *Allobophora chlorotica* (Savigny) and *Octolasion tyrtaeum* (Savigny) (Figure 2.4b), and the crop production site also contained an unidentified *Lumbricus* species. The process of identification began by measuring earthworm length and color. This allowed the species to be narrowed down. From there, the earthworm clitellum was examined to assist in determining the earthworm species; this is often the best feature for determining a particular species. A magnifying glass was used to inspect the area for final species determination. The clitellum is the region of epidermal swelling, which contains gland cells that secrete material to form a cocoon (Reynolds, 1977). In the clitellum region there are two structures that have unique placement or shape depending on species. These are the tubercula pubertatis(TP), a glandular swelling near the ventrolateral margins of the clitellum and the genital tumescences(GT), which are unique markings expressed as swellings, pits or grooves of the epidermis (Reynolds, 1977)

Figure 2.4 – a) *A. turgida* located in the DDT and dieldrin contaminated soil b) *O. tyrtaeum* (green muddy color) and *A. chlorotica* (pink/grey) located in the PBDE contaminated soil

As an example of the identification process, the main earthworm species at the two VA farm sites were small (<55mm) in length and had a muddy green color. Two VA area earthworms of this description are *A. chlorotica* and *Eisenella tetraedra* (Savigny). The clitellum of several of the specimens collected was examined using a magnifying glass. The earthworms exhibited small, sucker-like discs on the clitellum segments, which were TP. This means that the earthworms were *A. chlorotica,* otherwise known as the green worm (Reynolds, 1977). The *E. tetraedra* have long TP along the clitellum rather than the sucker-like discs seen on the samples (Reynolds, 1977). This procedure was followed for all earthworm samples.

The unidentified *Lumbricus* species mentioned were not able to be properly identified due to similarities between several of the species. For example, two of the potential types are *Lumbricus festivus* (Savigny) and *Lumbricus rubellus* (Hoffmeister). Both are similar size and color, medium size

around 80mm and darker color that was ruddy brown or red. The difference on the clitellum is that the *L. festivus* has GT on only half of the clitellum segments and the *L. rubellus* had GT on all segments of the clitellum (Reynolds, 1977). This difference was not able to be determined on the samples collected in this research.

After identification, the earthworms collected at the farms where biosolids were applied went through an additional step, known as depuration. This involved removing the earthworms from the soil and placing them on moist filter paper for 24 hours. These conditions allow approximately 95% of the gut contents to be evacuated (Jager et. al., 2005). The larger DDT and dieldrin research project procedure called for the earthworms to not be depurated.

All earthworms were rinsed with deionized (DI) water, placed in clean glass jars, and frozen at -15 °C. The earthworms were later freeze-dried for one week at 25 °C, and 5-10 mtorr pressure (Virtis freeze-dryer). Once all of the moisture was removed during freeze drying, the worms were ground. This was accomplished by placing each sample into a stainless steel blender and adding dry ice to assist in grinding and to reduce loss of sample. For the DDT and dieldrin contaminated field, all of the earthworms from each sub-plot were ground into one sample. For the fields where biosolids were applied, all of the earthworms from each field were ground into one sample. Samples were then stored in a freezer at -20°C until extraction.

2.2.3 Soil processing

Soil samples were sieved to 4mm. The DDT and dieldrin contaminated field was sieved on site and the PBDE contaminated soil was sieved two days after collection in the laboratory. Sieving removed any non-organic matter (rocks etc.) or plant matter that would not be used as food by the earthworms or contain extractable/available POP concentration. Soil was then mixed to create a more homogenous sample. The DDT and dieldrin contaminated soil was mixed by rolling each bucket across a tarp four times, stopping at each end to invert the bucket twice. For the PBDE contaminated soil, the two buckets from each site were emptied into a concrete mixer, and mixed for 20 minutes. The mixer was stopped every 5 minutes and any soil stuck on the sides of the mixer was cleared by hand. Once well mixed, soil samples were stored in a freezer at -20℃ until extraction, to minimize or eliminate any degradation of contaminants.

Soil moisture was determined for each sub-plot and each field where biosolids were applied by spreading approximately 10 g of soil in a thin layer on an aluminum weigh boat, and baking the soil at 100℃ for at least 4 hours. Previously, soil with 25% moisture was dried for 8 hours, sacrificing one replicate every half hour, and no change in weight was seen after 4 hours. The difference in weight was used to calculate soil moisture content. Six replicates for each of the fields where biosolids were applied, three from each bucket, and four replicates for each sub-plot of the DDT and dieldrin contaminated soil were used. The average was determined and utilized in extraction preparation. The data was quite consistent between replicates. For the soil from the fields where biosolids

were applied the differences were 0.43 and 0.48% respectively. The DDX soil moisture percent differences ranged from 0.14 to 2.05%.

2.3 Soil and Earthworm Concentration Analysis

2.3.1 Extraction

Soil and earthworm samples were extracted using a Dionex Accelerated Solvent Extractor (ASE) 350. The parameters of the extraction method were: preheat time: 5 minutes; temperature: 120℃; pressure: 2000 psi; static time: 10 minutes; solvents used: 20% Acetone, 80% Hexanes; flush: 60%; purge time: 200 seconds; cycles: 2. All solvent HPLC grade, Fisher Scientific, Pittsburgh, PA, USA. Samples were stored in -20℃ freezers before sample preparation for extraction. Earthworm and soil samples were allowed to reach room temperature before extraction preparation.

Using the moisture content of the soil, 2.0 g (dry weight) of soil was weighed into an aluminum weigh boat. Based on the moisture content, enough hydromatrix (Agilent Technologies, Santa Clara, CA, USA) an inert diatomaceous earth sorbent, was blended with the soil sample using a mortar and pestle. The hydromatrix sorbs the water, keeping it from being extracted. A 22mL stainless steel ASE cell was prepared for each sample replicate. An ASE cellulose filter (Thermo Scientific, Sunnyvale, CA, USA) was placed on the bottom of the cell, and a stainless steel cap was screwed on to keep the filter in place. The filter was covered with oven baked sand (JT Baker purified, washed and ignited, VWR, Philadelphia, PA, USA). The ground soil and hydromatrix was then added. More oven baked sand was used to fill the cell. Another cellulose filter was

placed on top, and another cap was screwed onto the top of the cell. Cells were loaded onto the ASE, and 60 mL amber collection vials were loaded onto the collection carousel. For PBDE analysis, 10 replicates for each of the two fields were extracted. For the DDT and dieldrin contaminated samples, three replicates for each sub-plot were extracted.

2.3.2 Analysis

After extraction any remaining water in the soil extracts had to be removed before samples could be analyzed using the gas chromatography/mass spectrometry (GC/MS). The soil sample extract was filtered through anhydrous sodium sulfate to achieve this additional drying. This was done by adding a plug of glass wool to a long-stemmed glass funnel, and adding approximately 5 g of sodium sulfate. The sodium sulfate was wet with approximately 5mL of hexane before filtering the sample extract. The sample was filtered into a clean 60mL amber collection vial.

Earthworm samples required lipid content analysis. The lipids were removed from the sample extract so that they would not interfere with the GC/MS analysis. Sample extracts were reduced to 4.0 mL using a Zymark TurboVap evaporator with a water bath set to 40°C. The 60mL sample collection vials were placed into the TurboVap and Nitrogen gas was blown on the samples at a pressure of 0.4-0.6 psi for approximately 30 minutes. Samples that were dried beyond the 4.0 mL mark were brought back to 4.0 mL using hexanes. One mL of the sample was transferred to a pre-weighed GC vial, and then re-weighed. After

24 hours, the solvent had evaporated and the sample was weighed again to determine the weight of the lipids.

The 3.0 mL of PBDE contaminated extract remaining was evaporated to dryness using the TurboVap. Five mL of acetonitrile was then added to the collection vials. The POPs of interest were dissolved in the acetonitrile but the lipids were not. The samples were vortexed for 15 minutes, and the solution was transferred to a clean 60mL amber collection vial. A second 5 mL aliquot of acetonitrile was added, vortexed and transferred to the final collection vial. The lipids remaining in the original collection vial were discarded.

The soil extracts and 10mL acetonitrile earthworm extracts were evaporated to complete dryness with Nitrogen gas at a pressure of 0.6-0.8 psi for 45 minutes to 1 hour. Each sample was then reconstituted with 2.0mL of hexanes and was vortexed for approximately 2 minutes. An internal standard used for GC/MS calibration was added to each sample. An internal standard helps compensate for many random and systematic errors by plotting the ratio of the analyte's signal to the internal standard's signal during calibration. There are several characteristics to look for when selecting a compound to use as an internal standard. The compound should provide a signal similar to the analyte's signal(s), but be distinguishable from the analyte's signal(s). The internal standard must not be in the sample matrix but should have the same matrix effects as the analytes (Skoog et. al., 2007). For the DDT and dieldrin contaminated soil, 40µL of 540.584 ng/µL pentachloronitrobenzene was added to each sample, to give a final concentration of 10.812 ng/mL. The DDT and dieldrin

earthworm and EVA coated vial (discussed later) samples had 40µL of 27.03 ng/µL solution added to each extract sample, for a final concentration of 0.5406 ng/mL. For all of the PBDE contaminated samples, 10µL of 4µg/mL $^{13}C_{12}$ labeled polychlorinated biphenyl 138 (PCB 138) was added to each sample to give a final concentration of 40 ng/mL.

The samples were transferred from the 60mL amber collection vials to 2.0mL amber GC sample vials. Two different GC/MS configurations were used for analysis. For DDT and dieldrin, an Agilent 6890 GC was coupled with an Agilent 5973 MS detector in electron impact (EI) mode. The 5975 mass spectrometer (MS) was in electron impact (EI) ionization mode. The capillary column was a DB-5-MS with a length of 30m, inner diameter of 0.25mm, and film thickness of 0.25µm (Agilent J&W Scientific, Folsom, CA). The carrier gas was helium at a constant flow of 1.2 mL/min. The oven program was: 70℃ for 1 min, increase of 20℃/min to 210℃, hold for 1 min, increase 5℃/min to 280℃, hold for 1 min, increase 20℃/min to 300℃ and hold for 10min. The injection volume was 1µL, and a split/splitless inlet was used with a temperature of 210℃, pressure of 75.9 kPa and purge flow of 30mL/min for 1 min. The GC/MS interface was kept at a constant temperature of 280℃. Sample analysis was done using the internal standard method. The soil total concentration samples were analyzed with a six point calibration curve, and the earthworm and EVA coated vial samples were analyzed using a more dilute five point calibration curve.

PBDEs were analyzed as described in Andrade *et. al.* 2010. The procedure used an Aglient 6890 GC coupled with an Agilent 5975 MS detector in

negative chemical ionization (NCI) mode. The capillary column used was DB-5-MS with a length of 15 m, inner diameter of 0.25mm and film thickness of 0.1µm (Agilent J&W Scientific, Folsom, CA). The carrier gas used was helium with a constant flow of 1.3 mL/min The oven temperature program was: 48℃ temperature, increase of 25℃/min to 210℃, hold 10 min, increase 25℃/min to 310℃, hold for 9.52 min. The injection volume was 1µL. A programmable temperature vaporization (PTV) inlet was used with the temperature program: 51℃ increase 600℃/min to 300℃, hold 10 min. The injection pulse pressure was 280kPa until 2 min. Purge flow to the split vent was 500mL/min at 1.98 minutes. The GC/MS interface was kept at constant temperature of 300℃. An internal standard method was used for MS analysis. For soil concentration a six point calibration curve was used, and for the earthworm and TF-SPE samples a lower concentration six point calibration curve was used.

For the DDT and dieldrin analysis method, 6 analytes were included: 2,4'-DDE, 4,4'-DDE, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT and Dieldrin. The detection limit for the method was 0.8 µg/mL for the soil analysis and 0.05 µg/mL for the earthworm and EVA coated vial analysis. The PBDE congeners included in the analysis method were BDE 28, BDE 47, BDE 100, BDE 99, BDE 154, BDE 153, BDE 183 and BDE 209. For the TF-SPE and earthworm samples the detection limit was 0.08 ng/mL, and for the soil it was 0.8 ng/mL. For soil analysis the BDE 209 congener signal was present, however, the signal was lost during coated vial analysis. Due to extenuating circumstances, the lack of BDE 209 analysis for the

earthworm and EVA coated vial samples was not able to be remedied expediently, and could not be discussed here.

Part of the quality control for the analytical methods was the addition of a surrogate to the samples before extraction. A surrogate is a compound that has similar properties to the analytes of interest, but is not present in the sample (Kebbekus and Mitra, 1998). It is added to the sample before extraction and gives a measure of the performance of extraction method by giving the percent recovery. In this research, ^{13}C p,p'-DDT was used as the surrogate for the DDT and dieldrin analysis, and PCB 209 was used as the surrogate in the PBDE analysis. The concentration of surrogate that was spiked into the samples was approximately the mid-point of the calibration curve concentrations. For example, for DDT and dieldrin soil samples, the range of the standards used in the calibration curve were 1 ng/µL to 12 ng/µL, and the amount of surrogate used in the samples was approximately 6 ng/µL.

For PBDE analysis, the sample and spike surrogate recoveries for soil analysis were 92±13% (n=22). Replicate samples resulted in an average percent difference of 8.5±5.2% for all analytes. Sand spike recovery of all analytes was 82±4%. Average earthworm spike recovery for all analytes was 99.9±0.3%(n=1) and average earthworm sample surrogate recovery was 71.7±4.0% (n=4) Because the TF-SPE samples did not go through a traditional extraction, the surrogate would not serve the same purpose, and so is not discussed here.

For DDT and dieldrin analysis, recoveries were calculated separately for the Pot Study and Field Study samples, as different preparations of standards

were used for each. For soil analysis, replicate samples resulted in an average percent difference for all analytes of 7.3±5.7%(n=165) for the pot study and 4.1±3.4% (n=70) for the field study. The surrogate recoveries for samples and spikes were 92.7±8.0% (n=107) for the pot study and 73.0±14.3% (n=70) for the field study. Average sand spike recovery for all analytes was 93.7±4.5% (n=17) for the pot study and 91.9±7.9% (n=5) for the field study.

2.4 TF-SPE Procedure

TF-SPE was carried out by coating 20 mL vials with an EVA solution as per Meloche *et. al.*, 2009. A 6.21 g/L solution of EVA (ELVAX® 240 resin, DuPont, Wilmington, DE, USA) in dichloromethane (DCM) (HPLC grade, Fisher Scientific, Pittsburgh, PA, USA) was prepared. For every 100mL of solution, 10mg of SudanIV red dye was added to the solution. The dye was useful for assuring an even coating, and in loss prevention during polymer extraction. Twenty five µL of EVA solution and 5µL of a 2% dichlorodimethylsilane(CAS# 75-78-5, Chemtrec, Falls Church, VA, USA) solution was added to a 20mL amber vial, and the vial was capped. The silane solution assisted the polymer in adhering to the glass vial. The vial cap was slowly removed while rotating the vial slowly by hand. The DCM slowly evaporated, leaving a 0.48 µm thick coating in the vial. The vials were baked at 40℃ for 1 hour to cure the polymer coating.

Vials were prepared in advance, and stored at room temperature until sample addition. For each PBDE contaminated site, 135 coated vials were prepared and for each DDT and dieldrin contaminated sub-plot, 27 vials were prepared. The PBDE concentrations in the soil were relatively low, and multiple

vials were required for each replicate sample. Vials were extracted at 9 times points, ranging from 1 hour to 45 days.

After soil processing, the coated vials were loaded. Before the soil was added, the appropriate amount of water was added to the coated vial so all samples were 33% moisture. This was to eliminate moisture content as a variable. A 33 % moisture content was chosen for several reasons. First, this moisture content was previously used in Andrade *et al*. 2013 for EVA TF-SPE analysis of soil samples. Secondly, all samples initial moisture was less than 33%, so none of the samples had to be dried before TF-SPE. Thirdly, supervision of a small project examining the effects of moisture on the uptake of DDT and dieldrin to EVA which was carried out by a USDA high school intern, Kayla Harley showed 33% to be a good moisture content to use. As seen in Figure 2.5 dieldrin and p,p'-DDE show differing EVA uptake behavior with changing moisture content. p,p'-DDE appears to absorb at a higher rate at lower moisture content, however dieldrin shows lower absorption at low moisture content. Thirty three percent moisture shows decent uptake into EVA of both p,p'-DDE and dieldrin.

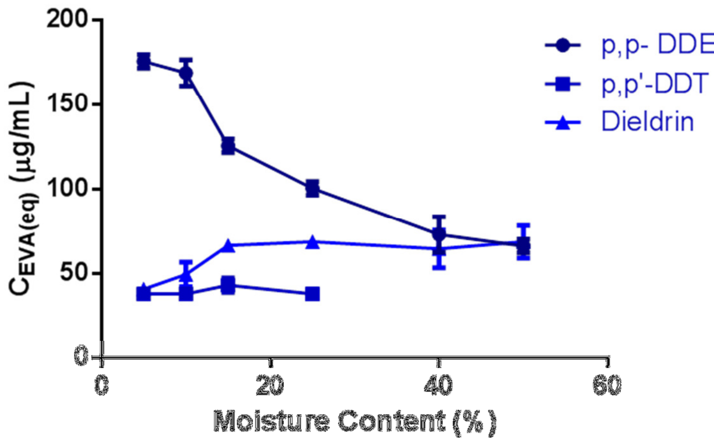


Figure 2.5 – USDA intern coated vial experimental results showing EVA concentration of p,p'-DDT, p,p'-DDE and dieldrin with respect to moisture content

Approximately 30 g (wet weight) of soil is needed to completely fill the vials. For vial loading, the empty coated vial was weighed, and then the appropriate amount of water that was needed to bring the soil moisture to a final amount of 33% was added. The vial was weighed again, and approximately 30 g of soil was added. The vial was gently tapped when needed to ensure a complete fill, and once filled was capped. Vials were left at room temperature until their assigned extraction time.

At the designated extraction time, the vials were emptied by vigorously shaking out the soil. The vial was rinsed several times with organic free water to remove any remaining soil particles. The vials were then centrifuged at 2650 rcf for 5 minutes. The residual water was removed using a pipette. An aliquot of 0.5 mL of hexanes was added to the vials, which were rolled on a Stuart SRT9D roller mixer for 5 minutes at 60 rpm. The hexanes was transferred to a collection vial, for the DDT and dieldrin contaminated samples an amber 2mL GC vial, and for the PBDE contaminated samples, a second amber 20mL

vial. This was because the lower contaminant concentration in the PBDE contaminated soil required multiple EVA coated vials to be used per analytical sample. Five EVA coated vial extracts were combined into one sample for analysis. After transfer, a second 0.5mL aliquot of hexanes was added to the vial and it was again rolled for 5 minutes and then transferred to the collection vial.

After extraction, the PBDE extracts had to be concentrated to 1 mL, this was done using a Meyer N-Evap analytical evaporator. Samples that were dried beyond the 1.0 mL mark were brought back to 1.0 mL using hexanes. Any DDX or dieldrin contaminated samples that had less than 1.0mL final volume was brought to 1.0 mL using hexanes. Internal standard was added to each vial, and samples were analyzed as previously discussed.

Chapter 3: ASSESSING BIOAVAILABILITY OF PBDES IN AGRICULTURAL SOILS TO NATIVE EARTHWORMS USING TRADITIONAL AND THIN-FILM SOLID-PHASE EXTRACTION.

Abstract

The introduction and buildup of PBDEs in agricultural soils has been measured by determining total congener concentrations for many years. In the present study the accumulation of PBDEs in native earthworms from two different agricultural sites was evaluated. A large difference in the accumulation of these contaminants in the earthworm samples was seen between sites, indicating the soil characteristics play a large role in the bioavailability of these contaminants. A TF-SPE method using EVA as a surrogate for the earthworms correlated well with the availability trends of the contaminants. The EVA polymer film showed higher sorptive capacities for PBDEs as compared to native earthworms. This difference in contaminant uptake between polymer and earthworms was greater for the brominated PBDEs than for similar chlorinated persistent organic pollutants. The potential for TF-SPE use to evaluate the availability of PBDEs was verified, but would need to be refined before wider application.

3.1 Introduction

Biosolids, by-products generated from wastewater treatment plants, have been applied to soils as an agricultural amendment for many years (Tenenbaum, 1997). This application provides a recycling route for nutrients and organic

matter. However, biosolids can introduce persistent organic pollutants, such as polybrominated diphenylethers (PBDEs), to farmlands. PBDEs have been found at high levels in biosolids from wastewater treatment plants around the world, with values being highest in the United States (Andrade et. al., 2010; {MOIRA:2007}. Once biosolids are applied, semivolatile, highly hydrophobic persistent organic compounds such as PBDEs find a reservoir in the soil with abundant data showing the persistence of these contaminants in agricultural soils with probable build up upon multiple applications (Andrade et. al. 2010).

The use of biosolids as fertilizers in agricultural soils is viewed as a sustainable practices as compared to landfilling or incineration (Tenenbaum, 1997). While regulations exist to set tolerance levels for heavy metals and pathogens, both in the US and Europe, providing acceptable levels for organic pollutants has been a difficult task as many uncertainties with respect to their fate and potential risks still remain.

Measuring the risk posed by PBDEs in agricultural soils is challenging. Agricultural soils are heterogeneous in nature and so is the commercial application of biosolids. Biosolids are applied by commercial spreaders as biosolid pieces which vary in size (Andrade et. al. 2010). This application method creates a very heterogeneous medium with variable incorporation of the biosolids into the soil. Thus, studies have illustrated that concentration measurements of PBDEs are highly variable spatially, which is not unexpected (Gorgy et. al., 2012; Andrade et. al., 2010). It is generally accepted that soil total concentration of persistent organic pollutants in soil is a poor measure of an organism's exposure

and potential ecological risks(Alexander, 2000). It is expected that potential risk of PBDEs in a biosolids-amended soil would be a function of soil parameters, environmental conditions, agricultural practices as well as the time since last application. Thus, there is a need for an alternative measure other than soil concentration.

The aim of the present study was to investigate the feasibility of the chemical extraction method to assess the bioaccumulation of PBDEs by native earthworms in soil from farm fields where biosolids have been applied. In this research the Bioaccumulation Factors (BAF) for native earthworms were calculated as the measured concentration in the earthworms divided by the measured concentration in the soil. The correlation between an ethylene vinyl acetate (EVA) coated vial thin-film solid-phase extraction (TF-SPE) method and the BAF values was examined. The TF-SPE and BAF correlation has been explored before for other persistent organic pollutants, such as DDT, dieldrin and PCBs (Andrade *et al.* 2013). This relationship is proposed as an easier means of evaluating variability and/or reduction in bioavailability of PBDEs in biosolids applied agricultural fields in the future.

3.2 Materials and Methods

3.2.1 Sites and Samples

Soil and earthworm samples were collected from two agricultural fields in Virginia, USA, one in Orange County and one in Fauquier County. The sites sampled in this study were previously examined for total PBDE concentrations in 2006 by Andrade *et al* 2010 and again in 2009 by Andrade, 2013. During the

initial study the first field site was being used for corn production and the second field site was being used as a pasture for cattle. Both fields were still being utilized in the same manner when sampled for this study. The previous research involved soil collection throughout the fields, with 5 or 9 samples averaged to produce PBDE concentrations for each field. The dominant congeners detected were BDE-47, BDE-99 and BDE-209. The two field sites used during this research were selected from among the 27 agricultural fields sampled in the initial study for two reasons; 1)The first was the relatively high level of contamination in the two fields, predominantly due to the multiple biosolids applications and 2) the second being the different management practices of the fields offered the chance for a field management comparison to be investigated. Field characteristics and biosolids application information for the two sampled field sites are summarized in Table 3.1.

Site Location	Field use at time of sampling	Time span of biosolids applications	Total amount of dry sludge applied (ton/acre)	Carbon content (%) from 2009	Predominant Soil Type
Orange County, VA	Pasture	1997-2009	34	3.09	Fluvanna loam
Fauquier County, VA	Corn Production, harvested	1995-2012	20	1.58	Catlett loam

Table 3.1 - Sample site characteristics

Soil and earthworms were collected from each site by removing soil in one 1.5 m^2 area to 15 cm depth. The soil was placed on a tarp and broken apart by hand. Any earthworms located in the soil were removed and placed in glass jars with a small portion of the soil. Two 19 L containers of soil were collected from throughout the 1.5m^2 section. Soil was sieved (4 mm), homogenized into one ~38 L sample using a concrete mixer and stored at -20℃ until analysis. The two predominant earthworm species found at both sites were identified as

Allobophora chlorotica (Savigny) and *Octolasion tyrtaeum* (Savigny). The corn production field also contained a significant percentage of an unidentified *Lumbricus* species. The earthworm samples were depurated by placing the samples on moist filter paper for 24hr. After depuration, the earthworms were rinsed with deionized water to remove adhered particles, freeze-dried at 25℃ and 5-10mtorr for 1 week, homogenized using a stainless steel blender and kept at -20℃ until analysis.

3.2.2 Soil and Earthworm Concentration Analysis

Soil moisture content was determined by baking soil at 100℃ for 4 hours, with replicate samples resulting in an average percent difference of 0.43 and 0.48% (n=6) for the pasture and corn production fields respectively. All PBDE processing and analysis was done in a laboratory space with window shades drawn and light filters to block wavelengths below 620 nm to minimize photodegradation of PBDEs.

Soil and earthworm samples were extracted using a Dionex Accelerated Solvent Extractor (ASE) 350. The parameters of the extraction method were: preheat time: 5 minutes; temperature: 120℃; pressure: 2000 psi; static time: 10 minutes; solvents used: 20% Acetone, 80% Hexanes; flush: 60%; purge time: 200 seconds; cycles: 2. Two g (dry weight) of soil was blended with hydromatrix (Agilent Technologies, Santa Clara, CA, USA) using mortar and pestle. The sample was loaded into a 22mL stainless steel ASE cell, bounded by sand (JT Baker purified, washed and ignited, VWR, Philadelphia, PA, USA) and ASE cellulose filters (Thermo Scientific, Sunnyvale, CA, USA) for extraction. Prior to

extraction, 2,2’,3,3’,4,4’,5,5’,6,6’-decachlorobiphenyl (PCB-209) was added as an extraction surrogate. Following extraction, soil samples were filtered through anhydrous sodium sulfate (Fisher Scientific, Pittsburgh, PA, US). Earthworm samples were reduced to 4.0 mL at 40℃ and 0.4-0.6 psi using a Zymark TurboVap Nitrogen evaporator. One 1.0mL portion of the sample was transferred to a clean pre-weighed GC vial. This portion of extract was allowed to evaporate for 24 hours in a fume hood, and was re-weighed. These values allowed for lipid content to be calculated. The remaining 3.0mL of sample extract was evaporated to dryness. One aliquot of 5 mL of acetonitrile was added to the collection vials which were then vortexed for 15 minutes. The acetonitrile solution was transferred to a clean 60 mL collection vial, and a second aliquot was added, vortexed and transferred.

The 10 mL earthworm samples and sodium sulfate filtered soil samples were then evaporated to complete dryness using the TurboVap at 40℃ and 0.6-0.8 psi, and reconstituted to 2.0mL using hexanes (HPLC grade, Fisher Scientific, Pittsburgh, PA, US). An internal standard, $^{13}C_{12}$ 2,2’,3,4,4’,5’-hexachlorobiphenyl ($^{13}C_{12}$ PCB 138)(Cambridge Isotope Laboratories, Inc., Andover, MA) was added, the samples were vortexed and transferred to 2.0mL amber GC vials.

Samples were analyzed using an Aglient 6890 GC coupled with an Agilent 5975 MS detector in negative chemical ionization (NCI) mode. The capillary column used was DB-5-MS with a length of 15 m, inner diameter of 0.25mm and film thickness of 0.1µm (Agilent J&W Scientific, Folsom, CA). The carrier gas was

helium with a constant flow of 1.3 mL/min. The oven temperature program was: 48℃ temperature, increase of 25℃/min to 210℃, hold 10 min, increase 25℃/min to 310℃, hold for 9.52 min. The injection volume was 1µL. A programmable temperature vaporization (PTV) inlet was used with the temperature program: 51℃ increase 600℃/min to 300℃, hold 10 min. The injection pulse pressure was 280kPa until 2 min. Purge flow to the split vent was 500mL/min at 1.98 minutes. The GC/MS interface was kept at constant temperature of 300℃. An internal standard method was used for MS analysis. For soil concentration a six point calibration curve was used, and for the earthworm samples a lower concentration six point calibration curve was used.

3.2.3 EVA Thin-Film Solid-Phase Extraction

TF-SPE was carried out by coating 20 mL vials with an EVA solution as per Meloche et. al., 2009. In brief, 250 µL of a 6.21 g/L solution of EVA (ELVAX® 240 resin, DuPont, Wilmington, DE, USA) in dichloromethane (DCM) and 5 µL of a 2% dichlorocimethylsilane solution were introduced into a 20mL amber vial. A 0.48 µm thick polymer film was created by allowing the DCM to slowly evaporate while rotating the vial by hand. Vials were baked at 40℃ for 1 hour to cure the polymer film. Vials were prepared in advance, and stored at room temperature until sample addition.

For each site, 135 coated vials were prepared. Five vials per sample replicate were necessary for detection limit of the analysis method to be breached. Vials were loaded by first pipetting in the adequate amount of water to bring the moisture content of the soil sample to 33%, and then filling with 30 g of

soil. Soil filled vials were left at room temperature until extraction. For extraction of the PBDEs from the EVA, all of the soil was removed from the coated vial, and the vial was rinsed several times with deionized water to remove any remaining soil particles. The vials were centrifuged at 2650 rcf for 5 minutes, and any remaining water was removed using a pipette. One 0.5 mL aliquot of hexanes was added to the sample vials which were then rolled for 5 minutes at 60 rpm using a Stuart SRT9D roller mixer. The first aliquot of hexanes from the 5 vials per replicate sample was transferred to a clean 20mL amber vial. A second aliquot of clean hexanes was added to the coated vials, and the vial rolling and hexanes transfer was repeated, giving a final extract volume of approximately 5.0mL. The replicate samples were then reduced to a final volume of 1.0 mL using a nitrogen evaporator. The same internal standard, $^{13}C_{12}$ PCB 138, was added to each sample which was then transferred to 2.0mL an amber GC vial and analyzed using the same method and standard line concentration as the earthworm samples.

3.3 Results and Discussion

3.3.1 Bioaccumulation of PBDEs in Native Earthworms

Soil concentrations are summarized and presented with historical soil concentration data in Table 3.2. In past research, the observation of PBDE concentration decrease after 3-4 years without subsequent biosolids application. This trend was again observed in the pasture field site during this study. The last application of biosolids to that site was in 2009. In contrast, the corn production field site received biosolids application in the spring of 2012. This explains the

increase in BDE-209 congener concentration seen at this site. The only commercial BDE mixture still in use is the DecaBDE product, which is predominantly (98%) BDE-209 (McDonald, 2002). The BDE-47 and BDE-99 concentrations from 2009 and 2012 were not significantly different at the corn production field site.

Site	PBDE concentrations in 2006 (µg/kg d.w.)			PBDE concentrations in 2009 (µg/kg d.w.)			PBDE concentrations in 2012 (µg/kg d.w.)		
	BDE-47	BDE-99	BDE-209	BDE-47	BDE-99	BDE-209	BDE-47	BDE-99	BDE-209
Pasture Field	15.4	33.0	78.7	9.18	14.04	255.74	5.35	6.05	210.73
Corn Field	2.73	4.82	BQL	2.39	3.61	66.20	1.74	2.85	107.40

Table 3.2- Predominant PBDE congener concentrations at sample sites from 2006, 2009 and the present study collection in 2012

The contribution from each congener to the total PBDE concentration is plotted for the two sites over the three sampling time points in Figure 3.1. This figure illustrates the previous phase out of lower brominated PBDE products, and the longer continued DecaBDE use. For the latest soil concentration analysis the BDE-209 congener made up 92.7 and 94.0 % of total congener concentration in the pasture and corn production fields respectively.

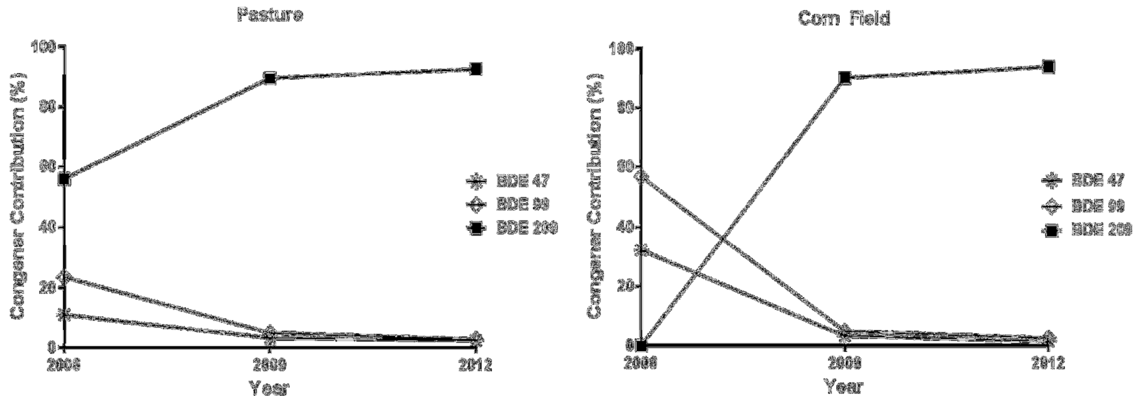


Figure 3.1 – Congener contribution to total PBDE concentration (%) over time at the two sampling sites.

Earthworm PBDE concentrations and lipid content from this study are summarized in Table 3.3. The lipid content of the earthworms was not statistically different between the two sites. The earthworm concentrations of PBDEs were within on the same scale at each field.

Site	Earthworm % lipids	Earthworm (ng/g d.w.)		Earthworm (ng/g lipid)	
		BDE 47	BDE 99	BDE 47	BDE 99
Pasture Field	8.95±0.38	3.69±0.21	4.01±0.11	41.35±4.09	44.83±3.14
Corn Field	8.47±0.54	3.92±0.11	5.61±0.12	46.42±4.26	66.38±5.71

Table 3.3 – Earthworm lipid content and PBDE concentrations (dry weight and lipid normalized).

The BAF was calculated for each site on a dry weight and a lipid normalized basis, and are plotted in Figure 3.2. Though the earthworm concentrations of BDE congeners were similar at both sites, the soil concentrations were not. As the BAF values show, the accumulation was much higher in the corn production field earthworms.

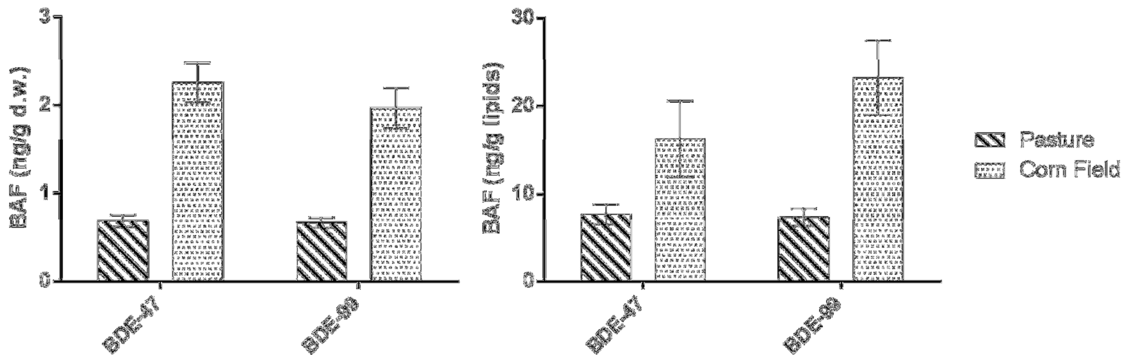


Figure 3.2 – BAF of BDE-47 and BDE-99 at the two sampling sites, dry weight and lipid normalized.

BAF values for the pasture field agreed well with previously published values, however the corn production field had BAF values approximately twice the levels previously seen (Matscheko et. al., 2002 ; Nyholm et. al., 2010; Sellstrom, 2005). The corn production field BAF levels have been reported previously in one sample site in southern Sweden by Matscheko et. al. 2002.

3.3.2 PBDE equilibrium during TF-SPE

Three factors should be met for the TF-SPE method to be properly used as a surrogate for bioaccumulation. First, the EVA should reach equilibrium with the soil, showing stable concentration in the EVA polymer. Secondly, the equilibrium time should be reasonable. Lastly, the EVA should not deplete the analyte concentrations in the soil (Mayer et. al., 2003; Wilcockson and Gobas, 2001). Less than 5% of the total amount of analytes in the soil should partition to the EVA film. During analysis, these factors were examined by calculating the time to reach 95% of equilibrium (t_{95}) for model fits and calculating the percent of analyte depletion in the coated vial soil.

The time dependent EVA analyte concentrations were fitted with a two-phase nonlinear model (Equation 3.1).

$$C_{EVA}(t) = C_{EVA(fast)}(1 - e^{-k_{fast}t}) + C_{EVA(slow)}(1 - e^{-k_{slow}t}) \quad (3.1$$

Where $C_{EVA(fast)}$ and $C_{EVA(slow)}$ are the concentrations in the EVA thin film that reflect the fast and slow components of the contaminant uptake and the kinetics of these components are described by their rate constants, k_{fast} and k_{slow} (Meloche et. al., 2009). As can be seen in Figure 3.3, this model was well suited for both BDE-47 (R^2=0.997-0.999) and BDE-99 (R^2=0.998-0.999).

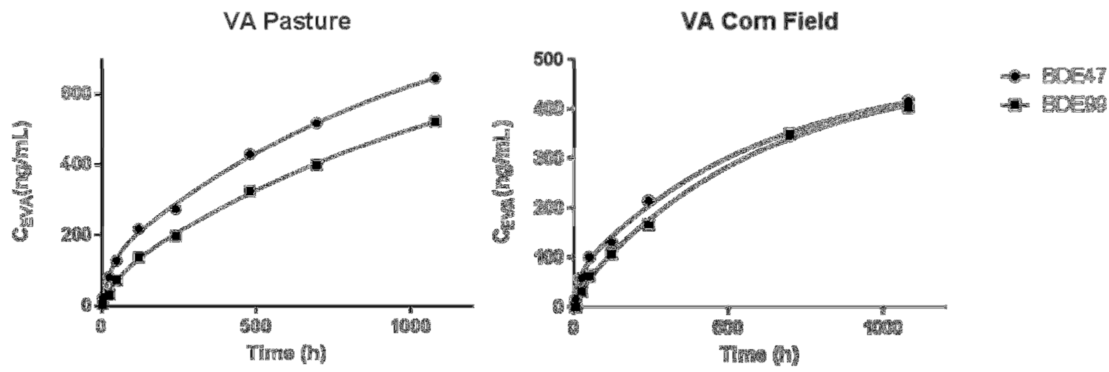


Figure 3.3 - Congener polymer concentration C_{EVA}(ng/mL) versus time (hours) for the two sampling sites. Lines are the model fits from the two-phase nonlinear models.

These models fit the equilibrium concentration values, $C_{EVA(eq)}$, for the congeners at both sites(r^2 ranged from 0.9973-0.9992). Due to underestimation of the equilibration time, the first two factors of TF-SPE methodology were not fully satisfied in this study. The t_{95} values were 2863 and 1674 hours for BDE-47 and 3648 and 1701 hours for BDE-99 at the pasture site and corn production site respectively. The longest incubation time used in this study was 1080 hours, lower than the all of the t_{95} values. The longer than expected t_{95} is most likely due

to the much larger brominated molecules partitioning at a slower rate than previously studied, smaller chlorinated molecules. One potential improvement in the method to help lower the equilibrium time for these contaminants involves slowly agitating platform to encourage equilibrium partitioning (Mayer et. al., 2003).

The third methodology factor, minimal contaminant depletion from the sample medium was satisfied during this study. The average percent depletion was 0.81±0.03% and 1.6±0.13% for BDE-47 and 0.58±0.02% and 0.90±0.06% for BDE-99. This could be partially due to lack of equilibrium, however the depletion is well below the 5% requirement, and would most likely not exceed it once equilibrium was reached.

3.3.3 Comparison of TF-SPE with earthworm bioavailability

The same trends seen in the BAF measurements are underscored again when examining the $C_{EVA(eq)}/C_{soil}$ ratio (Table 3.4). Again, higher accumulation of PBDEs from the corn production field soil was noted.

Site	C_{EVA} (ng/g)		C_{EVA}/C_{soil}	
	BDE 47	BDE 99	BDE 47	BDE 99
Pasture	965±109	890±131	180.5±11.5	147.0±11.9
Corn	505±47	509±44	209.5±7.4	178.8±1.2

Table 3.4 –TF-SPE EVA equilibrium concentrations of PBDEs from contaminated agricultural soil

The EVA appeared to be 20 times more sorptive than the earthworms from the pasture soil and 10 times more sorptive than the earthworms in the corn production soil for PBDEs on a lipid normalized basis. This difference in sortion is

compared to the near 1:1 sorptive capacity between EVA and earthworms for chlorinated persistent pollutants (DDX and dieldrin) in Figure 3.4. Beyond the longer equilibration time, the larger and heavier brominated molecules most likely partition into the EVA at a different proportion than the chlorinated contaminants from the previous investigation.

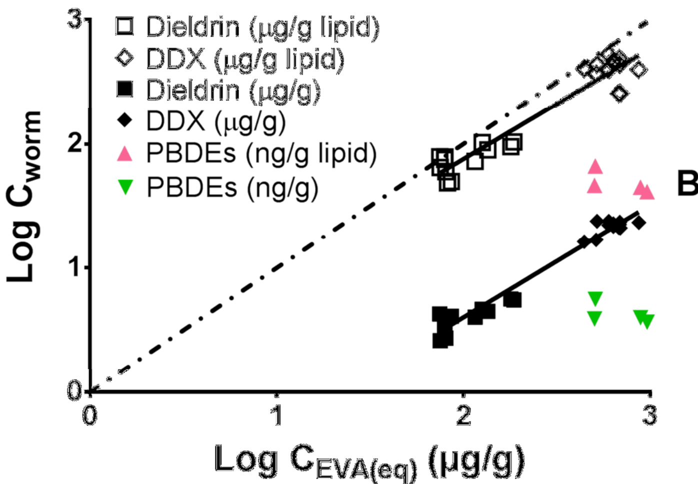


Figure 3.4 – (Andrade et. el. 2013) Previously reported persistent organic pollutant (DDX and dieldrin) correlation between the $\log C_{worm}$ and $\log C_{EVA(eq)}$ (on a dry weight and lipid basis). The PBDE correlations calculated in this study were added, on dry weight (green) and lipid basis (pink).

3.4 Conclusions

By only looking at the total concentration of PBDE contaminants in soil that has received biosolids application, a potential risk can be the underestimation or overestimation of accumulation of PBDEs. In this study the pasture site soil had 2-3 times higher total concentration of PBDEs compared to the corn production site, however the earthworm concentrations of the two sample sites were comparable. The two sites therefore show similar potential environmental risk, despite their soil concentration differences.

The potential use of a surrogate method to the traditional biological extractions was investigated, and showed some potential for future use. The contaminant is taken up following the same trends as the earthworm samples. The method can be improved by ensuring vial incubation times surpass the equilibration time or possible by agitating the vials during incubation. Because of the use of Biosolids as an agricultural amendment, and subsequent introduction of PBDEs into the agricultural soil, accumulation evaluation of these contaminants should be used instead of total concentrations measurements. The alternative TF-SPE method could make sampling across large areas feasible.

Chapter 4: Results and Discussion

4.1 Variability in Bioavailability in DDT & dieldrin contaminated soil

4.1.1 DDT and dieldrin pot study

When analyzing DDT and dieldrin contaminated soil, earthworms and TF-SPE samples the analytes 2,4-DDE and 4,4'-DDD were often below detection limit, and 2,4'-DDT was intermittently below detection limit. Therefore these three compounds are not discussed separately during the results and analysis section. These compounds were added to the 4,4'-DDT and 4,4'-DDE concentrations when discussed, which is referred to as DDX, the sum of all DDE,DDD and DDT species detected.

Results for the DDT and dieldrin pot study have been discussed in Andrade *et. al.* 2013. However, in this research a larger data set is discussed and compared to the previously reported results. The largest difference in data sets was the soil concentrations used during bioavailability analysis. Andrade *et. al.* 2013 soil concentration data was from soil collected before the 4L pots were loaded (time zero). A large portion of contaminated soil was taken from the abandoned orchard on the BARC site. The soil was mixed well giving a homogenous soil sample. Portions of the large sample were taken and mixed with their assigned amendment. Once the amendments had been mixed with the soil, initial time zero soil samples were taken of these bulk mixtures. After time zero sampling, the pots were loaded.

In this research the soil concentration data was determined from soil samples collected from each pot on the day the earthworms were harvested

(harvest). The time zero data allows faster analysis, as soil can be extracted and analyzed before the bioavailability study is complete. However, the amendments may not have been fully equilibrated with the soil. Therefore, analysis comparing the soil concentrations from time zero and harvest samplings were performed.

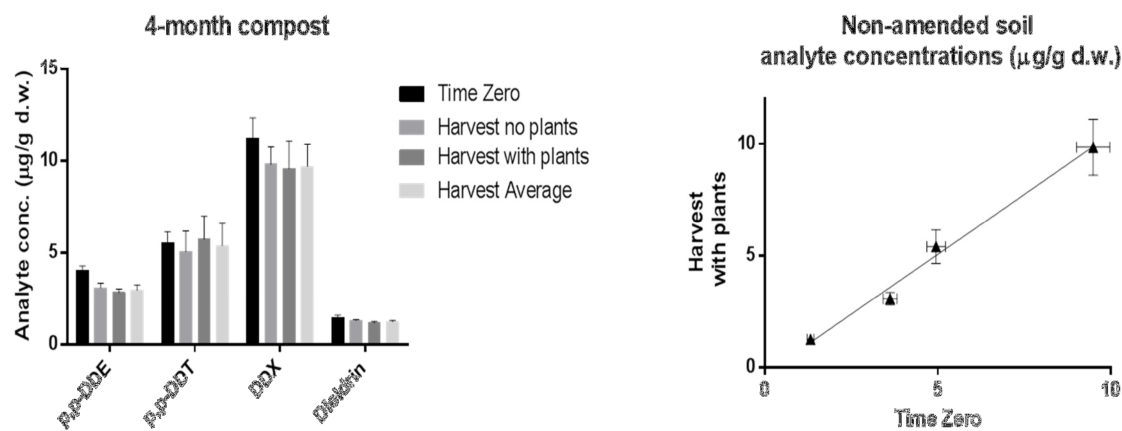


Figure 4.1.- A) Analyte concentrations for 4 month compost amended soil from time zero and harvest sampling. B) Correlation between time zero and harvest sampling concentrations for non-amended soil. Harvest concentration = 1.072 x Time Zero concentration – 0.2292 (r^2=0.9892).

The time zero and harvest soil concentrations were consistent for most of the amendment types. The two plots in figure 4.1 visually express this agreement. Figure 4.1 a. shows the average concentration of the time zero soil, each pot type (with plants and without) as well as the averaged harvest soil concentrations for the 4 month compost amendment. However, only the p,p-DDT remained consistent between the time zero and the averaged harvest soil for the 4 month compost amendment, as the p,p'-DDE, DDX and Dieldrin were significantly different ($p<0.05$). However, the difference in concentration did not significantly change the BAF values. Figure 4.1 b. plots the non-amended soil values for the time zero and harvest samplings. The values were consistent for

the non-amended data shown. For the other amendments, the farthest from the 1:1 relationship was seen in the 2 year compost with plants versus the two year compost time zero soil. The slope was 0.6632±0.1071. and for p,p'-DDE, DDX and dieldrin the time zero and harvest data sets were significantly different ($p<0.05$).

As previously discussed, the main goal of the pot study was to determine which, if any, of six soil amendments would reduce accumulation of DDT and it's byproducts and dieldrin in earthworms. Figure 4.2 summarizes the BAF (d.w.) values for each soil treatment used in the study: unamended soil and 5 different amended soil treatments. The sixth soil treatment used in the pot study was unsuitable for earthworm survival, and BAF values could not be obtained. On possible reason for this was the addition of limed biosolids raised the pH in the soil. All amendments showed a significant reduction ($p<0.05$) in BAF except for 2 year compost (112 dry t/ha) p,p'-DDT BAF, which is considered statistically the same as the unamended p,p'-DDT BAF, and for the biochar amended soil p,p'-DDT and dieldrin BAFs, which show a significant increase in from the unamended soil BAF values ($p<0.05$). As discussed in Andrade et. al. 2013 this behavior, though unexpected, has been observed before. This study's observations varied slightly from the statistical comparison of BAF values in Andrade et. al. 2013. The harvest soil data generally gave lower BAF values due to the higher control soil concentration values for the harvest soil. In particular, the p,p'-DDE concentration was significantly different from time zero to harvest ($p<0.05$).

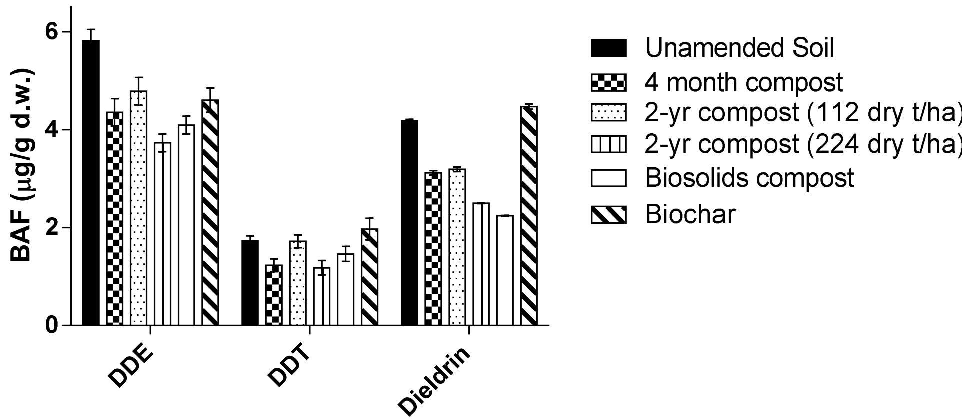


Figure 4.2 – BAF on a dry weight and lipids basis for the DDT and dieldrin pot study using harvest soil concentration data.

As previously mentioned the 4 mo. Compost and the 2 yr. compost both showed significant reduction and were relatively inexpensive and easily accessible, therefore they were chosen to use in the field plot study.

4.1.2 DDT and dieldrin field plot study time zero

The BARC pilot field plot study commenced in the fall of 2012 with the time zero soil and earthworm sampling to get an initial concentration and bioaccumulation measure for each sub-plot. In the spring of 2013 the field plots were installed, and the two chosen forms of manure compost were applied to randomly assigned sub-plots. The time zero sampling will provide general variability of bioavailability measurement throughout the field, and serve as a starting point for assessment of the performance of the compost as a bioavailability reduction strategy.

The soil contaminant concentrations, as well as surrogate recoveries, are listed in table 4.1. Unlike the pot study soil concentrations data, the surrogate recoveries for the field plot time zero samples were relatively low. EPA Method 1618 covers the analysis of DDT, DDE, DDD and dieldrin in soil, and requires surrogate recovery to be within the ranges of 79-119 % for DDT, 54-126% for DDE and 48-158% for dieldrin. Most of the samples will need to be re-extracted for the surrogate recovery to meet the DDT requirement. However, all of the surrogate recovery values highlighted in green in table 4.1 are still within the DDE acceptable range, with only two sub plot sampling sites below that range (table 4.1 in blue). The objective of this portion of the study was to assess the bioavailability of contaminants in the DDT and dieldrin contaminated site using the EVA TF-SPE method. Because the trends over the field site and not the exact concentrations were the focus, the low surrogate recoveries will not significantly affect the results.

Plot	Average concentration values in ug/g dry weight				Standard Deviation values in ug/g dry weight				Recovery (%)
	4,4'-DDE	4,4'-DDT	Total DDX	Dieldrin	4,4'-DDE	4,4'-DDT	Total DDX	Dieldrin	
1A	5.77	11.25	19.40	1.20	0.25	0.64	1.58	0.05	85.9
1B	4.52	8.20	15.38	0.97	0.19	0.28	0.09	0.01	76.6
1C	3.89	5.80	11.20	0.64	0.09	0.29	0.09	0.03	68.1
1D	2.67	3.65	7.34	0.81	0.14	0.19	0.30	0.05	65.5
2A	1.13	1.48	2.61	BDL	0.01	0.02	0.04	BDL	60.2
2B	BDL	0.85	0.85	BDL	BDL	0.03	0.03	BDL	*53.3*
2C	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	54.9
2D	0.87	0.94	1.81	BDL	0.01	0.01	0.02	BDL	72.2
3A	2.40	2.56	4.96	BDL	0.03	0.07	0.09	BDL	72.6
3B	5.66	7.14	14.43	1.05	0.05	0.18	0.63	0.08	70.5
3C	4.15	4.87	10.84	0.81	0.07	0.27	0.36	0.03	86.4
3D	3.99	4.54	10.32	0.94	0.05	0.19	0.23	0.06	82.2
4A	2.37	3.49	7.47	BDL	0.03	0.15	0.12	BDL	70.0
4B	2.69	3.09	7.34	BDL	0.20	0.59	0.92	BDL	68.0
4C	2.78	2.50	6.65	BDL	0.03	0.04	0.07	BDL	*47.3*
4D	3.16	3.41	7.76	BDL	0.02	0.16	0.20	BDL	94.3
5A	1.20	1.61	3.74	BDL	0.05	0.11	0.16	BDL	88.4
5B	BDL	0.92	1.77	BDL	BDL	0.04	0.05	BDL	85.6
5C	2.35	4.44	7.97	BDL	0.15	0.98	1.14	BDL	85.9
5D	1.23	1.38	3.52	BDL	0.01	0.04	0.06	BDL	87.0
6A	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	72.0
6B	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	76.6
6C	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	67.3
6D	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	65.3
7A	3.11	3.51	7.51	BDL	0.08	0.12	0.16	BDL	71.2

Table 4.1 – Contaminant concentrations in time zero soil samples collected from the BARC contaminated site.

The soil concentrations for field plot 6 were all below the detection limit for the analysis method. Field plot 7, sub plots B-D samples were not yet extracted, as the low surrogate recovery must be investigated and mitigated. Therefore, only plots 1-5 are discussed here.

The time dependent EVA analyte concentrations were fitted with a two-phase nonlinear model (Figure 4.3). A two-phase model fit was provided a better fit for p,p'-DDT, p,p'-DDE and dieldrin at both sub plot 1A and sub plot 3C than a one phase model. In previous research the one-phase model provided a better fit for DDT (Andrade et. al. 2013), however in this study the DDT r^2 values were 0.9894 and 0.9955 for the 2-phase model and 0.9865 and 0.9821 for the one-

phase model. As previously discussed, three factors were examined to determine the validity of use of the EVA TF-SPE method. The t_{95} was calculated for the p,p'-DDT, p,p'-DDE and dieldrin for two of the field sub-plot sites, 1A and 3C. The p,p'-DDT t_{95} values were 1696 and 1766 hours, the p,p'-DDE values were 1419 and 993 hours and the dieldrin values were 550 and 357 hours. The p,p'-DDT t_{95} values surpassed the 1080 hours used in this experiment. However, because the data had a good two-phase model fit, and longer t_{95} values have been seen before with p,p'-DDE (Adrade et al. 2013), the equilibrium values are considered accurate. The average percent depletion ranged from 0.13 – 1.26%, well below the 5% limit.

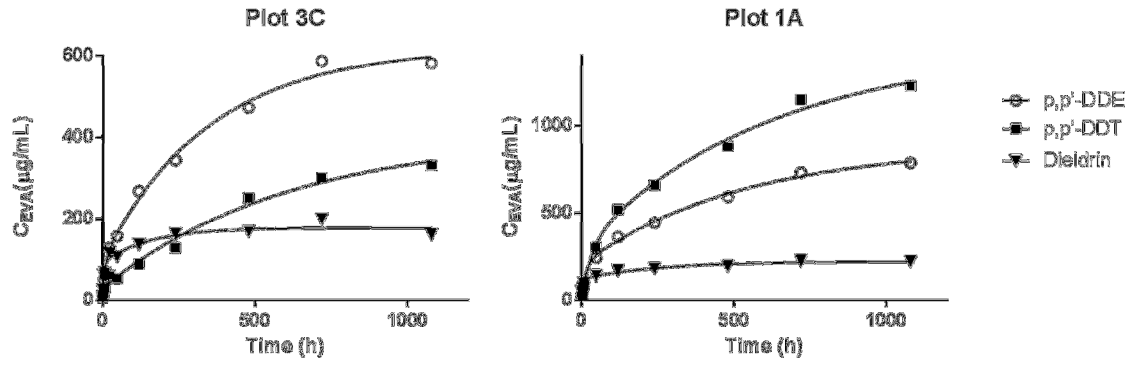


Figure 4.3 – Two phase nonlinear model fits for two sub plots in the DDT and dieldrin contaminated site for three contaminants.

Using the equations generated by the time-dependent model fits, the $C_{EVA(eq)}$ values for the rest of the sub-plots were estimated. EVA concentrations for the 45 day time point were determined for all sub-plot sites. The $C_{EVA(eq)}$ values were used as a surrogate measurement for the earthworm contaminant accumulation. There has been shown to be a correlation between the $\log C_{EVA(eq)}$

values and $\log C_{earthworm}$ values (Andrade et. al. 2013) which is shown in Figure 3.4. Because of this correlation, the $C_{EVA(eq)} / C_{soil}$ ratio provides a measure of the BAF trends for the earthworms. These values are shown in Figure 4.4 alongside the C_{soil} values for the sub plots. A few interestin observations can be noted. Plot 4 shows higher total concentration values, but plot 3 shows higher accumulation tendencies.

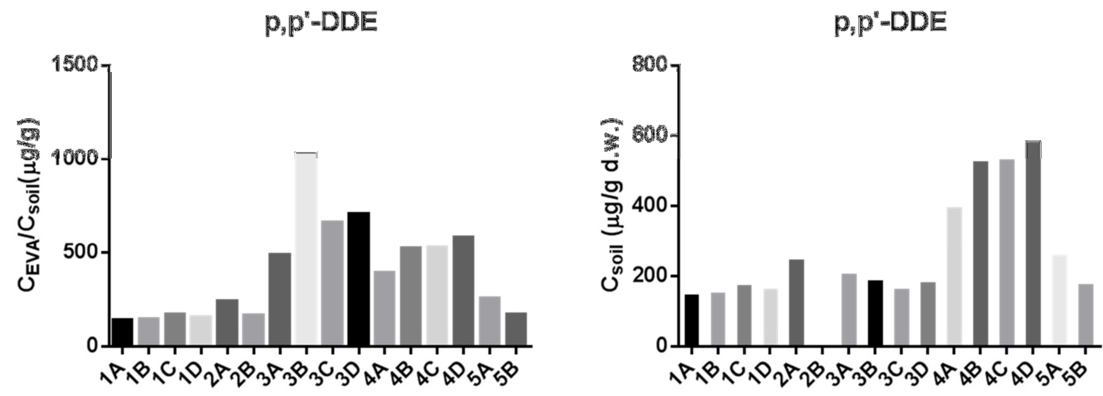


Figure 4.4 – a) Bioaccumulation tendencies of subplots. Equilibrium EVA and total soil concentration ratio is a surrogate for earthworm BAF values. b) Total concentration values for p,p'-DDE in field plot sites.

This initial profile for the DDT and dieldrin contaminated site field plots can provide information about potential amendment performace, as well as highlight the variation across a contaminated site. The higher the initial bioavailability, the more potential for the *in situ* remediation efforts to have an effect.

Chapter 5: Conclusions

Bioavailability in soil is highly variable, and the measurement of persistent organic pollutants in soil must address the availability to organisms and not just the total concentration. Many traditional total concentration extraction methods for POPs are lengthy and exhaustive. For example, one standard method used for DDT and dieldrin analysis is EPA method 3540 using soxhlet extraction, in which each sample is extracted for around 16 hours (Morrison et. al. 2000). The accelerated extraction method used in this research was much faster, taking approximately 45 minutes per sample to extract. The method was validated during the DDT and dieldrin pot study and during the soil analysis for the PBDE contaminated soil. The surrogate compounds used were recovered to an acceptable amount, and spiked samples gave high levels of recovery. Use of this faster and simpler total concentration method helps shorten analysis time for POP contaminated soil.

The use of polymer passive sampling to mimic earthworm bioavailability is promising. The TF-SPE method used in this study has been reported to be suitable for measuring chlorinated POP accumulation in earthworms and this research showed the efficacy of the method for measuring brominated contaminants (PBDEs). The mathematical model fit to the time-dependent polymer concentrations allowed the equilibrium concentrations to be determined, which correlated well to the earthworm concentrations. A larger number of data

points would need to be collected to definitively determine differences from the chlorinated compounds, but the data from this research showed that the PBDEs may not be as available to the earthworms as chlorinated contaminants. This is most likely due to their larger size. However, the PBDEs appear to be taken up in the polymer at a similar amount as the chlorinated compounds. This demonstrates the higher sorptive capacity of the EVA for PBDEs compared to the earthworm uptake of PBDEs. The TF-SPE method is much easier and cheaper than the traditional earthworm biological extractions also used in this study. Even with the requirement of multiple vials per sample replicate for the PBDE TF-SPE methodology, it would be feasible to measure the bioavailability of a large number of soil samples. This would not be likely with biological extraction, because it would be extremely difficult to collect enough earthworm samples.

The EVA TF-SPE method evaluation of the bioavailability of the DDT and dieldrin contaminated soil site showed the high variability of bioavailability. The assessment also highlighted the difference in variation across the field of total concentration and bioavailability. The bioavailability does not follow the same trends as the total concentration, which emphasizes their need to be evaluated separately. The results of this research demonstrate the efficacy of the EVA TF-SPE method for assessing the bioavailalability of POPs in soil. The method can be used to evaluate remediation efforts, gather information on the differences in bioavailability in agricultural soils, and be used to assess differences in bioavailability with different field management practices.

Appendix A : Ions monitored for chromatographic analysis

Compound	Ions
BDE-28	79, 81, 161
BDE-47	79, 81, 161
BDE-100	79, 81, 161, 403
BDE-99	79, 81, 161, 405
BDE-154	79, 81, 161, 430
BDE-153	79, 81, 161, 430
BDE-183	161, 483, 561
BDE-209	484, 486
13C12 PCB-138	338, 372
PCB-209	464, 482

Compound	Ions
pentachloronitrobenzene	142, 237, 249, 295
o,p'-DDE	246, 248, 176, 318
p,p'-DDE	246, 318, 248, 176
Dieldrin	79, 81, 82 263
p,p'-DDD	235, 165, 237, 176
o,p'-DDT	235, 165, 237, 199
p,p-DDT	247, 249, 177, 188
13C12 p,p'-DDT	235, 237, 165, 212

Appendix B: Moisture content in DDT and dieldrin contaminated soil samples from Field Time Zero collection

Plot	Moisture Content (%)	MC Standard deviation (n=6)	% difference
1A	20.85	0.43	2.05
1B	19.94	0.15	0.75
1C	20.36	0.10	0.49
1D	19.35	0.09	0.49
2A	14.85	0.06	0.37
2B	15.39	0.08	0.50
2C	15.27	0.18	1.16
2D	15.88	0.13	0.80
3A	18.86	0.12	0.63
3B	18.47	0.09	0.51
3C	17.16	0.15	0.87
3D	19.76	0.11	0.54
4A	19.59	0.14	0.69
4B	19.18	0.17	0.89
4C	18.14	0.11	0.63
4D	18.99	0.24	1.27
5A	16.26	0.14	0.89
5B	15.35	0.17	1.11
5C	14.92	0.20	1.32
5D	15.49	0.17	1.07
6A	15.88	0.06	0.38
6B	19.34	0.12	0.60
6C	20.54	0.08	0.39
6D	20.03	0.13	0.67
7A	14.70	0.18	1.21
7B	16.02	0.07	0.46
7C	14.69	0.02	0.14
7D	15.13	0.09	0.56

Appendix C: Moisture Content in DDT and dieldrin contaminated soil samples from initial Pot Study

Sample	Moisture Content (%)
CTR-T0-2-R1	14
COMA-T0-1-R1	19.8
COMB-T0-1-R1	16
COMC-T0-1-R2	20.7
COMD-T0-1-R2	16
CHAR-T0-2-R2	14.2
LBR-T0-2-R1	18.6

Sample	Moisture Content (%)
CTRW1-1	21.2
CTRW2-1	15.6
CTRW3-1	15.2
CTRW4-1	16.1
CTRWP1-1	12.0
CTRWP2-1	5.5
CTRWP3-1	6.7
CTRWP4-1	13.1
COMAW1-1	22.2
COMAW2-1	19.8
COMAW3-1	20.3
COMAW4-1	21.3
COMAWP1-1	10.7
COMAWP2-1	7.7
COMAWP3-1	9.4
COMAWP4-1	10.4
COMBW1-1	18.9
COMBW2-1	19.0
COMBW3-1	20.4
COMBW4-1	18.8
COMBWP1-1	10.4
COMBWP2-1	10.0
COMBWP3-1	9.7
COMBWP4-1	9.1
COMBWR1-1	7.6
COMBWR2-1	7.4
COMBWR3-1	6.0
COMBWR4-1	7.9
COMCW1-1	21.5
COMCW2-1	21.3
COMCW3-1	22.9
COMCW4-1	21.5

Sample	Moisture Content (%)
COMCWP1-1	11.4
COMCWP2-1	9.9
COMCWP3-1	9.2
COMCWP4-1	11.6
COMDW1-1	20.2
COMDW2-1	20.6
CHARW3-2	19.1
CHARW4-2	17.8
CHARWP1-2	7.2
CHARWP2-2	4.2
CHARWP3-2	6.4
CHARWP4-2	10.1
LBRW1-2	21.2
LBRW2-2	19.6
LBRW3-2	20.1
LBRW4-2	20.6
LBRWP1-2	25.3
LBRWP2-2	28.0
LBRWP3-2	28.3
LBRWP4-2	26.6
CTR1-2	17.9
CTR2-2	19.5
CTR3-2	18.4
CTR4-2	13.3

Appendix D: DDT and dieldrin contaminated soil Pot Study Time Zero sample concentrations

Sample Code	Concentration values in μg/kg dry weight							
	2,4'-DDE	4,4'-DDE	4,4-DDD	2,4'-DDT	4,4'-DDT	Total DDx	Dieldrin	Surrogate Recovery (%)
CTR-T0-1-R1	BDL	3699.95	BDL	890.73	5170.79	9761.46	1274.43	80.1
CTR-T0-2-R1	BDL	3387.03	BDL	871.20	4760.51	9018.74	1275.69	*79.0*
CTR-T0-3-R1	BDL	3341.38	BDL	842.95	4510.22	8694.55	1129.73	*77.6*
CTR-T0-3-R2	BDL	3526.89	BDL	915.45	4861.52	9303.87	1296.09	81.7
CTR-T0-4-R1	BDL	3511.46	BDL	898.93	4485.30	8895.69	1226.67	*77.9*
CTR-T0-5-R1	BDL	3866.89	BDL	1020.94	5004.21	9892.05	1433.55	84.6
COMA-T0-1-R2	BDL	4430.12	BDL	1100.03	6480.17	12010.32	1440.04	103.8
COMA-T0-2-R1	BDL	3853.46	BDL	948.39	5241.10	10042.95	1317.76	92.8
COMA-T0-2-R2	BDL	4255.67	BDL	1068.91	5744.16	11068.75	1378.60	100.6
COMA-T0-3-R1	BDL	4178.77	819.76	1359.60	5968.24	12326.37	1629.52	108.2
COMA-T0-3-R2	BDL	3982.02	880.45	1380.70	5662.87	11906.04	1560.79	102.2
COMA-T0-4-R1	BDL	4075.17	981.25	1491.89	5737.28	12285.60	1531.94	92.2
COMA-T0-4-R2	BDL	3772.07	950.52	1280.70	4832.65	10835.93	1530.84	92.6
COMA-T0-5-R2	BDL	3753.41	BDL	910.83	4714.28	9378.52	1221.11	83.6
COMB-T0-1-R1	BDL	3580.60	1206.83	1346.47	4388.48	11200.61	1625.73	89.0
COMB-T0-1-R2	BDL	4039.75	1329.92	1479.91	4879.70	12419.23	1909.88	108.0
COMB-T0-2-R1	BDL	3977.39	1355.70	1505.23	4954.29	12480.44	1973.74	99.8
COMB-T0-3-R2	BDL	3991.37	1377.02	1456.85	4600.05	12113.81	1905.88	94.2
COMB-T0-4-R1	BDL	3921.52	1446.87	1426.92	4240.83	11724.65	1885.92	87.6
COMB-T0-5-R1	BDL	4458.86	1496.26	1755.61	5755.62	14164.62	2004.99	102.2
COMB-T0-5-R2	BDL	4126.28	1538.61	1468.68	4605.85	12428.79	1878.31	96.4
COMC-T0-1-R1	BDL	3084.95	BDL	1477.58	4971.85	9534.38	1487.56	84.9
COMC-T0-2-R1	BDL	3088.74	BDL	1569.36	5437.78	10095.87	1459.40	98.4
COMC-T0-3-R1	BDL	2915.92	BDL	1517.87	4633.51	9067.30	1388.06	84.4
COMC-T0-4-R1	BDL	3145.10	BDL	1587.53	5731.07	10463.69	1457.73	100.9
COMC-T0-5-R1	BDL	3195.78	BDL	1627.85	6121.91	10945.54	1498.02	105.3
COMD-T0-1-R1	BDL	3645.86	1464.01	1275.11	3891.43	10276.41	1983.50	96.4
COMD-T0-2-R1	BDL	3639.01	1559.58	1279.65	3599.02	10077.26	1979.46	85.5
COMD-T0-3-R1	BDL	3764.39	1597.62	1288.08	3714.47	10364.56	1997.02	95.6
COMD-T0-4-R1	BDL	3477.59	1588.90	1249.14	3457.61	9773.24	1808.75	76.0
COMD-T0-5-R1	BDL	3681.16	1660.52	1280.40	3661.16	10283.25	1970.62	90.9
CHAR-T0-1-R1	BDL	3564.04	1297.83	1467.55	4252.89	10582.31	1467.55	82.2
CHAR-T0-1-R2	BDL	3638.61	1276.01	1505.29	4396.24	10816.14	1495.32	80.0
CHAR-T0-2-R1	BDL	4048.09	1289.39	1619.24	5167.57	12124.29	1609.24	98.8
CHAR-T0-2-R2	BDL	3960.00	1300.00	1590.00	5070.01	11920.01	1590.00	91.4
CHAR-T0-4-R1	BDL	4302.81	1367.71	1647.25	5001.64	12319.40	1747.08	97.6

CHAR-T0-4-R2	BDL	4285.04	1378.40	1658.08	5034.17	12355.70	1698.03	99.0
CHAR-T0-5-R1	BDL	3689.21	1379.71	1529.67	4249.09	10847.68	1539.67	85.0
CHAR-T0-5-R2	BDL	4238.73	1429.57	1649.51	5118.47	12436.28	1659.50	101.8
CHAR-T0-3-R1	BDL	4140.94	1347.05	1606.49	5019.02	12113.51	1696.29	97.2
LBR-T0-4-R1	BDL	4988.28	3982.66	826.40	2399.55	12196.90	3922.92	88.2
LBR-T0-1-R2	BDL	4419.39	3182.36	837.99	2683.56	11123.30	3431.76	93.2
LBR-T0-2-R1	BDL	4219.74	3184.71	826.03	2498.01	10728.49	2468.15	96.2
LBR-T0-2-R2	BDL	4466.91	3310.30	857.49	2642.26	11276.97	3878.64	96.2
LBR-T0-3-R2	BDL	4500.85	3452.98	848.27	2624.66	11426.76	3922.02	87.6
LBR-T0-5-R1	BDL	4150.54	3541.93	BDL	2534.23	11004.93	3202.70	*75.1*
LBR-T0-5-R2	BDL	5337.85	4272.27	1045.66	3366.03	14021.80	3804.21	*74.3*

Appendix E: DDT and dieldrin contaminated soil Pot Study Harvest samples concentrations

Sample Name	Concentration values in µg/kg dry weight							
	2,4'-DDE	4,4'-DDE	4,4-DDD	2,4'-DDT	4,4'-DDT	Total DDx	Dieldrin	Surrogate Recovery (%)
CTRW1-1	BDL	3335	BDL	1198	5701	10234	1448	99.3
CTRW1-2	BDL	3252	BDL	1198	5498	9948	1423	88.7
CTRW2-1	BDL	3291	BDL	1173	5458	9921	1441	94.2
CTRW3-1	BDL	3246	882	1265	8214	13607	1327	96.1
CTRW4-1	BDL	3004	BDL	1121	5197	9322	1272	92.2
CTRWP1-1	BDL	3096	BDL	1158	5183	9437	1258	93.2
CTRWP2-1	BDL	2760	BDL	1066	4444	8271	1176	87.7
CTRWP3-1	BDL	2995	801	1132	6161	11089	1202	95.5
CTRWP4-1	BDL	3430	BDL	1277	5843	10549	1385	96.1
COMAW1-1	BDL	3059	820	1130	4929	9938	1320	96.2
COMAW2-1	BDL	2849	910	1050	4009	8818	1240	81.7
COMAW3-1	BDL	4518	1459	2899	12064	20940	1879	96.0
COMAW4-2	BDL	3343	BDL	1098	6237	10677	1337	94.1
COMAWP1-1	BDL	2615	BDL	968	4920	8503	1118	82.9
COMAWP2-1	BDL	2761	BDL	967	4984	8712	1086	85.7
COMAWP3-1	BDL	2856	BDL	1038	5382	9276	1208	92.2
COMAWP4-1	BDL	3079	BDL	1079	7626	11785	1259	91.5
COMBW1-1	BDL	3105	BDL	1433	4966	9503	1443	83.8
COMBW2-1	BDL	2970	BDL	1106	5034	9110	1176	87.2
COMBW2-2	BDL	2907	BDL	949	4745	8602	1179	86.0
COMBW3-1	BDL	2963	BDL	968	4828	8758	1227	82.9
COMBW4-1	BDL	2783	BDL	901	4526	8210	1121	82.4
COMBWP1-1	BDL	3156	BDL	989	5143	9288	1348	83.8
COMBWP2-1	BDL	2208	BDL	899	3547	6654	959	95.5
COMBWP3-1	BDL	2961	BDL	1010	5172	9144	1261	85.7
COMBWP4-1	BDL	2899	BDL	1040	4679	8619	1220	93.2
COMBWR1-1	BDL	3105	BDL	992	4908	9006	1242	83.0
COMBWR2-1	BDL	3047	BDL	1628	6034	10709	1359	99.6
COMBWR2-2	BDL	3067	BDL	1633	5507	10208	1374	101.6
COMBWR3-1	BDL	3019	BDL	1584	5549	10152	1235	93.2
COMBWR4-1	BDL	2919	BDL	1529	5697	10145	1239	102.6
COMCW1-1	BDL	3269	BDL	1595	6308	11172	1395	105.6
COMCW2-1	BDL	3171	BDL	1635	5713	10519	1446	98.5
COMCW4-1	BDL	3214	BDL	1577	5549	10340	1387	97.4
COMCWP1-1	BDL	2863	BDL	1536	5227	9626	1177	94.2
COMCWP2-1	BDL	2981	BDL	1595	8256	12832	1236	96.1

COMCWP3-1	BDL	2804	BDL	1517	4890	9211	1247	89.5
COMCWP4-1	BDL	2720	BDL	1510	4900	9130	1200	89.6
COMCWP4-2	BDL	2941	BDL	1541	5032	9514	1261	87.0
COMDW1-1	BDL	3350	BDL	1525	5264	10139	1824	86.7
COMDW2-1	BDL	3130	BDL	1475	4995	9600	1695	84.3
COMDW3-1	BDL	3283	BDL	1636	5458	10377	1886	94.2
COMDW4-1	BDL	3298	BDL	1709	5906	10913	1989	102.9
COMDWP1-1	BDL	3386	BDL	1748	6222	11355	1868	108.7
COMDWP2-2	BDL	3069	BDL	1664	5420	10153	1793	102.1
COMDWP3-1	BDL	3252	BDL	1711	5913	10875	1931	105.0
COMDWP3-2	BDL	3003	BDL	1651	5375	10029	1641	97.1
COMDWP4-1	BDL	3269	BDL	1714	5721	10705	1784	100.5
CHARW1-1	BDL	3401	BDL	1661	5662	10725	1521	105.3
CHARW2-1	BDL	3120	BDL	1645	5483	10248	1376	101.3
CHARW3-1	BDL	3373	BDL	1677	5768	10818	1567	102.7
CHARW4-1	BDL	3222	BDL	1646	5625	10493	1486	102.9
CHARWP1-1	BDL	3271	BDL	1660	5836	10766	1461	99.6
CHARWP1-2	BDL	3464	BDL	1383	5872	10719	1364	97.2
CHARWP2-1	BDL	3163	BDL	1287	5178	9628	1217	90.2
CHARWP3-1	BDL	3133	BDL	1317	5258	9707	1307	90.8
CHARWP4-1	BDL	3302	BDL	1361	5573	10235	1321	94.9
CTR1-1	BDL	3541	BDL	1426	6293	11260	1466	95.7
CTR2-1	BDL	3861	BDL	1500	6702	12063	1580	98.0
CTR3-1	BDL	3685	BDL	1448	6191	11323	1518	97.1
CTR4-1	BDL	3423	BDL	1377	5878	10679	1407	91.8

Appendix F: DDT and dieldrin contaminated soil Pot Study Earthworm samples

Sample Name	Lipid (%)	Conc of Analyte in Worm Sample (µg/g dry wt)					Surr Rec (%)
		4,4'-DDE	4,4'-DDD	2,4'-DDT	4,4'-DDT	Dieldrin	
CTRW1-1	3.67	17.04	4.92	1.31	7.86	4.51	73.5
CTRW1-2	4.48	15.48	4.59	1.21	6.31	4.46	66.1
CTRW2-1	4.36	17.09	4.41	1.31	7.91	5.07	79.8
CTRW2-2	4.47	14.28	3.80	1.10	5.89	4.28	61.7
CTRW3-1	4.61	14.26	4.04	1.16	6.10	4.16	61.3
CTRW3-2	4.61	14.08	3.92	1.23	7.60	4.21	74.1
CTRW4-1	5.99	18.10	4.71	1.08	7.23	4.92	69.3
CTRW4-2	7.00	18.86	4.74	1.14	8.98	5.08	80.8
CTRWP1-1	5.98	22.95	5.95	2.60	10.31	7.12	79.3
CTRWP1-2	6.91	22.56	5.88	2.49	9.11	7.15	62.1
CTRWP2-1	8.07	20.14	5.70	2.50	10.55	6.50	77.3
CTRWP2-2	7.18	19.47	5.21	2.50	10.32	6.65	73.9
CTRWP3-1	4.39	19.06	4.05	2.77	13.68	5.43	103.4
CTRWP3-2	5.67	19.38	4.31	2.77	13.42	5.86	107.8
CTRWP4-1	5.82	18.80	4.30	2.76	14.18	6.06	115.2
CTRWP4-2	5.83	19.86	4.37	2.82	15.65	5.96	106.4
COMAW1-1	10.43	14.47	2.61	1.55	10.44	4.29	114.6
COMAW1-2	8.71	14.36	2.64	1.52	9.67	4.42	108.6
COMAW2-1	7.27	9.74	1.93	1.08	7.17	3.07	94.0
COMAW2-2	7.30	9.66	2.01	1.03	5.95	3.02	83.0
COMAW3-1	6.00	9.42	1.97	1.16	6.47	3.06	86.0
COMAW3-2	6.48	10.27	2.04	1.19	7.48	3.31	98.8
COMAW4-1	9.49	13.56	3.72	2.71	13.93	5.74	117.6
COMAW4-2	10.06	13.47	3.57	2.72	13.63	5.70	119.4
COMAWP1-1	8.21	14.03	2.98	2.39	7.87	4.41	87.2
COMAWP1-2	6.45	12.59	2.87	2.34	7.70	4.30	93.6
COMAWP2-1	8.51	16.17	2.08	1.25	6.02	3.97	95.2
COMAWP2-2	7.35	14.54	2.02	1.25	5.57	3.84	78.9
COMAWP3-1	7.95	17.09	2.26	1.28	7.38	4.39	103.8
COMAWP3-2	8.01	15.92	2.23	1.25	6.17	4.20	93.8
COMAWP4-1	6.15	14.27	1.67	1.08	5.49	3.44	84.4
COMAWP4-2	*6.74*	*14.25*	*3.18*	*0.83*	*3.39*	*3.32*	*45.7*
COMBW1-1	4.23	11.92	2.11	1.92	8.85	3.35	98.2
COMBW1-2	4.39	11.57	2.15	1.99	8.13	3.39	98.0
COMBW2-1	4.87	13.42	2.34	2.06	10.58	3.74	105.4
COMBW2-2	4.45	11.83	2.04	2.06	9.02	3.27	98.8
COMBW3-1	4.15	11.73	2.04	2.08	10.33	3.48	112.0

COMBW3-2	4.13	10.38	3.41	1.78	14.14	3.64	112.8
COMBW4-1	5.19	13.13	2.48	1.99	10.10	3.87	103.2
COMBW4-2	5.59	12.57	2.33	1.95	9.27	3.44	90.2
COMBWP1-1	6.23	13.63	2.72	1.79	6.83	3.41	88.6
COMBWP1-2	5.62	12.39	2.25	1.77	6.37	3.23	85.6
COMBWP2-1	4.84	10.97	2.24	1.74	6.62	3.05	84.2
COMBWP2-2	5.78	11.37	2.26	1.72	5.53	3.11	77.9
COMBWP3-1	6.18	13.61	3.14	2.42	7.28	3.93	88.4
COMBWP3-2	6.47	15.26	3.25	2.48	8.87	4.18	99.6
COMBWP4-1	5.32	12.43	2.30	1.75	6.01	3.14	78.1
COMBWP4-2	5.86	13.11	2.45	1.76	5.56	3.27	71.5
COMBWR1-1	6.74	15.55	2.82	0.82	5.79	3.32	82.6
COMBWR1-2	6.99	17.58	3.04	0.91	7.43	3.92	109.8
COMBWR2-1	7.67	17.21	5.01	1.54	9.75	5.17	116.8
COMBWR2-2	7.73	17.69	5.15	1.54	10.36	5.37	100.8
COMBWR3-1	8.70	16.38	5.69	2.77	9.42	6.06	115.4
COMBWR3-2	7.78	17.15	5.66	2.75	9.79	6.41	109.4
COMBWR4-1	6.35	17.97	3.33	1.09	9.79	4.82	117.8
COMBWR4-2	5.43	18.11	3.14	1.14	11.08	4.63	105.2
COMCW1-1	4.65	10.98	2.16	0.94	8.51	3.23	104.6
COMCW1-2	4.74	11.31	2.02	1.03	9.16	3.09	104.2
COMCW2-1	3.12	8.80	1.85	0.99	7.46	2.54	114.6
COMCW2-2	*4.92*	*11.25*	*2.06*	*1.08*	*8.47*	*3.21*	*121.8*
COMCW3-1	3.62	8.63	1.31	0.34	5.35	2.13	83.0
COMCW3-2	3.55	8.00	1.21	0.28	4.30	2.12	73.5
COMCW4-1	5.21	10.35	1.33	0.34	5.23	2.55	77.9
COMCW4-2	5.13	9.96	1.35	0.34	5.11	2.54	78.1
COMCWP1-1	4.19	9.18	1.60	0.24	5.16	2.58	87.0
COMCWP1-2	5.22	10.69	1.62	0.35	5.45	2.95	83.0
COMCWP2-1	5.75	15.89	1.68	0.40	8.68	3.75	113.8
COMCWP2-2	6.35	13.91	1.84	0.27	5.83	3.49	88.0
COMCWP3-1	5.66	12.07	3.03	0.27	7.02	4.04	107.6
COMCWP3-2	6.38	11.17	2.87	0.21	5.96	3.67	93.6
COMCWP4-1	5.83	13.89	1.94	0.67	9.05	4.98	101.6
COMCWP4-2	5.48	13.49	1.84	0.69	8.24	4.88	91.0
COMDW1-1	5.73	16.02	1.72	1.80	10.61	4.46	116.6
COMDW1-2	5.73	12.87	1.62	1.59	8.25	3.79	88.2
COMDW2-1	5.51	13.09	1.24	1.45	8.44	4.10	95.4
COMDW2-2	6.00	12.71	1.43	1.36	7.86	3.75	83.2
COMDW3-1	5.12	13.58	1.31	1.45	8.67	3.89	98.2
COMDW3-2	4.95	12.76	1.29	1.38	8.67	3.85	98.4
COMDW4-1	5.39	15.74	1.64	1.85	9.56	4.70	99.4
COMDW4-2	5.74	16.29	1.73	1.86	9.38	4.78	95.6

COMDWP1-1	6.84	16.39	2.87	2.33	10.19	5.41	103.6
COMDWP1-2	7.09	16.14	2.87	2.34	9.98	5.42	102.6
COMDWP2-1	5.19	13.48	2.88	2.45	8.95	5.22	106.0
COMDWP2-2	5.10	14.93	2.92	2.60	11.53	5.63	121.8
COMDWP3-1	7.14	9.79	1.28	1.08	4.92	3.02	65.9
COMDWP3-2	6.42	9.89	1.46	1.05	4.67	3.14	67.1
COMDWP4-1	4.81	8.09	1.19	0.89	3.67	1.95	66.3
COMDWP4-2	4.91	9.83	1.29	0.97	4.59	2.37	79.5
CHARW1-1	5.21	13.19	2.52	2.14	7.80	4.32	83.6
CHARW1-2	4.60	11.53	2.48	2.15	9.24	4.27	94.0
CHARW2-1	5.08	10.71	2.48	2.02	7.61	4.20	80.2
CHARW2-2	5.62	12.37	2.61	2.13	9.16	4.46	68.7
CHARW3-1	6.27	14.03	3.43	2.80	9.58	5.32	86.0
CHARW3-2	5.70	16.61	3.80	2.95	12.38	6.16	103.4
CHARW4-1	6.70	17.38	6.49	5.05	13.34	8.72	111.4
CHARW4-2	5.43	12.67	6.17	4.90	10.17	7.35	67.1
CHARWP1-1	6.10	12.34	3.62	2.66	6.94	4.76	74.9
CHARWP1-2	6.38	13.29	3.59	2.74	8.53	5.13	83.6
CHARWP2-1	7.93	19.94	6.98	4.71	10.26	8.89	84.8
CHARWP2-2	8.56	17.76	6.82	4.71	10.20	8.83	85.0
CHARWP3-1	*5.64*	*16.81*	*3.19*	*2.25*	*12.69*	*5.73*	*118.8*
CHARWP3-2	*5.96*	*17.78*	*3.37*	*2.29*	*15.81*	*6.03*	*125.0*
CHARWP4-1	*5.36*	*17.42*	*3.39*	*2.31*	*15.44*	*8.51*	*121.8*
CHARWP4-2	*5.54*	*16.86*	*3.23*	*2.27*	*15.13*	*6.06*	*124.2*

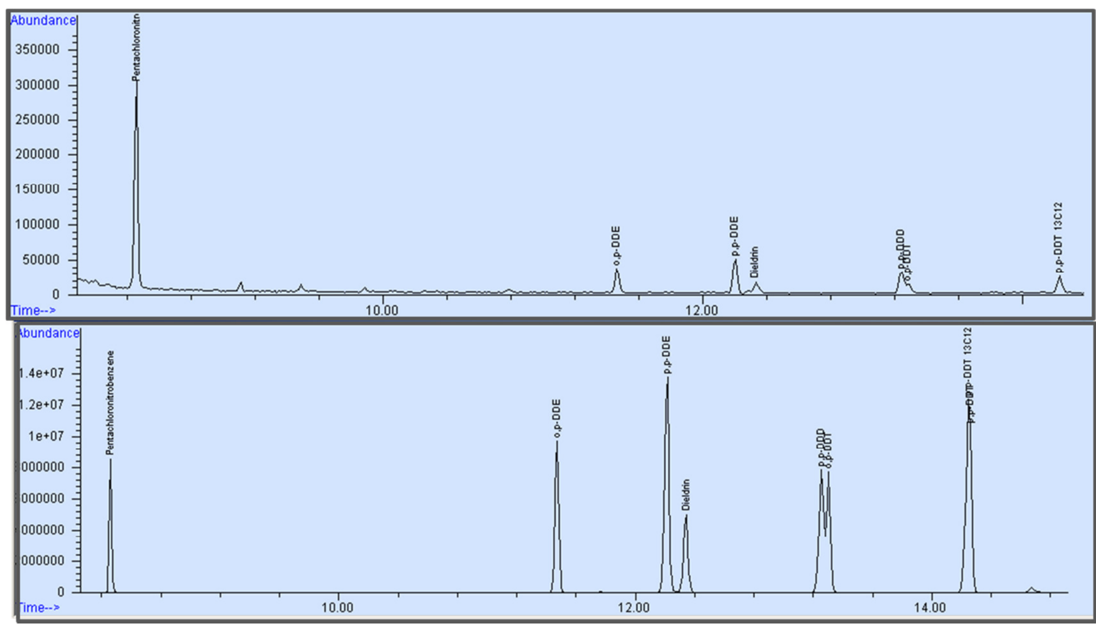
Appendix G: PBDE contaminated soil sample concentrations

Sample	μg/kg soil								
	BDE 28	BDE 47	BDE 100	BDE 99	BDE 154	BDE 153	BDE 183	BDE 209	% Recovery
T1A	BDL	1.81	1.05	3.03	0.51	0.75	BDL	86.61	82.85
T1B	BDL	1.83	1.12	3.15	0.58	0.80	BDL	99.11	97.68
T1C	BDL	1.83	1.04	3.13	0.53	0.74	BDL	150.03	102.73
T1D	BDL	1.87	1.10	3.06	0.55	0.75	BDL	124.33	87.38
T1E	BDL	1.47	0.79	2.31	BDL	0.61	BDL	123.24	76.03
T2A	BDL	1.69	1.07	2.86	0.50	0.75	BDL	98.66	93.60
T2B	BDL	1.80	1.02	2.95	0.55	0.72	BDL	95.19	101.55
T2C	BDL	1.71	0.98	2.69	0.50	0.68	BDL	94.54	83.45
T2D	BDL	1.65	1.01	2.66	0.50	0.70	BDL	107.26	93.80
T2E	BDL	1.71	1.03	2.68	0.53	0.69	BDL	94.99	87.90
G1A	BDL	5.04	2.09	5.70	0.81	1.26	1.17	160.25	83.65
G1B	BDL	5.10	2.18	5.60	0.81	1.27	0.67	252.17	93.45
G1C	BDL	5.05	2.15	5.56	0.82	1.20	0.69	160.87	82.58
G1D	BDL	5.52	2.34	6.05	0.91	1.39	0.76	239.98	88.45
G1E	BDL	5.15	2.32	5.87	0.92	1.27	0.69	219.74	90.90
G2A	BDL	5.60	2.41	6.32	0.96	1.35	0.68	204.35	101.35
G2B	BDL	5.23	2.26	5.88	0.90	1.33	0.70	192.86	95.93
G2C	BDL	5.40	2.40	6.21	0.87	1.35	0.69	219.11	103.38
G2D	BDL	5.65	2.52	6.62	0.99	1.39	0.68	210.85	101.50
G2E	BDL	5.73	2.55	6.70	1.00	1.37	0.69	247.16	128.70

Appendix H: PBDE contaminated earthworm sample concentrations

	ng/mL							
Sample	**BDE 28**	**BDE 47**	**BDE 100**	**BDE 99**	**BDE 154**	**BDE 153**	**BDE 183**	**% Surrogate Recovery**
G-E-R1	3.76	5.79	2.75	6.15	0.62	0.24	0.04	65.9
G-E-R2	4.37	5.31	3.09	5.88	0.64	0.23	0.03	75.4
T-E-R1	0.07	6.04	3.44	8.60	0.65	0.35	0.41	72.7
T-E-R2	0.11	5.73	3.39	8.23	0.61	0.27	0.00	72.6

Appendix I: Chromatogram Output for the highest and lowest standards for each analysis method.



DDT and dieldrin analysis: lowest standard=0.05ng/mL, highest standard=12 ng/mL.

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