ABSTRACT

Title of Document: ASSESSING THE FATE AND BIOAVAILABILITY OF HYDROPHOBIC ORGANIC POLLUTANTS IN AGRICULTURAL SOILS.

Natasha Almeida e Andrade, PhD, 2012

Directed By: Professor Alba Torrents
Department of Civil and Environmental Engineering

and

Dr. Laura McConnell
US Department of Agriculture

Persistent organic pollutants have been the cause of concern for many decades; however, little information is available about their environmental fate. One goal of this work was to assess whether land application of biosolids represents a source of persistent organic pollutants to agricultural soils. To address this goal, we developed a methodology to quantify low levels of the flame retardants polybrominated diphenyl ethers (PBDEs) in biosolids and soils and conducted field studies to determine the fate and persistence of PBDEs upon the land application of biosolids. We found that biosolids can take up to one year to completely incorporate into the soil matrix after application and biosolids-bound chemicals are released during this

time. PBDEs profiles in soils that receive biosolids applications are similar to PBDEs profiles in biosolids and both reflect commercial formulations of these flame retardants, indicating that biosolids are a source of these chemicals to soil. Residence time of these chemicals was reported for the first time and it was estimated at 16 yr. for the sum of BDE-47 and BDE-99. An abiotic methodology to assess bioavailability of aged soil residues was developed and results were compared to earthworms. The study illustrated that the polymer-based abiotic methodology can be used to assess the bioavailability of soil-bound hydrophobic organic chemicals to earthworms. Measured soil-polymer equilibrium concentration ratios of organic pollutants correlated strongly with earthworm bioaccumulation factors using the same soils. A laboratory protocol to introduce the concept of fugacity and bioavailability to undergraduate and graduate environmental science and engineering students was developed based on the methodology developed for research. The experiment provided an excellent opportunity for students to become familiar with the laboratory protocols and techniques for quantitative analysis as well as graphical analysis of data. The totality of this work improves knowledge of the fate of two classes of organic pollutants in soils. This work substantially adds information and understanding of chemical behavior to the general environmental engineering field. Although this unique experiment provided original and essential pieces of information, additional research is crucial to address the difficulties involved in assessing the environmental behavior of organic pollutants.

ASSESSING THE FATE AND BIOAVAILABILITY OF HYDROPHOBIC ORGANIC POLLUTANTS IN AGRICULTURAL SOILS.

By

Natasha Almeida e Andrade

Dissertation submitted to the Faculty of the Graduate School of the
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Advisory Committee:
Professor Dr. Alba Torrents, Chair
Dr. Laura McConnell, Co-advisor
Dr. Allen Davis
Dr. Kaye Brubaker
Dr. Mary Ann Ottinger, Dean's Representative

Dedication

This work is dedicated to my family, my mother Sandra, my father Fernando, and my brother Rafael, which in every way made me the person I am today. Being away from home for such long years was only possible because of their love and support. Thank you for all the sacrifice I put you through to achieve my dream. A saudade assombrosa que sinto de vocês só é suportável por saber que vocês nunca estão distantes de mim, mesmo com muitos quilometros entre nós.

Há momentos na vida em que sentimos tanto a falta de alguém que o que mais queremos é tirar esta pessoa de nossos sonhos e abraçá-la.

Sonhe com aquilo que você quiser.
Seja o que você quer ser, porque você possui apenas uma vida e nela só se tem uma chance de fazer aquilo que se quer.

Tenha felicidade bastante para fazê-la doce.
Dificuldades para fazê-la forte.
Tristeza para fazê-la humana.
E esperança suficiente para fazê-la feliz.

As pessoas mais felizes não têm as melhores coisas.
Elas sabem fazer o melhor das oportunidades que aparecem em seus caminhos.

A felicidade aparece para aqueles que choram.
Para aqueles que se machucam.
Para aqueles que buscam e tentam sempre.
E para aqueles que reconhecem a importância das pessoas que passam por suas vidas.

O futuro mais brilhante é baseado num passado intensamente vivido.
Você só terá sucesso na vida quando perdoar os erros e as decepções do passado.

A vida é curta, mas as emoções que podemos deixar duram uma eternidade.
A vida não é de se brincar porque um belo dia se morre.

Clarice Lispector

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I would like to thank my friends Fernanda and Julia for support when things were not going well and cheers when the projects were successful. Lastly, I would like to thank Daniel Gralewski. You helped me understand so much about life and work, even becoming an environmental engineer at times to help me through the process. Você é realmente a melhor pessoa que apareceu na minha vida. Te amo.

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Chapter 1 – Study Objectives and Outcomes

The main goal of this work was to assess whether land application of biosolids represents a source of persistent organic pollutants to agricultural soils. The research group also aimed to improve our capabilities to evaluate the potential environmental risks associated with hydrophobic chemicals in biosolids and soils. The reach these goals, this research developed methodologies for the detection and evaluation of organic pollutants bioavailability. This work presents both field and laboratory experiments and it contains hypothesis-driven research as well as investigation-driven research. While on the hypothesis-driven research there is a clear path of experimental design that leads to the answer of a testable hypothesis, on the investigation-driven research, results of the research are analyzed with a broad set of questions that are not necessarily testable.

Specific objectives were:

1. Develop methodologies to quantify low levels of the flame retardants polybrominated diphenyl ethers (PBDEs) in biosolids and soils.
2. Start a repository of biosolids and soil samples and a database of persistent organic pollutants. While this work concentrated in analyzing collected samples for PBDEs, other members of the research group are utilizing

archival samples to assess the presence and persistence of other chemicals of concern.

3. Conduct field studies to determine the fate and persistence of PBDEs upon the land application of biosolids.
4. Develop an abiotic methodology to assess bioavailability of aged soil residues to earthworms.
5. Develop a laboratory protocol to introduce the concept of fugacity and bioavailability to undergraduate and graduate environmental science and engineering students.

While analytical methodologies have been developed and are presented throughout the work, for each of the objectives 2-5, a series of questions and hypotheses have been postulated to reach the objectives.

1.1 – Objective 2

Presence and trends of organic pollutants in biosolids

General questions:

a) With the ban on Penta-BDE in 2005, how has the profile of PBDEs changed in biosolids since 2005 and how is it expected to change after phasing-out of Deca-BDE?

b) Are concentrations of PBDEs found in the studied wastewater treatment plant in comparable levels to others throughout the world?

Testable Hypothesis:

a) Penta-BDE concentrations in biosolids will decrease over the sample collection period by 10% (2005 to 2011).

Significance and Experimental Design

While there is a serious concern over the presence of organic pollutants in biosolids and their fate upon land application of biosolids, the amount of data available to increase scientists' capability to anticipate their presence and levels is very limited. This work initiated a long-term (over 6 years and ongoing) collection and storage of biosolids samples for current and future analysis of persistent organic pollutants of concern. New chemicals are being introduced into consumer products and old chemicals of concern are banned, therefore, tracking concentrations of organic pollutants in biosolids over a long period of time may provide insight on usage and release of organic pollutants from consumer products to the environment. To address this issue, we collected and analyzed biosolids samples every two months from a large Mid-Atlantic wastewater treatment plant (WWTP) from 2005 to 2011. Experimental design details and sample processing can be found in Chapter 3. The data obtained from this long-term analysis of biosolids will fill research gaps not only

for the target analytes of this research, polybrominated diphenyl ethers (PBDEs), but also for other chemicals, as the repository of samples collected by the present author is being utilized by other member of the research group, which will provide a rich dataset for researchers. Results for PBDEs in biosolids samples can be found in Chapter 3.

1.2 – Objective 3

Presence, persistence, and estimation of half-life of PBDEs in soils after land application of biosolids

General questions:

a) How long does it take for the incorporation of biosolids into the soil matrix after land application?
b) What is the PBDE profile in soils after single and multiple biosolids applications?
c) Examining data from multiple sample collections after a single application, what is the estimated residence time of selected PBDEs, i.e., BDE-47, BDE-99, and BDE-209 in soil?
d) What is the estimated residence time of PBDEs from farms that are commercially purposed as opposed to research field experiment?
e) Which processes are likely controlling the fate of PBDEs in soils?

Testable Hypothesis:

a) Calculated topsoil residence time of the sum of BDE-47 and BDE-99 will be within 20% of previously estimated residence time of 12.7 years (Andrade *et al.*, 2010).

Significance and Experimental Design

The analysis of organic pollutants in environmental samples can be challenging and PBDEs are particularly difficult to analyze given their large molecular size, hydrophobicity, and the fact that they represent a mixture of different compounds. The development of a reliable methodology to analyze PBDEs in soils and biosolids was one of the main goals of this research project. The approach utilized by the author was to modify existing methodologies and optimize for suitable available instruments. Previous methodologies did not work for this study because they either required a long extraction process (usually by soxhlet extraction) or they required a reliable Accelerated Solvent Extractor, which was unavailable at the method development period. The modifications came in the form of simplifying the method to a basic vortex extraction, which offered complete control over the process, and a simple clean-up of the dirty soil and biosolids matrix. The methodology was successful as it was reproducible, produced good recoveries of organic chemicals, and it was simple and comparatively fast.

With a good and working methodology in place, the questions of how biosolids are incorporated into the soil and how chemicals behave in the soil after biosolids application could be addressed. Two kinds of field experiments were set up in this project. The first was a follow up of a field survey that occurred in 2006, where soil samples were collected from farms that received single or multiple biosolids applications. In 2009, the same farms were sampled once again and data from the two collection periods were compared. The second experiment focused on one single biosolids application in a much smaller field, which was monitored over time for three consecutive years. Details of the experimental design, sampling, and sample processing can be found in Chapter 4, where results are also provided.

Both experiments combined provided a unique perspective in biosolids land application and also made it possible to estimate topsoil residence time in a realistic situation. These data can be used to enhance our understanding of the fate and behavior of the target chemicals and also provide a perspective on their environmental risk. It also provided insight into the heterogeneity of such systems with a clear indication of large temporal and spatial variability. The large number of samples collected and analyzed in this work indicates that one needs to be cautious in generalizing conclusions from small experiments.

1.3 – Objective 4

Bioavailability of DDT family and dieldrin in contaminated soil

General questions:

a) How does the bioavailability methodology developed in the laboratory compare to using organisms such as earthworms?
b) Does the application of organic amendments increase soil organic matter and thus decrease the bioavailability of chemicals to earthworms?

Testable Hypotheses:

a) Bioavailability obtained through the thin-film solid phase extraction (TF-SPE) methodology will present values that are within 10% bioavailability determined using earthworms.
b) The bioavailability of soil aged DDT will decrease by 10% with the application of organic amendments at a rate of 5% by mass.

Significance and Experimental Design

It is well established that the use of bioavailability instead of total contaminant concentration is more appropriate when estimating environmental risk of soil bound organic pollutants. As the bioavailable fraction of harmful chemicals in the soil decrease (the chemicals become immobile in the soil organic matter), their associated

risk to wildlife also decreases. To measure bioavailability, the use of biological assays has been usually employed; however, they can be costly and time-consuming. The development and optimization of a chemical extraction methodology that can be used as a surrogate for biological bioavailability, was the motivation of this portion of the project. The author optimized an existing fugacity-based methodology and compared results with earthworm assay methodology. Details of the methodology, sample processing, and experimental design can be found in Chapter 5.

A highly and historically contaminated soil was examined with the chemical and biological assays. Organic matter amendments were added to the contaminated soil, and the effects of the resulting increased organic carbon on the bioavailability of organic contaminants were analyzed. A detailed set of results is available in Chapter 5. The results of this project provide a better understanding of how organic contaminants behave in soil after a long period of aging. Also, results show that the interactions between contaminants, soil, and organic amendments is complex, and stress the need for further research in this area.

1.4 – Objective 5

Application of bioavailability experiment as a teaching tool

The general goal for this portion of the research project was to address the teaching of an ever-evolving environmental science research that generates new and

improved methodologies at a fast pace. Exposing students to new technologies keeps the knowledge gain during undergraduate and graduate work up-to-date and engaging. The author utilized the methodology developed to estimate bioavailability to organic pollutants in soil to develop a laboratory class for undergraduate and graduate students. The laboratory class was executed throughout two semesters to two different student groups and results of this implementation were analyzed. The process of class development, implementation, and results is discussed in Chapter 6. During doctoral degree training, exposure to all aspects of academic life is important. This project stressed the need of updated teaching methodologies, the skill of transforming part of research into a teaching tool, as well as the enhanced understanding of the leaning abilities of students.

Chapter 2 – Introduction

2.1 – Hydrophobic Organic Pollutants

Hydrophobic organic pollutants have been of concern for many decades due to their high chance of accumulation and persistence in the environment. These chemicals are divided into various groups and they are mostly man-made compounds. Two groups of hydrophobic organic pollutants are the focus of this research, polybrominated diphenyl ethers and pesticides.

2.1.1 – Polybrominated Diphenyl Ethers

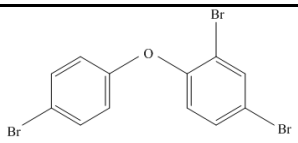
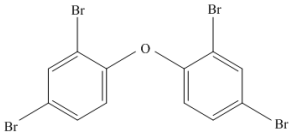
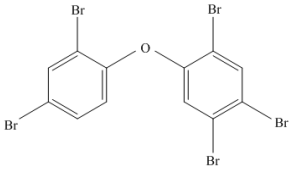
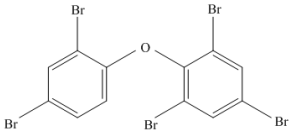
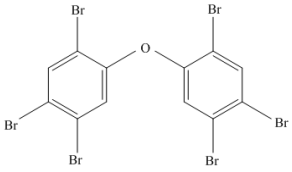
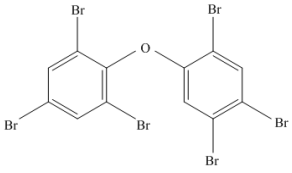
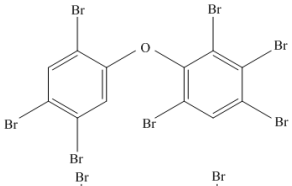
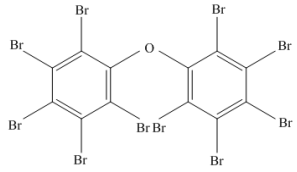
Physical-chemical characteristics

Polybrominated diphenyl ethers (PBDEs) are a class of chemical compounds in which up to 10 bromine atoms are attached to a diphenyl ether molecule. There are 209 different possible combinations, forming different compounds depending on the number and position of the bromine atoms. Each one of the 209 possible compounds is called a congener. PBDEs are hydrophobic (Table 2.1) and resistant to degradation. For these compounds, water solubility and vapor pressure decrease with increasing degree of bromination. Also, as the number of bromine atoms are

increased so does the hydrophobicity of the molecule; the molecule increases in size without a gain in polarity. A study measured the Henry's law constants for congeners BDE-28, BDE-47, BDE-100, BDE-99, BDE-154, BDE-153, and BDE-209 and found that there is a strong dependence of Henry's law to temperature, and this dependence varies with degree of bromination and the structural position of bromines (Cetin and Odabasi, 2005). Values varied from 0.04 to 4.83 Pa m^3 mol^{-1} at 25℃, where BDE-209 held the lowest value and BDE-28 held the highest.

All the properties combined clearly indicate that these compounds are likely to strongly bind to organic matter and are likely to have a low tendency to volatilize. These characteristics are even clearer for the highly brominated congeners, indicating that the BDE-209 has a high chance of being persistent in the environment.

Table 2.1 – Physical and chemical properties of the compounds of interest for this study (± standard errors). K_{oa} = n-octanol/air partition coefficient; P_L = supercooled liquid vapor pressure (Pa); H = Henry's Law constant measured at 25°C (Pa m^3 mol^{-1}); K_{ow} = n-octanol/water partition coefficient. References: (1) Hui-Ying *et al.*, 2007; (2) Wania *et al.*, 2002; (3) Wong *et al.*, 2001; (4) Cetin and Odabasi, 2005; (5) Braekevelt *et al.*, 2003.

Abbreviation	Structure	$logK_{oa}$	$logP_L$	H	$logK_{ow}$
BDE - 28		9.7[1]	-2.93[1]	4.83 ± 0.67[4]	5.94 ± 0.15[5]
BDE - 47		10.34[2]	-3.5[3]	0.85 ± 0.35[4]	6.81 ± 0.08[5]
BDE - 99		11.28[2]	-4.17[3]	0.6 ± 0.11[4]	7.32 ± 0.14[5]
BDE - 100		11.40[1]	-4.47[1]	0.24 ± 0.06[4]	7.24 ± 0.16[5]
BDE - 153		12.15[2]	-5.07[3]	0.26 ± 0.08[4]	7.90 ± 0.14[5]
BDE - 154		12.18[1]	-5.18[1]	0.08 ± 0.04[4]	7.82 ± 0.16[5]
BDE - 183		12.89[1]	-5.84[1]	logH= 1.535[1]	8.27 ± 0.26[5]
BDE - 209		15.73[1]	-8.4[1]	0.04 ± 0.01[4]	10.33[5]

Production

PBDEs receive commercial names in addition to their IUPAC names by industry and consumers. These compounds are produced as a mixture of congeners; rarely are they sold separately. Commercialized mixtures were: penta-BDE (mixture of tri-BDE, tetra-BDE, penta-BDE, and hexa-BDE), octa-BDE (mixture of hexa-BDE, hepta-BDE, octa-BDE, and nona-BDE), and deca-BDE (mixture of octa-BDE, nona-BDE, and deca-BDE), which is the only one still in production. The mixtures have different compositions depending on the manufacturer.

PBDEs belong to a group of chemicals known as brominated flame retardants (BFRs). Industry uses BFRs globally in hundreds of products, such as foam mattresses, televisions, computers, plastics, textiles, and more, in order to reduce their flammability (de Wit, 2002). The global production of BFRs increased from 107,000 metric tons in 1989 to 203,000 metric tons in 1999 (Alaee *et al.*, 2003) with a large percentage of the global production directed to North America (de Wit, 2002). As the use of plastics increases so will the demand for these chemicals.

The total global demand for brominated flame retardants data is very difficult to acquire. According to the Freedonia Group, Inc. (2012), the growth in flame retardant demand will push demand numbers to 2.2 million metric tons in 2014 (Table 2.2). Although the demand slowed down from 2004 to 2009 mainly due to economic problems in North America and Europe, demand never decreased in other parts of the world and is on the rise once again throughout all continents. Penta-BDE

and Octa-BDE have not been produced since 2005, but Deca-BDE is still in production and the date of the voluntary phasing out production by industry keeps being delayed, being now estimated to 2014.

Table 2.2 – World Flame Retardant Demand. Reference: Freedonia Group, Inc. (2012)

Location	**2004**	**2009**	**2014**	**2004-'09**	**2009-'14**
	Demand in 1000 tons			% Annual Growth	
World Flame Retardant Demand	1528.9	1653.0	2220.0	1.6	6.1
North America	480.2	390.8	511.5	-4.0	5.5
Western Europe	392.0	360.7	427.5	-1.7	3.5
Asia/Pacific	546.6	758.5	1090.0	6.8	7.5
Other Regions	110.1	143.0	191.0	5.4	6.0

Toxicity

PBDEs have similar structure to polychlorinated biphenyls (PCBs), which are well known pollutants with adverse effects on biota. PBDEs could present health risks for humans because they are hydrophobic and partition to fatty tissue in humans and animals. The low brominated congeners are known to bioaccumulate (accumulation in the same trophic level), biomagnify (accumulation in different trophic levels) (Voorspoels *et al.*, 2007), to easily adsorb, and to be more bioactive

than the deca-BDE (McDonald, 2002). It is reported that all PBDE products can potentially cause thyroid effects and neurobehavioral effects, while the deca-BDE presented some carcinogenic potential in female and male rats (McDonald, 2002).

PBDEs' structures are similar to the thyroid hormone (Figure 2.1), therefore once inside the organism, PBDEs can mimic the role of the hormone, deregulating the production of the same, which later causes the disruption of the endocrine system. Lilienthal *et al.* (2006) showed that offspring of female rats that received penta-BDE (10 mg/kg body weight) during pregnancy had a decrease in sex steroids and feminization of adult males. Rats and mice are usually the choice of toxicology effect studies for they could be used to represent effects in humans. Staskal *et al.* (2005) shows that metabolic capacity and exposure for different species (rats and mice) may significantly influence the congener profile inside the body, which raises the question of which species would better represent humans in toxicological studies.

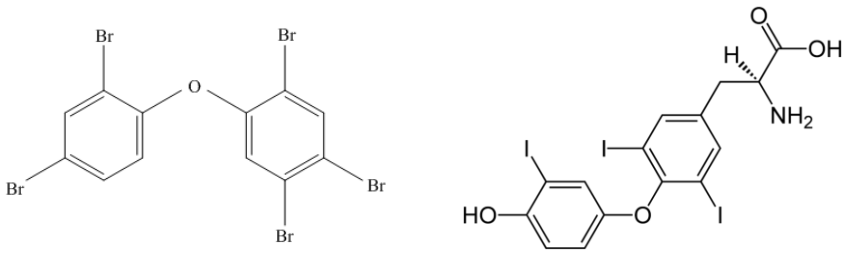


Figure 2.1 – Congener BDE-99 (left) and the thyroid hormone Triiodothyronine (T_3) (right) molecular structures. BDE-99 can potentially substitute the thyroid hormone and cause endocrine system disruption.

Toxicity in humans is not well studied and limited data are available. Two studies on skin sensitization proved no sensitization for deca-BDE, which was the only congener analyzed (Darnerud *et al.*, 2001; Hardy, 2002). Workers from factories that manufacture PBDEs presented higher levels of hypothyroidism, but the effects could not be attributed to PBDEs (Darnerud *et al.*, 2001). Although there are a few studies with humans and many studies with animals, the risk for human health offered by PBDEs cannot be well established at this time, and more studies in this area are needed.

Distribution

PBDEs are added to different polymers but they are not chemically bound to the polymer backbone and thus are easily released to the environment. They are generally released to the environment in two different ways: volatile release from consumer products or with wastewater effluents or solids. There are many studies demonstrating that PBDEs are volatilized from consumer products. PBDEs have been reported in house dust and indoor air (Stapleton *et al.*, 2005; Harrad *et al.*, 2006; Harrad *et al.*, 2008; Hazrati and Harrad, 2006; Jones-Otazo *et al.*, 2005; Mandalakis *et al.*, 2008; Schecter *et al.*, 2005), illustrating that humans are susceptible to ingestion of these chemicals in different environments.

Studies that analyzed mammals like polar bears (Dietz *et al.*, 2007; Gebbink, 2008) and humans (Sjodin *et al.*, 2001) show PBDEs in their systems. For humans,

the highest concentration is found in breast milk (Antignac *et al.*, 2008), which increases the concern that children may be exposed as infants. Trudel *et al.*, (2011) showed results that indicated that infants PBDEs doses are 5 times higher than adult's doses and toddlers and teenagers' doses are 3 times higher than adults'. The average concentration in dust inside a house varies according to the microenvironment analyzed. Allen *et al.*, (2008) concluded that the concentrations in the main living areas of houses were higher than the concentrations in bedrooms. A report by Mandalakis *et al.* (2007) shows concentration of PBDEs in indoor air from computer/electronics shops are usually higher than the concentrations found in indoor air from houses or furniture stores.

A study indicates that electronics are a bigger source of PBDEs to indoor air than furniture (Chen *et al.*, 2008). Lorber (2008) suggests that the majority of the intake of PBDEs by humans is derived from the use and the presence of electronics rather than food ingestion. Dermal exposure was also cited by Staskal *et al.* (2005); in their study they confirmed that approximately 62% of the BDE-47 administered to female mice was absorbed through skin.

Environmental fate

In the environment, PBDEs have been detected in all environmental compartments; however, their transformation in specific compartments is still largely unknown. Besides its toxicity, the potential to bioaccumulate, persistence in the

environment, and the potential for long-range atmospheric transport are used to assess appropriate restrictions for use of these chemicals (Gouin and Harner, 2003). Studies show that the transport of PBDEs is difficult to model accurately (Gouin and Harner, 2003). It appears that there are parameters and processes that are not included in current models governing the partitioning of PBDEs in the many compartments of the environment.

The photodegradation of BDE-209 has been observed (Ahn *et al.*, 2006; Sanchez-Prado *et al.*, 2005; Eriksson *et al.*, 2004) and is considered important for the environmental fate of this compound because deca-BDE formulation is the major industrial PBDE product. Ahn *et al.* (2006) has shown photodegradation in soil and this degradation was dependent on the amount of light received, the type of soil where the PBDEs are present, and the amount of PBDEs that are adsorbed into organic matter. The products of the photodegradation of deca-BDE are less brominated congeners. The products found by Rayne *et al.*, (2006) when they analyzed the photodegradation products of the hexa brominated congener, BDE-153, were seven different penta and tetra-brominated congeners. While analyzing the photodegradation products of BDE-209, Bezares-Cruz *et al.* (2004) found that the products were a wide range of PBDEs, from nona brominated to tetra brominated congeners.

Anaerobic biodegradation studied by Gerecke *et al.* (2005) indicates that deca-BDE biodegrades to nona and octa-BDEs "within a period of 238 days in experiments

with sewage sludge as the inoculum". The calculated half-life for BDE-209 by Gerecke *et al.* (2005) was 700 days. Another study (Welsh, 2008) shows that bacteria present in sewage sludge was capable of performing anaerobic debromination of BDE-209 at environmental relevant levels (40ppb), but does not mention rate of degradation. A comparison between biodegradation in a sediment environment and in a biomimetic environment (a laboratory procedure that mimics the natural environment) was performed by Tokarz *et al.* (2008). This study investigated BDE-209, BDE-47, and BDE-99. The debromination of BDE-209 to mainly hexa brominated compounds occurred fast in the biomimetic system (five minutes), while the debromination in sediment occurred slowly and significant increase in the products was observed after 3.5 years of incubation. This study also used the biomimetic acquired reaction rate to predict the reaction rate in an environment where sorption is an important factor. The predicted values are near to the values obtained by the sediment experiments, although the rates for the latter varied by an order of magnitude. This experiment shows that the anaerobic degradation of BDE-209 could play an important role in the presence of lower brominated BDEs in the environment (Tokarz *et al.*, 2008).

Other studies report reductive debromination (He *et al.*, 2006; Rayne *et al.*, 2003; Vonderheide *et al.*, 2006) as well and conclude that the transformation of the higher brominated compounds into the lower brominated compounds is slow; therefore the persistence of these chemicals could be relatively large. The pathway of

debromination had never been systematically studied, due to difficulties in detecting some of the congeners and co-elution in GC columns, until a different method of analysis was used (Robrock *et al.*, 2008). Seven congeners (main components of an octa-BDE mixture and BDE-47 and BDE-99) underwent degradation by three dehalogenating cultures. Also noteworthy was that the extent of removal of bromines from the BDE molecules was orders of magnitude smaller than the removal of chlorines from PCBs (Robrock *et al.*, 2008).

Soil and biosolids concentrations

PBDEs levels in European soils started to increase in the 1970s (Hassanin *et al.*, 2005), reached the highest level in the year 2000 and then concentrations started to decline in response to restrictions on the use of PBDEs in Europe. Schuster *et al.* (2011) analyzed soils contaminated with PBDEs in 1998 and in 2008 and concluded that 2008 concentrations were 66 ± 102% lower than 1998 concentrations. A study that analyzed soil from remote areas representing background soil concentrations (Hassanin *et al.* 2004), found that concentrations could reach up to 12 μg/kg d.w. (Σ all PBDEs congeners). Soils that have been amended with biosolids have also been analyzed by several groups. Generally, data is reported for top layer of the soil, because PBDEs are not expected to move down into the soil layer. Recently, Gorgy *et al.* (2012) analyzed contaminated soil at different depths and at two different biosolids application rate. Regarding application rate, results are in accordance to

what Andrade *et al.*, (2010) has found, a larger application rate results in larger soil PBDE concentrations. At the larger application rate of 80t/ha, the top layer of soil (0.05-0.25m) had the highest PBDE concentration, followed by the second layer (0.25-0.45m). The general trend for all their experiments was a decline in PBDE concentration with soil layer depth, which is in agreement with Xia *et al.* (2010) findings.

Xia *et al.* (2010) also reported that a field that received annual biosolids applications for 33 years at three different rates, heavily accumulated PBDEs. For the highest cumulative loading of 2,218 mg dry biosolids/ha, concentration of PBDEs in the top layer (0-15cm) was 658 µg/kg dry weight, while concentration in the second layer (15-30cm) was 105 µg/kg dry soil.

An extensive review of concentrations of PBDEs and other organic contaminants in sewage sludge or biosolids was recently published (Clarke and Smith, 2011). BDE concentrations vary widely throughout the world and U.S. and Australia have the highest concentrations. Also, BDE-209 is the dominant congener for the majority of the studies reported in the review. Recently, eight Italian WWTPs were investigated for PBDEs in sludge and concentrations ranged from 158.3 to 9427 ng/g dry weight, with BDE-209 also being the dominant congener (Cincinelli *et al.*, 2012). Historical sludge samples (1975-2008) from a WWTP in Chicago were analyzed for PBDEs and authors reported that, as expected, BDE-209 dominated the congener profile, and that concentrations of this congener rapidly increased from the mid-1990s to mid-2000s.

They found high levels of PBDEs in samples from 2004 to 2007, with averages of 1080, 6630, and 7800 µg/kg for $\sum penta - BDE$, $\sum deca - BDE$, and $\sum PBDE$ respectively.

Nylund *et al.*, (1992) investigated sewage sludge as a source of PBDEs to the Baltic Sea and observed the tetra and penta-brominated PBDEs at concentrations ranging from 3.4-19 µg/kg d.w. per congener. A more extensive study in the Netherlands measured PBDEs in solids associated with the influent and effluent waters from 4 different WWTPs (de Boer *et al.*, 2003). Surprisingly, suspended particle PBDE concentrations in the effluent waters were often higher than in the influent, especially for the most hydrophobic decabrominated PBDE. The authors speculated that the effluent contained only the finest particles with the highest organic carbon content and the highest concentrations of PBDE. This may indicate that the influent water contained more inorganic material that 'diluted' the particle phase PBDE concentrations.

Biosolids samples from 22 wastewater treatment plants in Sweden resulted in concentrations from 0.3 to 11 µg/kg wet weight for different (BDE-47, BDE-85, BDE-99, BDE-100, BDE-138, BDE-153, BDE-154, and BDE-209) congeners, with BDE 209 having the highest concentrations and BDE 138 having the lowest (Oberg *et al.*, 2002). In Germany, samples were collected and analyzed from 11 wastewater treatment plants (Knoth *et al.*, 2004). The concentration for the sum of BDE 28, 47,

99, 100, 153, 154, and 183 congeners ranged from 12.5 to 288 µg/kg d.w., and the concentration of BDE 209 ranged from 97.1 to 2217 µg/kg d.w. In Spain, sewage sludge samples were collected in five WWTPs and concentrations found were between 197 and 1185 µg/kg d.w. and the mean value was established at 572 µg/kg d.w. (Eljarrat *et al.*, 2008). A more recent study in Kuwait analyzed sludge samples from three WWTPs and reported mean concentrations (∑PBDEs) in the range of 5.7-1599 µg/kg. This study showed a high concentration variability and the authors also observed a seasonal trend related to temperature effects (Gevao *et al.*, 2008). In Australia, 16 WWTPs were surveyed for PBDEs and the average sludge concentration was 1137 µg/kg (Clarke *et al.*, 2008). The same study collected samples in 2005 and 2006 to analyze for seasonal variations, but samples presented differences only between WWTPs and not between years.

In the U.S., Hale *et al.*, (2001a), found PBDEs in biosolids in eleven samples from WWTPs in Virginia, Maryland, New York, and California. The concentrations ranged from 1100-2290 µg/kg d.w. for the penta-brominated PBDEs and 85-4890 µg/kg d.w. for the decabrominated PBDE congener indicating that input was high. In California, reported concentrations from one plant ranged from 0.06 to 1.44 µg/kg d.w. for different congeners and that congeners from the penta-BDE commercial formulation corresponded to 88% of the total PBDE concentration in the effluent while BDE-209 contributed to 6% of the total PBDE concentration (North. 2004).

While there is a lot of information on PBDE levels in the environment, the

information is spotty and generally from small sample sizes. There is a large variability of levels of PBDEs in both soils and biosolids and to the author's knowledge there is no data for soil half-life of these chemicals.

2.1.2 – DDT Family and Dieldrin

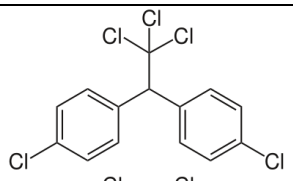
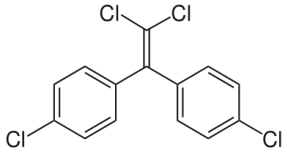
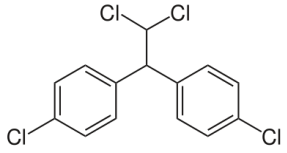
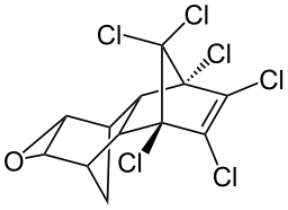
Physical-chemical characteristics and production

1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT; 4,4'-DDT) was the first of the modern synthetic insecticides, and it was developed in the 1940s (EPA[f]). In 1962, when DDT use was the highest, Silent Spring (Carson, 2002) was published questioning its widespread use. It continued to be a very commonly used pesticide in the US until it was banned in 1972. It was first introduced and used with great success in the second half of World War II to control mosquitoes that transmitted malaria and typhus among civilians and troops, and it has been credited for the control of malaria around the world. The commercial mixture of DDT is a mixture of three forms: 4,4'-DDT (85%), 2,4'-DDT (15%), and trace amounts of 2,2'-DDT (EPA[f]). The technical mixture may also contains DDT's degradation products: 1,1-dichloro-2,2-di(4-chlorophenyl) ethylene (4,4'-DDE) and 1,1-dichloro-2,2-di(4-chlorophenyl)ethane (4,4'-DDD) (Table 2.3).

1,2,3,4,10,10-hexaclhoro-6,7-epoxy-1,4,4α, 5,6,7,8,8α-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene (Dieldrin) is also a synthetic insecticide that was

commonly used in the US, especially from 1050s to 1970s on crops. Dieldrin stopped being used for crops in 1970 and stopped being used to kill termites in households in 1987 (ATSDR, 2002a).

Table 2.3 – Chemical formula, structure, CAS #, and molecular weight of DDT, DDE, DDD, and dieldrin.

Name	Chemical Formula	Chemical Structure	CAS #	Molecular Weight
4,4'-DDT	$C_{14}H_9Cl_5$		50-29-3	354.49
4,4'-DDE	$C_{14}H_8Cl_4$		72-55-9	318.03
4,4'-DDD	$C_{14}H_{10}C_{l4}$		72-54-8	320.05
Dieldrin	$C_{12}H_8Cl_6O$		60-57-1	380.91

Toxicity

Toxicity of DDT, its metabolites, and dieldrin is well documented by EPA and the World Health Organization. The banning of the insecticides is mostly due to their toxicity to wildlife. Although levels in wildlife have been declining since 1970s, they can still be detected in many parts of the US due to their environmental persistence.

Animal studies have shown that DDT and its metabolites can cause cancer, respiratory arrest in case of acute exposure, endocrine disruption, and immunocompetence (ATSDR, 2002b). Dieldrin is also toxic to animals, causing effects mainly in the nervous system and kidneys. There is some evidence that it may cause birth defects and cancer in mice (ATSDR, 2002a).

In humans, DDT can cause nausea, confusion, headache, vomiting, anemia, and other symptoms (ATSDR, 2002b). No reproductive effects have been reported in humans, and although EPA classifies it as a carcinogen, there is inadequate evidence that it may cause cancer in humans. Dieldrin causes convulsions and other nervous system effects and may cause kidney damage in people exposed to large amounts of the chemical. Similar effects caused by DDT like vomiting, nausea, and headaches are also caused by dieldrin in moderate amounts. According to EPA, dieldrin is a probable human carcinogen.

Environmental fate

Even though DDT and dieldrin are no longer used in the US (DDT is still used in many developing countries to combat malaria), various contaminated agricultural sites exist with high levels of DDT, DDE, DDD, and dieldrin. Because of physical and chemical properties, DDT and dieldrin can reach any environmental compartments, although these chemicals favor partition to organic-rich locations. DDT and dieldrin are very persistent in the environment in soils as well as aquatic

environments. The potential of run-off from soil is very small, therefore higher concentrations are found in soil, where the half-life is 2 to 15 years for DDT and about 5 years for dieldrin (ATSDR, 2002a and b).

Bioavailability

Bioavailability of an organic compound in any environmental phase is the degree at which this compound is available to take part in chemical and biological reactions. Only part of the total concentration of an organic compound is available, depending of the physical and chemicals characteristics of both the compound and the environmental phase in which the compound is located. When assessing risk of organic pollutants, it is important to differentiate between total environmental concentrations and chemical and biological bioavailability. Scientists agree that bioavailability is more representative of the risk associated with a chemical than its concentration (Alexander, 2000; Reichenberg and Mayer, 2006). Although a variety of methods to measure bioavailability have been developed and tested, there is not one method that has been established as the most suitable, therefore bioavailability data remains difficult to be compared and introduced in risk assessment schemes (Reichenberg & Mayer, 2006).

Hydrophobic contaminants bioavailability can be obtained generally with two approaches, measuring contaminant concentrations in organisms dwelling in the contaminated media (Lanno *et al.*, 2004), or using a chemical assay to extract the

bioavailable portion from the contaminated media (Mäempää *et al.*, 2011; Tang *et al.*, 1999). Mild solvent extractions, tenax-aided desorption, cyclodextrin extraction, and solid-phase micro extractions have been used as chemical assays (Hunter *et al.*, 2011). To ensure that chemical extraction is representative of bioavailability, measurements are usually compared to living organisms' concentrations and the resulting relationship provides insight on the usefulness of the methodology. To evaluate the bioavailability of DDT, its metabolites, and dieldrin in soil, researchers usually report concentrations in earthworms and sometimes compare those concentrations to ones found with different chemical extraction methodologies.

Bioavailability of organic chemicals can be reported as concentrations in earthworms or as bioaccumulation factors (BAFs), which are defined as the concentrations in the earthworms divided by the initial soil concentration. Alternatively, uptake by earthworms can be reported as a percentage of the total soils concentration that was taken up by earthworms. Tang *et al.* (1999) reports that *Eisenia foetida* accumulated 27.8, 24.6, and 30.4% of DDT, DDE, and DDD respectively from a sassafras silt loam contaminated soil aged 49 yr. They also report that for a chester loam soil also aged 49 yr., accumulation in the earthworms was 3.53, 18.4, 6.68% for DDT, DDE, and DDD respectively, which indicates that bioavailability to earthworms is very dependent in soil type. They also analyzed soils that were aged for a much shorter period of time in the laboratory and in general, concluded that DDT, DDE, and DDD were more assimilated by worm dwelling in

soils containing unaged contaminants, soils with lower organic matter content, and soils containing higher pollutant concentrations. Reduction in the bioavailability of DDT was also observed for other laboratory-aged soils with different levels of organic carbon contents when compared to freshly spiked soils (Vlcková and Hofman, 2012).

Another study also investigated the aging effect when earthworms were collected from a contaminated field over a period of 11 yr. (Beyer and Gish, 1980). BAFs were measured at treatment, 5.5 yr. after treatment and 11 yr. after treatment. For dieldrin, BFAs were 15, 8, and 3.9 for the different times, while DDT, they were 0.25, 0.37, and 0.56 and for DDE BAFs were 16, 9.9, and 6. One conclusion was that generally, bioavailability decreases with aging time, however, for DDT, bioavailability increased with time. Also, bioavailability of DDE is much larger than bioavailabity of DDT, which is a cause for concern, given that DDE has a more harmful effect in birds that would consume these contaminated earthworms. The fact that DDE is more bioavailable than DDT was also observed by Tomaszewski *et al.* (2008), which reported DDT, DDE, and DDD levels in mussels living in contaminated sediments.

The uptake by Eisenia foetida of DDT, DDE, DDD, and dieldrin was measured in a 30 yr.-aged soil and compared to an unaged soil. Uptake of DDT increased from 2.02 to 2.85% and of DDD increased from 1.15 to 4.80%, while uptake of DDE decreased from 1.56 to 1.11% and of dieldrin decreased from 19.9 to 12.8% from the aged soil to the unaged soil (Morrison *et al.*, 2000). The combination of these studies

suggest that there are inumerous variables influencing the bioavailabity of these chemicals in soils, which include but are not limited to: soil organic matter content, soil type, pollutant concentration, earthworm species, aging of soil contamination, and residence time of earthworms in contaminated soil (Kelsey *et al.*, 2008; Kelsey *et al.*, 2005; Gaw *et al.*, 2012).

2.2 – Biosolids

2.2.1 – Overview

Biosolids are the byproduct of wastewater treatment that meets criteria set by EPA for land application. A wastewater treatment plant (WWTP) consists of a continuous set of processes designed to treat wastewater collected from residential, commercial, and industrial areas. The different processes are named treatments and they can be classified as preliminary, primary, secondary, and tertiary treatments (Figure 2.2). A preliminary screening procedure that removes large particles from the water precedes the primary treatment. Primary treatment itself removes smaller solids that are suspended in the wastewater. This process is conducted in tanks that are called clarifiers. Ferric chloride is added to the process for the studied biosolids to improve phosphorus removal. The solids removed from the primary treatment are further treated. The secondary treatment reduces biochemical oxygen demand and

removes additional suspended solids. Plants use activated sludge, trickling filters, and rotating biological contactors to achieve this goal and the solids removed in this step are added to the primary sludge to be further treated. The tertiary treatment kills the majority of pathogens remaining in the wastewater. Plants use chlorination, ozonation, or ultra-violet light for tertiary treatment. Once the sludge is gathered, it receives additional treatment. The sludge goes through thickening, stabilization, disinfection, and dewatering before it can be disposed of. Stabilization can be achieved by lime addition, composting, or by aerobic or anaerobic digestion. Once treated, the sewage sludge can be called biosolids and the quality of the biosolids produced is dependent on the treatment it receives along with the origin of the sewage sludge.

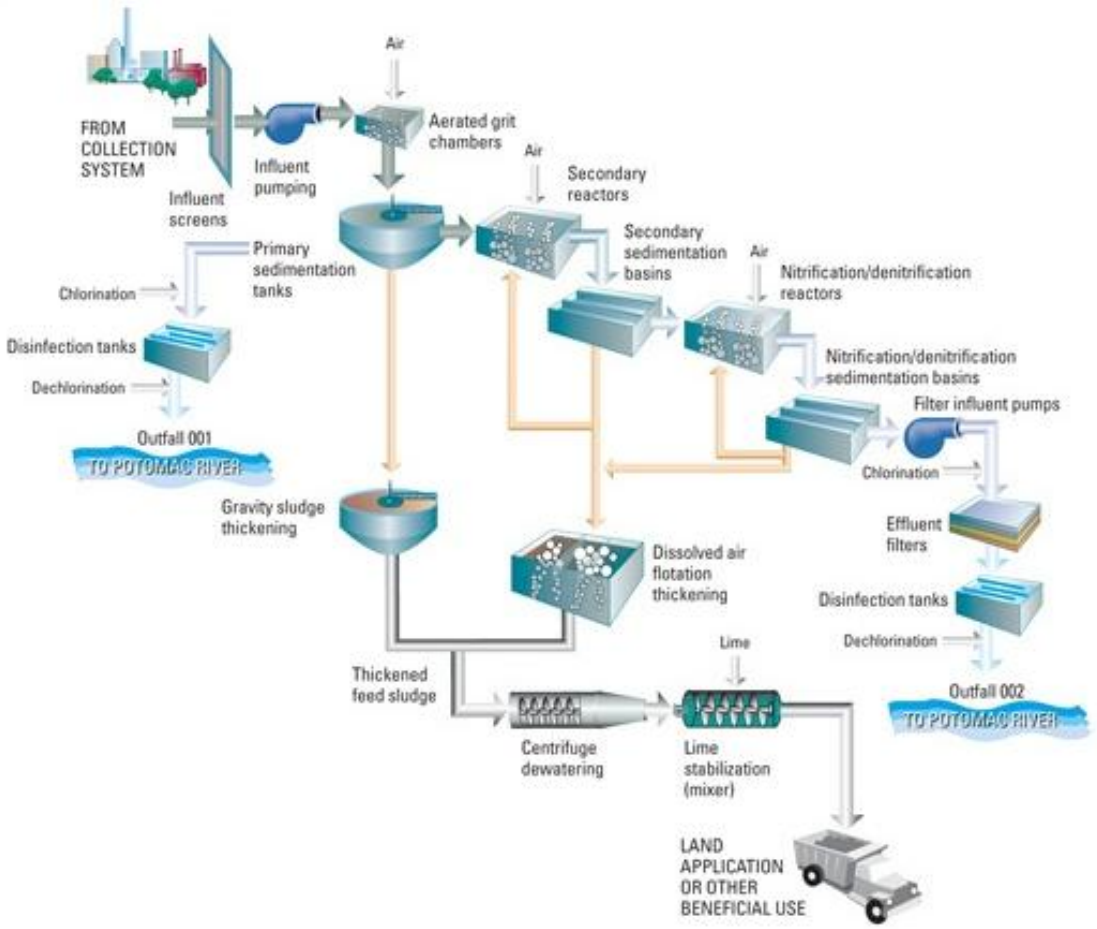


Figure 2.2 – General layout of the sampled WWTP showing primary and secondary treatments with chlorination and dechlorination. Source: (DCWater, 2012)

Biosolids contain high levels of nutrients and are used as fertilizer, for soil remediation projects, or as a soil conditioner. According to the National Biosolids Partnership biosolids are land applied in all 50 states (NBP, 2008). Beneficial use programs for land application of biosolids are important avenues for disposal of solids outflows from WWTPs. In a recent study, Singh and Agrawal (2008) provide a summary on the potential benefits and risks of application of biosolids to agricultural

lands. Their report mentions beneficial soil conditioning properties of biosolids along with changes in the physical, chemical, and biological properties of soils (Singh and Agrawal, 2008). The risks for the soil and crops were mainly accumulation of heavy metals after biosolids' application. Increase in yield was observed in a number of studies and the extent of yield increase was dependent upon soil type, application rates, and crop (Singh and Agrawal, 2008). Mantovi *et al.* (2005) confirmed that the crop as well as the type of biosolids applied influences the yield and in general yields are increased with biosolids application. Biosolids can be effectively used for land remediation efforts (Brown *et al.*, 2003) and they reduce dramatically the use of chemical fertilizers by farmers. This decrease in the use of fertilizers provides an important economic benefit for farmers. There are estimates that savings can be from US$60 to US$160 per acre (Obreza and O'Connor, 2003). These savings vary according to the type of biosolids being applied, the application rate of the biosolids, the type and properties of the soil receiving the biosolids, the crop being planted, and the expected yields. Muraro *et al.* (2001) estimated that a farmer could save from 12% to 63% of the total costs of fertilizers.

U.S. EPA estimates that 6.9 million dry tons of sewage sludge were produced in 1998 and 60% of the sewage sludge was considered biosolids that were beneficially used, with 41% being land applied. The 40% of the sewage sludge produced that were not beneficially used were disposed through incineration, surface disposal, and other methods. Also, the U.S. EPA estimated that the amount of sewage sludge in 2010

was around 8 million dry tons, and in addition, it is estimated that the land application percentage is 55% of the total biosolids produced in the US, with about 74% of that amount being used in agriculture (Beecher *et al.*, 2007).

Domestic and industrial wastewaters contain a variety of synthetic compounds in trace amounts that are only partially removed from the liquid phase by conventional treatment processes. Most of their removal is through their incorporation into the solids portion of the waste stream, i.e., biosolids. Thus there is an increased concern that along with nutrients, biosolids contain organic pollutants which may have toxic or bioaccumulative properties. Some of these chemicals have been labeled Emerging Organic Pollutants (EOP), those chemicals which have recently been identified in environmental compartments and may have significant negative effects on ecosystem health. The research community and environmental groups are concerned that many of the EOPs such as antibacterials, pharmaceuticals, and flame retardants are not currently being regulated by EPA, FDA, or others. According to the literature (de Boer *et al.*, 2003, Hassanin *et al.*, 2004, Ikonomou *et al.*, 2002, Knoth *et al.*, 2004, North. 2004), these chemicals have been detected in the environment and it has been postulated that land application of biosolids may represent a major source of these pollutants.

2.2.2 – Regulatory Status

The U.S., European Union, and Canada regulate land application of biosolids. The U.S. biosolids regulations are contained in 40 CFR Part 503 (EPAg, 1993). Part 503 specifies rules for maximum metal concentrations, pathogens concentrations, and vector attraction reduction. U.S. EPA did not consider background levels of heavy metals to set the ceiling concentrations. In general, levels of heavy metals in U.S. soils are lower than the ceiling concentrations resulting in accumulation of heavy metals over time, although leaching processes can be an important removal pathway for pollutants (Harrison *et al.*, 1999). McGrath *et al.* (1994) estimated that since the ceiling concentrations allowed in the U.S. are much higher than the concentrations allowed in the European Union, the cumulative levels of heavy metals would be approximately an order magnitude higher in the U.S.

Although Part 503 regulates the application of biosolids on a federal level, states are allowed to have their own regulations which may exceed requirements of Part 503. The regulations in the U.S. were established using a risk assessment approach. Some have suggested that this risk assessment performed should now be revised (Harrison *et al.*, 1999) and have questioned if risk assessment alone is enough to create regulations (Schoof and Houkal, 2005). U.S. regulations also do not regulate any organic pollutant. The inclusion of some organic pollutants (PCBs, dioxins, and furans) to Part 503 has been discussed but not finalized (Harrison *et al.*, 1999).

In Canada, the regulations are at a territorial level rather than at the federal level. Some provinces in Canada use the U.S. regulations as basis for their regulations while others have developed their own. In the European Union, the regulations for land application of biosolids can be found in 18 articles from a 1986 Directive Council of the European Communities) that has been amended many times. The European Union has a more conservative approach in determining the ceiling concentrations of pollutants allowed in the sewage sludge and is planning on regulating many organic pollutants that the U.S. is not considering. However, the pathogen limits in the European laws are not as well established as set by Part 503 (Iranpour *et al.*, 2004).

Chapter 3 – Presence of PBDEs in Biosolids – Trends over a 6-yr Period

3.1 - Abstract

Treated sewage sludge, also known as biosolids, is generally land applied as fertilizer. Although biosolids contain a large concentration of nutrients, concern exists over the presence of highly persistent organic contaminants. In this study, biosolids samples were collected every two months over a 6-yr period from a large metropolitan Mid-Atlantic wastewater treatment plant. Samples were analyzed for a class of flame retardants, polybrominated diphenyl ethers. Results show that BDE-209, BDE-47, and BDE-99 were the major congeners in the biosolids samples. The total concentration of PBDEs over the collection period of 2005 to 2011 was 1790 ± 528 µg/kg d.w. BDE-209 concentrations averaged 1490 ± 503, while the sum of BDE-47 and BDE-99 averaged 255 ± 78.0 µg/kg d.w. Over the 6-yr period, concentrations of BDE-209 varied with wastewater influent flow, while BDE-47 and BDE-99 concentrations decreased, representative of the production ban of these congeners in the US.

3.2 – Introduction

Sewage sludge production in the world continues to increase as population increases. Methods for disposal of this by-product of wastewater treatment are usually land application, incineration, landfilling, and composting. It is debated which method is the most efficient and appropriate and it is generally agreed that the most sustainable disposal method is land application. However, the world-wide reports on the presence of organic pollutants in land applied biosolids is disquieting. Therefore, it has become increasingly important to monitor biosolids that are destined to land application to prevent environmental contamination.

Incidents as the one reported in Milwaukee, where polychlorinated biphenyls (PCBs) contaminated biosolids were applied in several city parks and later triggered soil remediation efforts should be prevented (Hale et al., 2012). While this was a chronic incident; many are going unrecognized. There are claims that low level concentrations of persistent organic pollutants in the aquatic environment are due to land application of biosolids (Hale *et al.*, 2001a). Thus there is a need to monitor biosolids and assess the levels of persistent organic pollutants. To accomplish and justify the monitoring of biosolids, it is important to have cost-effective analytical methodologies and show that observed trends can be used to predict environmental concentrations and possibly be applied to risk assessments.

In this study, a single metropolitan wastewater treatment plant (WWTP) was

monitored over a 6-yr period. This large plant has the capacity to treat 370 million gallons of wastewater per day and it produces 1,200 wet tones of biosolids daily. Biosolids were collected every two months and samples analyzed for polybrominated diphenyl ethers (PBDEs). PBDEs are flame retardants that are used in a variety of consumer products. Three commercial formulations were originally produced, the penta-BDE, octa-BDE, and the deca-BDE. Today, only the deca-BDE formulation is still in production and there are efforts to completely stop its production. PBDEs are known endocrine disruptors, toxic to wildlife, and possible carcinogens. They have been identified in biosolids throughout the world, with the highest levels observed in the United States and Australia (Clarke and Smith, 2011). BDE-209 is the fully brominated congener and the main component of the deca-BDE commercial formulation, while BDE-47 and BDE-99 are the major components of the penta-BDE commercial formulation, which contains a variety of congeners, from tetra- to hepta-brominated compounds. BDE-209 is the congener detected in the highest concentrations in environmental samples.

The objectives of this study were to establish a repository of biosolids samples, to monitor one WWTP that receives residential, industrial, and storm water influent, and to determine if biosolids concentrations over a 6-yr period could detect temporal and seasonal trends for PBDEs. Also, the hypothesis 'the concentration of BDE-47+BDE-99 decreases by 10% over the collection period' was tested.

3.3 – Materials and Methods

3.3.1 – Target Compounds

Eight PBDE congeners were selected for analysis (Table 3.1). These analytes were chosen for their intense routine presence in environmental samples as well as their presence in commercial formulations of PBDEs (de Boer *et al.*, 2003, Hassanin *et al.*, 2004, Ikonomou *et al.*, 2002, Knoth *et al.*, 2004, North., 2004).

Table 3.1 – Chemical name, abbreviation, CAS#, molecular formula, and molecular weight of PBDEs analyzed in this study.

General Term	Name of Compound	Abbreviation	CAS#	Molecular Formula	Molecular Weight
Tri-BDE	2,4,4' - tribromodiphenyl ether	BDE - 28	41318-75-6	$C_{12}H_7Br_3O$	406.89
Tetra-BDE	2,2',4,4' - tetrabromodiphenyl ether	BDE - 47	5436-43-1	$C_{12}H_6Br_4O$	485.79
Penta-BDE	2,2',4,4',5 - pentabromodiphenyl ether	BDE - 99	60348-60-9	$C_{12}H_5Br_5O$	564.69
Penta-BDE	2,2',4,4',6 - pentabromodiphenyl ether	BDE - 100	189084-64-8	$C_{12}H_5Br_5O$	564.69
Hexa-BDE	2,2',4,4',5,5' - hexabromodiphenyl ether	BDE - 153	68631-49-2	$C_{12}H_4Br_6O$	643.58
Hexa-BDE	2,2',4,4',5',6 - hexabromodiphenyl ether	BDE - 154	207122-15-4	$C_{12}H_4Br_6O$	643.58
Hepta-BDE	2,2',3,4,4',5',6 - heptabromodiphenyl ether	BDE - 183	207122-16-5	$C_{12}H_3Br_7O$	722.51
Deca-BDE	decabromodiphenyl ether	BDE - 209	1163-19-5	$C_{12}Br_{10}O$	959.21

All samples extracts were analyzed using an Agilent 6890 gas chromatograph (GC) coupled with an Agilent 5975 mass selective detector (MSD) in negative

chemical ionization (NCI) mode. An Agilent capillary column (DB-5-MS) had a length of 15m, nominal diameter of 0.25mm, and nominal film thickness of 0.1µm (J&W Scientific, Folsom, CA). The carrier gas used was helium with a constant flow of 1.6 mL/min. The oven temperature program was as follows: 48^{o}C for 3 minutes, 20^{o}C/min to 210^{o}C, 25^{o}C/min to 310^{o}C, 310^{o}C for 5 minutes. Extract injection volume was 1µL and the syringe volume was 10µL. A PTV (Programmable Temperature Vaporizing) inlet was used with the following temperature program: 48^{o}C for 0.45 minutes and then ramped at a rate of 600^{o}C/min to 300^{o}C and held for 23 minutes. The inlet also had a pulse pressure of 280kPa. The GC-MS interface was kept at a temperature of 300^{o}C. Sample concentrations were quantified using the internal standard method and a five point calibration curve. We monitored each compound using at least two ions (Appendix A). We successfully identified the compounds using specific ion proportions with a relative 30% window for error and a 0.1 minute retention time window.

3.3.2. – Sample Collection

Biosolids samples were collected approximately every two months from July 2005 until June 2011 from a Mid-Atlantic wastewater treatment plant (WWTP). Biosolids are stabilized with addition of lime (approximately 15% on a dry weight basis). Stabilized biosolids are transferred to large storage tanks and then loaded on

to trucks that transport the biosolids to farms that receive land application. The samples for this project were collected from the transfer lines that direct the biosolids to the storage tanks. Samples for PBDE analysis were obtained using the plant's sampling system and were then transferred to 250mL amber, wide-mouth jars and were kept frozen (-30^{o}C) until processing. All samples were analyzed for moisture content (Appendix B).

3.3.3 – Sample analysis

The laboratory lights (overhead and hood) were covered with a light filter that blocks light with wavelengths below 240nm and the windows were kept covered so minimal natural light would come into the lab. Biosolids samples were kept frozen and in the dark until preparation for extraction and were then thawed overnight and allowed to reach room temperature. Two aliquots were removed from the sample jar. A 1.0-g soil sample was pre-weighed, transferred to an aluminum tray, baked at 100 ^{o}C for 4 hours, and then re-weighed to determine moisture content. A second 1.0-g aliquot was weighed and dried with approximately 30 g of anhydrous sodium sulfate (J.T. Baker, Phillipsburg, NJ) using a mortar and pestle. Samples were split in two approximately equal parts and placed into two 50 mL Teflon centrifuge tubes. Each tube received 15 mL of dichloromethane (DCM) and 5µL of a 4 ppm solution of PCB 209 that was used as extraction surrogate, which is a chemical similar to target

analytes that is added to the beginning of the extraction process to provide recovery information for the methodology.

The sample was rapidly mixed using a vortex mixer (Fisher Scientific, Fairlawn, NJ) at a speed of 2500 rpm for 2 minutes. Samples were then centrifuged for 5 minutes at the speed of 5000 rpm, and the solvent was decanted. Samples were extracted a second time with 10 mL of DCM, centrifuged, and the solvent combined with that from the first extraction. The extract was concentrated to a 1mL using a gentle stream of N_2. Extract was cleaned up using a 2g alumina Superclean N-alumina SPE cartridge (pre-rinsed with 6mL of DCM). Target analytes were eluted with 6mL of DCM and the extract was concentrated to 1mL, solvent-exchanged to hexane and further concentrated to 500µL before they were transferred to the final 2-mL amber glass vials. An internal standard (10µL of a 4-ppm solution) of $^{13}C_{12}$ 2,2',3,4,4',5' – hexachlorobiphenyl ($^{13}C_{12}$ PCB 138) was added to the GC vial.

3.3.4 – Quality Control

Results were statistically analyzed using two tailed *t*-tests, one-way ANOVA, and model fits, which were performed using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA, USA).

For biosolids that were collected over a period of six years from the WWTP, average sand surrogate (PCB-209) recoveries (n=6) were 90.7 ± 5.34% while matrix

surrogate recoveries (*n*=58) averaged 73.0 ± 7.29%. Differences between samples that were processed in duplicates ranged from none to 14.7% difference for all eight congeners analyzed. The biosolids applied to experimental field was sampled prior to application (*n*=6) and matrix surrogate recoveries averaged 81.4 ± 2.22%. Duplicates for these samples ranged from none to 9.54% difference for all congeners.

3.4 – Results and Discussion

3.4.1 – General Trend

Biosolids samples were taken from a Mid-Atlantic WWTP to establish an average concentration over a period of six years and to investigate the impact of the interruption in production of the penta-BDE commercial formulation in concentrations of PBDEs in biosolids that are land-applied. A complete list of results is available in Appendix C. Congeners that were below detection limit (BDL) or below quantification limit (BQL) were attributed values of one half of the method detection limit (MDL) for those congeners in order to enable calculations (EPA, 1997).

The contribution of each congener to the total PBDE concentration is important, as it can illustrate the source of PBDEs (Appendix D). The fully brominated BDE-209

was the dominant congener, representing 82.1 ± 5.88% (*n*=62) of the total PBDE concentration (mass based) of biosolids samples collected from the WWTP from July 2005 until June 2011. Other significant congeners were BDE-47 and BDE-99, which combined represented 14.9 ± 5.13% (*n*=62) of the total concentration. Other congeners contributed only slightly to the total PBDE concentration of the biosolids samples (Table 3.2).

Table 3.2 – Congener contribution to the total PBDE concentration in biosolids samples. Value is reported as the mean ± standard deviation, *n*=62 (mass based) for all collection years.

	Congener Contribution to Total Concentration (%)
BDE 28	0.28 ± 0.14
BDE 47	7.09 ± 2.39
BDE 100	1.16 ± 0.65
BDE 99	7.85 ± 2.75
BDE 154	0.48 ± 0.15
BDE 153	0.50 ± 0.15
BDE 183	0.40 ± 0.12
BDE 209	82.1 ± 5.88

From 2005 to 2011, congener contribution changed. It was expected that the contribution of BDE-47 + BDE-99 would decrease due to the ban of the penta-BDE commercial formulation and the contribution of BDE-209 would increase due to its consequent increased use. In 2005, the contribution of BDE-47 + BDE-99 to the total concentration was 23.8% (*n*=4) and this contribution decreased to 12.0% (*n*=6) in 2011, a 33.0% difference, which is in agreement to what was expected. For BDE-

209, the change in contribution is not as pronounced, from 72.4% contribution in 2005 (n=4) up to 85.0% (n=6) in 2011, an 8.0% difference. This suggests that monitoring biosolids can be a useful tool to predict effects of product bans and also to predict environmental concentrations.

The profile of the congeners suggests that the biosolids leaving the WWTP come mostly from the commercial formulations utilized in consumer products (Figure 3.1). This trend has been observed by others in the US as well as other parts of the world. BDE-209 is the dominant congener in all studies that analyze for this congener, generally contributing to more than 70% of the total concentration. In Italy, a survey of eight WWTPs resulted in BDE-209 contributing between 75% and 99.8% of the total concentration of all plants (Cincinelli *et al.*, 2012). Hale *et al.* (2012) reported that penta- and deca-BDE commercial mixtures main congeners were the major contributors in sewage sludge samples collected since the 1970s in a Chicago plant. Same trend was observed by many other researchers (North, 2004; Knoth *et al.*, 2007; Eljarrat *et al.*, 2008).

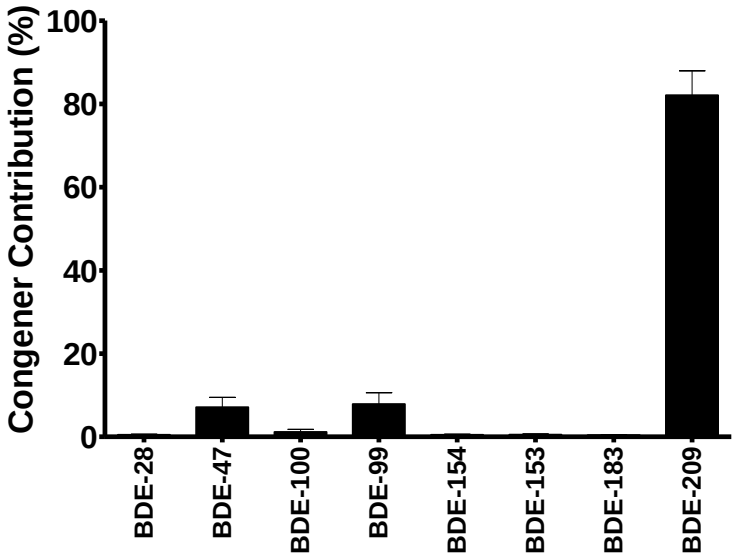


Figure 3.1 – Congener profile of biosolids samples collected from 2005 to 2011 from the large Mid-Atlantic WWTP analyzed in this study (*n*=62).

The total concentration of PBDEs over the collection period of 2005 to 2011 was 1790 ± 528 µg/kg d.w. (*n*=62). The concentration of BDE-209 averaged 1490 ± 503 while the sum of BDE-47 and BDE-99 averaged 255 ± 78.0 µg/kg d.w. Other congeners had much lower concentrations as expected (Table 3.3).

Table 3.3 – Average PBDE concentration (µg/kg d.w.) plus standard deviation (*n*=62) in biosolids samples collected from 2005 to 2011.

	Concentration (µg/kg d.w.)
BDE-28	7.66 ± 0
BDE-47	121 ± 36.1
BDE-100	20.5 ± 11.1
BDE-99	134 ± 42.1
BDE-154	7.97 ± 0
BDE-153	8.23 ± 0
BDE-183	6.64 ± 0
BDE-209	1490 ± 503
BDE-47 + BDE-99	255 ± 78.0
SUM of all BDEs	1790 ± 528

The concentrations found in the WWTP samples collected for this study are in agreement with other studies and results are in the same order of magnitude of most studies. In Italy, a recent study reported mean concentrations of 2763 µg/kg d.w. for eight different WWTPs with PBDEs concentrations ranging from 158 to 9427 µg/kg d.w., which indicates a high variability among plants (Cincinelli *et al.*, 2012). In a survey of 16 plants in the United States, containing biosolids that were aerobically and anaerobically digested, as well as biosolids that were composted, PBDE concentrations ranged from 71 to 1020 µg/kg d.w., which is smaller than generally found in the US, due to the fact that BDE-209 was left out of the analysis (Xia *et al.*, 2010). In Chicago, the average concentration (all BDEs) from 2004 to 2007 was 7800 µg/kg d.w., which is higher compared to our study, but in the same order of magnitude of this study and others around the world (Hale *et al.*, 2012).

In Germany, samples from 11 wastewater treatment plants resulted in PBDE

concentration in biosolids of 12.5 to 288 µg/kg d.w. (sum of BDE 28, 47, 99, 100, 153, 154, and 183 congeners) and 97.1 to 2217 µg/kg d.w. for BDE-209 (Knoth *et al.*, 2004). In Spain, sewage sludge samples from five WWTPs yielded concentrations between 197 and 1185 µg/kg d.w. and the mean value was established at 572 µg/kg d.w. (Eljarrat *et al.*, 2008). In Australia, 16 WWTPs were surveyed for PBDEs and the average sludge concentration was 1137 µg/kg (Clarke *et al.*, 2008).

3.4.2 – Temporal trends and WWTP flow relationship

Over the collection period, concentrations of BDE-209 were variable; however, there was no indication that it was increasing or decreasing. Concentrations of BDE-47 + BDE-99 suggest a decline over the years (Figure 3.2) until 2008 and stabilize at a lower level until the last sampling event in 2011. The decline of the major congeners of the penta-BDE commercial formulation ($p<0.0001$, $R^2 = 0.69$, Figure 3.3) may be due to its ban in 2005, despite the existence and continued use of products containing this formulation. From 2005 to 2006, there is a decline in concentration of 16.6% ($p<0.0001$); the change in concentration for the following years was not statistically significant, however from 2006 to 2007 the decline in concentration was of 8.78%; from 2007 to 2008 the decline in concentration was of 10.5%; from 2008 to 2009 concentrations decreased by 4.7%; from 2009 to 2010, the only period when concentrations increased, they increased by 10.1%; and finally,

from 2010 to 2011, concentrations decreased by 14.4%. The total decrease in concentration throughout the sampling period in the concentration of BDE-47 + BDE-99 was of 42.4%. Hale *et al.* (2012) also analyzed biosolids samples to identify temporal trends for organic contaminants. They observed an exponential increase in concentrations of the major congeners of the penta-BDE commercial formulation from the mid-1970s until they peaked in the mid-1990s, after which, they leveled off. Concentrations of BDE-209 may decrease in the future, when this product has been completely substituted by another flame retardant.

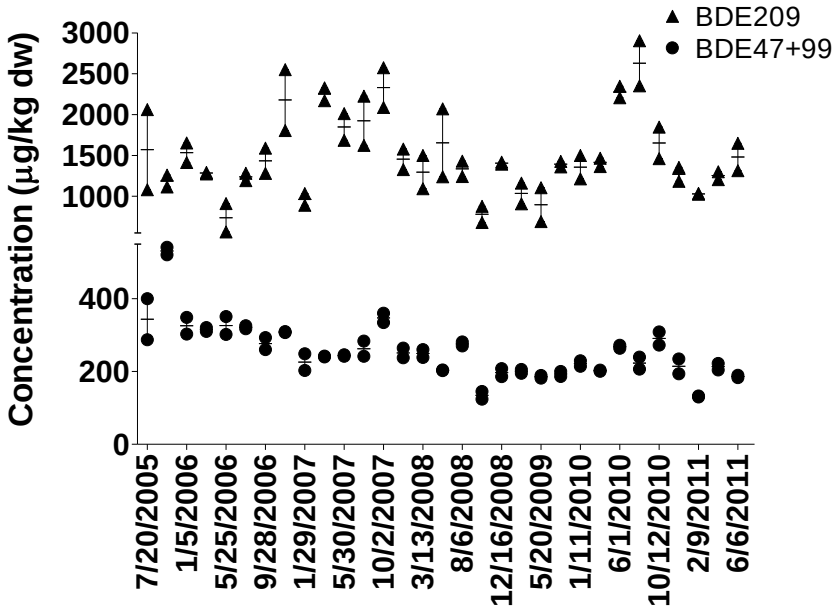


Figure 3.2 – Concentrations of selected BDE congeners in biosolids samples over the collection period. Each point represents a range of values obtained from two duplicates for each sampling day.

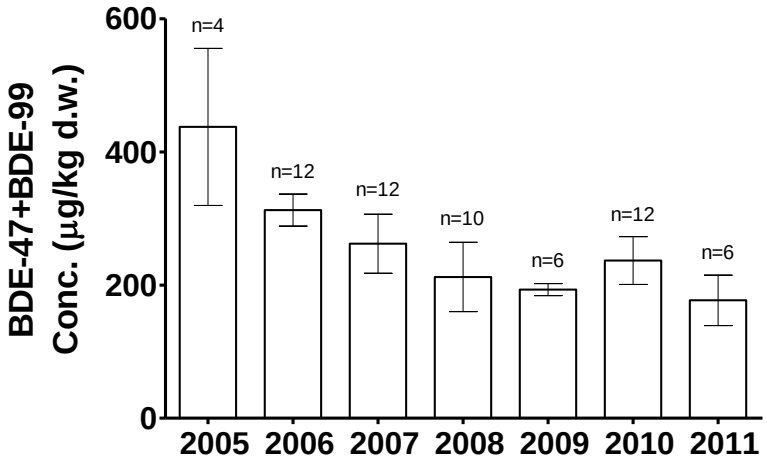


Figure 3.3 – Sum of concentrations of BDE-47 and BDE-99 over the biosolids collection period. Bars represent yearly average concentrations with standard deviation. Means are statistically different (p<0.05). Penta-BDE formulation ban occurred in 2005.

Since the concentration of the BDE-209 appears to vary in a cyclic pattern, possible factors that influence such concentrations were analyzed. BDE-209 levels do not vary according to season, as high concentrations were observed during Spring and also during Fall of different years. The relationship between PBDEs congeners' concentrations and the average of the influent flow to the WWTP (Appendix E) was analyzed. Averages of the flow were analyzed as follows: flows for the two days before the biosolids collection were averaged (Figure 3.4) and flows for the two days before collection and the day of biosolids collection were averaged (Figure 3.5).

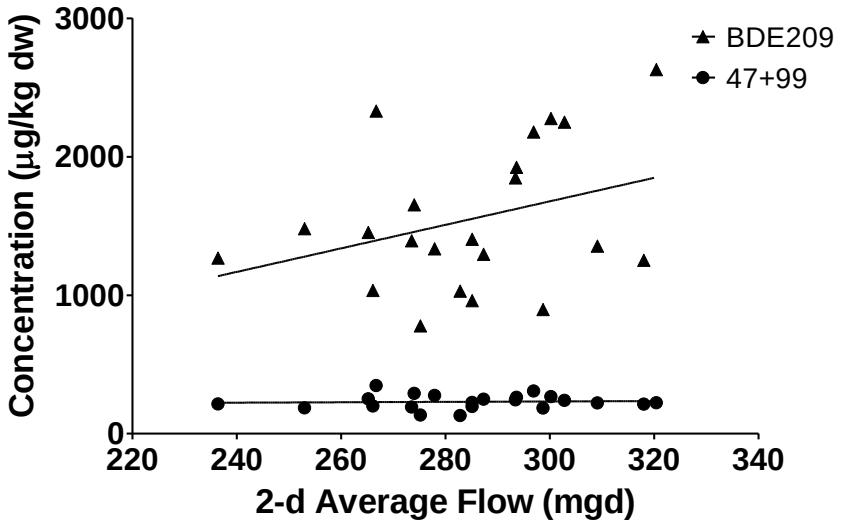


Figure 3.4 – Relationship between concentrations of selected BDE congeners and a 2-d average of influent flow (two days before biosolids collection).

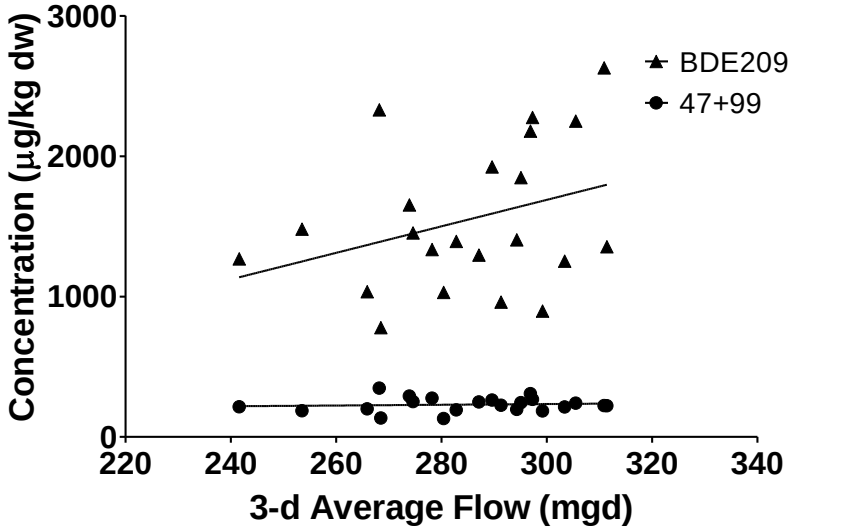


Figure 3.5 – Relationship between concentrations of selected BDE congeners and a 3-d average of influent flow (two days before biosolids collection and the day of biosolids collection).

Concentrations of the BDE-47 + BDE-99 do not correlate strongly with influent flow ($p>0.05$). In the case of these chemicals, the long-term trend is more evident. However, BDE-209 concentrations do correlate to the influent flow to the plant, including the sample collection day or not. The relationship is statistically significant ($p<0.05$), however the correlation is not strong mostly due to the high variability of the concentrations; $R^2 = 0.1003$ for the relationship with the 2-d average flows and $R^2 = 0.098$ for the relationship with the 3-d average flows. The surveyed WWTP receives wastewater mostly from residential regions, however, at the center of the collection ducts, there are connections to the storm water collection system, and therefore the plant receives a considerable amount of storm water as well. A higher influent flow to the plant can be due to heavy rain periods, which can wash off and bring a higher input of total suspended particles that accumulate in the biosolids. Since PBDEs are hydrophobic, the congeners will remain attached to the suspended particles, which are settled down into the sewage sludge during wastewater treatment.

3.5 – Conclusions

A long-term survey of biosolids samples can be used to monitor usage and release of organic chemicals from consumer products. It is important to monitor treated sewage sludge, particularly stabilized biosolids that are utilized as fertilizers and used for land application. Over the sampling period of the target WWTP, PBDEs were

detected in all samples and average concentration of all sampling years were within what was expected and measured in other parts of the world. Also, congener profile changed along the years, with a decrease in BDE-47 + BDE-99 contribution associated with an increase in BDE-209 contribution. Approximately one year after the ban of the penta-BDE commercial formulation, a decrease in concentration of the major congeners of the formulation was observed in biosolids samples, supporting the postulated hypothesis. More emphasis needs to be placed in monitoring plants and also in developing possible stabilization methods that can break down organic pollutants.

Chapter 4 – PBDEs in Agricultural Soils

4.1 – Abstract

Polybrominated diphenyl ethers (PBDEs) are flame retardants that are known to be endocrine disruptors. Their presence and persistence in various environmental compartments generates concern among scientists and risk analysts. Despite the efforts to stop production of the commercial formulations, deca-BDE is still in production and environmental levels are on the rise. One possible source of PBDEs to the environment is land applied biosolids, however, little is known about the environmental fate of PBDEs in agricultural soils that receive biosolids applications. Two studies were set up to enhance the knowledge of the behavior of these chemicals in soil. The first, a small-scale field that received a single biosolids application, was monitored for three consecutive years. The second was a one-time survey of large-scale fields used for commercial purposes that received multiple or single biosolids applications. According to results from the experimental field, the topsoil residence time of BDE-47 and BDE-99 is approximately 1 yr., while results from the large-scale fields yield a residence time of 16 yr. The discrepancy in residence time from the two studies was attributed to intrinsic soil heterogeneity, differences in soil type, variability of biosolids application, soil management practices, among other factors.

4.2 – Introduction

While land application of biosolids is considered to be the most sustainable way to handle biosolids (Singh and Agrawal, 2008), there are some concerns, which include biosolids as a possible source of hydrophobic organic chemicals to the soil. One group of chemicals detected in biosolids, and consequently in soils receiving biosolids applications, is the polybrominated diphenyl ethers (PBDEs). PBDEs are flame retardants that are applied to a variety of consumer products, including plastics and electronics (de Wit., 2002). Of all PBDEs that were once produced, only one commercial formulation is still in production: the deca-BDE formulation, which consists mostly of the fully brominated congener, BDE-209 and other nona- and a few octa-BDEs (Hale *et al.*, 2012). However, the penta-BDE formulation, although not produced anymore, is still being released to the environment, as a variety of consumer products that received this flame retardant are still in use by the population.

It has been established that the application of biosolids to soils increases yield and farmers benefit from it financially (Mantovi *et al.*, 2005). However, it has also been established that biosolids application can increase PBDEs, and other chemicals of concern, concentrations in receiving soils (Andrade *et al.*, 2010; Eljarrat *et al.*, 2008; Lozano *et al.*, 2012). Although PBDEs are believed to be very persistent, very few studies have been conducted to determine their topsoil residence time in different environmental media. PBDEs' water solubility is low; thus it is expected that once

they reach soil they would not move to deeper layers. Recently, Gorgy *et al.* (2012) analyzed contaminated soil at different depths and at two different biosolids application rates. Regarding application rate, results are in accordance to what Andrade *et al.*, (2010) has found, a larger application rate results in larger soil PBDE concentrations. At the larger application rate of 80t/ha, the top layer of soil (0.05-0.25m) had the highest PBDE concentration, followed by the second layer (0.25-0.45m). The general trend for Gorgy *et al.* (2012) experiments was a decline in PBDE concentration with soil layer depth (with detectable concentrations down to the 0.85-1.05 m of soil), in agreement with Xia *et al.* (2010) findings.

Xia *et al.* (2010) also reported that a field that received annual biosolids applications for 33 years at three different biosolids application rates, heavily accumulated PBDEs. For the highest cumulative loading of 2,218 mg dry biosolids/ha, concentration of PBDEs in the top layer (0-15cm) was 658 µg/kg d.w., while concentration in the second layer (15-30cm) was 105 µg/kg d.w.; strongly suggesting the restricted downward mobility of these chemicals.

While there are some published studies on the fate and levels of PBDEs in soils that have received biosolids applications, such studies only provide information about one point in time, making it difficult to evaluate the fate of these chemicals in the environment. According to the EPA, environmental persistence and persistence in a particular medium are different. In this study, we focus in the environmental persistence in topsoil only, which includes any molecular transformation in the soil

and also transport from soil to other environmental media and/or soils outside of the field. As stated in Chapter 2; PBDEs are not expected to degrade under aerobic conditions (Wong *et al.*, 2012) and thus topsoil concentrations over time should not be attributed to degradations. Therefore, this study determines the topsoil residence time for PBDEs, which in general includes all possible transformations of PBDEs and transfers from our studied field to other environmental media.

The main objective of this study was to determine, in a realistic situation, the residence time of selected PBDE congeners in topsoil following biosolids applications with our main goal to assess possible buildup upon multiple applications of biosolids. Results from an experimental small-scale field with a controlled single biosolids application that was monitored for three consecutive years are compared with results from a survey of approximately 30 large-scale fields that were sampled in 2006 (Andrade *et al.*, 2010) and in 2009. Commercial large-scale fields that were managed by farmers were divided in three categories: fields that received multiple biosolids applications, fields that received a single biosolids application, and fields that never received application. Analyzing results from both experimental and large-scale fields provides insights on soil residence times and the complex behavior of organic pollutants in soils. Our extensive sampling also provides a clear indication of special variability in such studies and cautions results from studies with very limited samples.

4.3 – Materials and Methods

4.3.1 – Small-scale Field Experimental Design

A 0.24-ha field (Sunnyside fine sandy loam, Table 4.1 and 4.2) located in Beltsville, MD and managed by the University of Maryland extension service, received a single lime-stabilized Class B biosolids application in July 18, 2006.

Table 4.1 – Soil characteristics before biosolids application (soil analysis on 11/30/2005).

Parameters	**Value**
Soil pH	6.2
Phosphorus (P)	60 ppm
Potassium (K)	76 ppm
Calcium (Ca)	812 ppm
Magnesium	79 ppm
Organic Matter	1.6 %
Cation Exchange Capacity	4.5 meq/100g

Table 4.2 – Soil characteristics after biosolids application (soil analysis on 12/29/2006).

Parameters	**Value**
Soil pH	7.0
Phosphorus (P)	141 ppm
Potassium (K)	88 ppm
Calcium (Ca)	1615 ppm
Magnesium	84 ppm
Organic Matter	1.7 %
Cation Exchange Capacity	7.2 meq/100g

Applied biosolids originated from the same WWTP where biosolids samples were collected for this project. Triplicate samples of the biosolids were collected before application. A manure spreader applied 72.3 wet tons/hectare of biosolids on the field. The field was divided into eight subplots vertically that were alternately tilled and left untilled following application (Figure 4.1). Tilling (approximately 10 cm deep) was performed one day after biosolids application. Each column was divided in five equally-sized sampling quadrants (7.6 m x 6.1 m), which produced a total of 40 samples for each pre-established sampling time plus two control samples taken outside the field. Samples were taken in a systematic manner to facilitate spatial analysis.

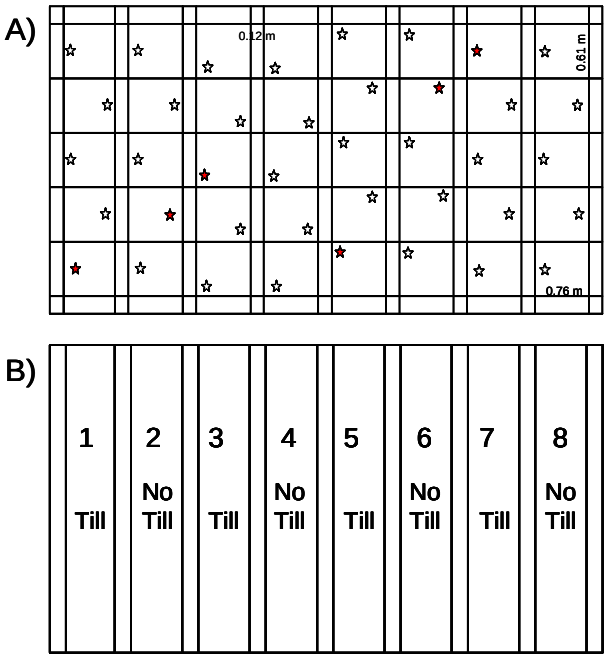


Figure 4.1 – Area plot design. A) Stars represent sample collection point (*n*=40). Numbers represent height (0.61 m) and width (0.76 m) per square. Total area plot was divided in 40 squares. 0.12 m is the width for the line that was set up between columns. Red stars represent the deep core samples locations collected one year after biosolids application. B) Represent the columns that were tilled and columns left untilled.

Soil samples (composite of three samples on top 10-cm) were taken before application (May 30, 2006), on application day (July 18, 2006), two weeks (August 1, 2006), two months (September 21, 2006), four months (November 14, 2006), 8 months (March 14, 2007), 1 year (July 18, 2007), 2 years (June 27, 2008), and 3 years (July 31, 2009) after biosolids application. Soybeans, alfalfa, and corn were planted in the first, second, and third years (Figure 4.2).

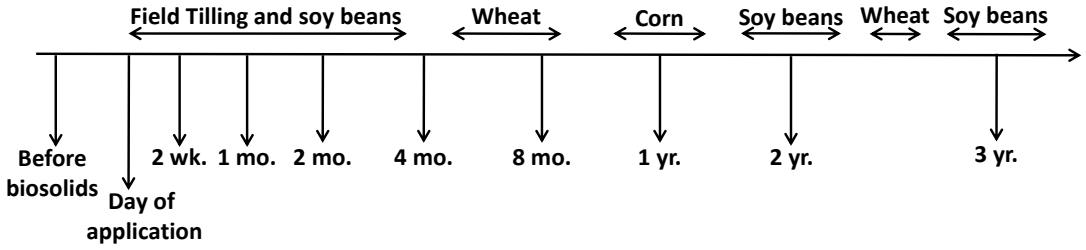


Figure 4.2. Timeline of sample collection and field management for the 3yr. experimental plot.

On July 18, 2007, six samples to a depth of 80 cm were taken from the field and two deep-core samples were taken from the control sites (Figure 4.1 A). Deep-core samples were divided in three individual sub-samples, from 0-25 cm, 25-50 cm and 50-75 cm for analysis.

4.3.2 – Large-scale Fields Experimental Design

Soil samples were collected on May 25th and May 26th of 2009 from farms in Virginia that had been previously sampled in 2006 (Andrade, 2008). A total of 27

fields (Appendix F) were targeted for sample collection with the following profile: a) 13 sites that received multiple biosolids application over the years; b) 10 sites that received a since biosolids application; and c) 4 sites that had never received biosolids application.

All selected fields, except for two (MA2 and MA10, which were planted with corn), were pasture fields for cattle to graze or hay fields (Appendix G). All the fields that have received biosolids application have received at least one biosolids application from the same WWTP where the biosolids samples were collected. Sample collection points were geolocated and recorded using a field GPS instrument (Trimble, Westminster, GeoExplorer Series). The sample collection sites were the same as the ones sampled in 2006 to allow for a direct comparison. The spatial analysis was performed using ArcMap (ESRI GIS and Mapping Software, Vienna, VA). All the satellite imagery was obtained from USDA Geospatial Data Gateway.

Surface soil samples were collected to a depth of approximately 10 cm using a N-2 Handle (Clements Associates Inc. (JMC Soil Samplers), Newton, JMC N-2 Handle PN003) sampler with attached zero-contamination tube (Clements Associates Inc. (JMC Soil Samplers), Newton, PN014) (Figure 4.3, 4.4). Soil samples were a composite of three cores that were each collected in a 30 cm diameter area around the collection site (Figure 4.5). The number of samples collected per field varied with the size and shape of the field (Appendix F). The zero contamination plastic liners were labeled appropriately and were stored temporally on ice until they could be

transferred to a freezer (-30℃). The soil samples were kept frozen until processing.

Figure 4.3 – N2-Handle sampler used to collect the soil samples in this study.

Figure 4.4 – The zero-contamination plastic tube is inserted in the metal bottom part of the sampler to hold the composite soil samples.

Figure 4.5 – Three-core sample collection with filled zero contamination tube.

4.3.3 – Sample Processing

Soil samples were kept frozen and in the dark until preparation for extraction, thawed overnight, and allowed to reach room temperature before processing. Samples were then sieved to remove grass, rocks, worms, etc. Like in biosolids processing, soils samples were divided into two aliquots, one for moisture content measurements (5-g sample) and one for analysis (10-g sample). The analysis aliquot was dried with anhydrous sodium sulfate (J.T. Baker, Phillipsburg, NJ) with a mortar and pestle, split into two approximately equal parts and placed into two 50 mL Teflon centrifuge tubes. Each tube was extracted with 15 mL and 10 mL of DCM for the first and second extractions respectively. PCB 209 was used as extraction surrogate.

Extraction was performed with a vortex mixer (Fisher Scientific, Fairlawn, NJ) at a speed of 2500 rpm for 2 minutes and samples were centrifuged for 5 minutes at the speed of 5000 rpm to decant the solvent. Extract was concentrated using a gentle stream of N_2, cleaned up using a 2-g alumina Superclean N-alumina SPE cartridge (pre-rinsed with 6mL of DCM), eluted with 6mL of DCM, and concentrated again to 1-mL. The extract was then exchanged to hexane and samples were further concentrated to 500µL and transferred to 2-mL amber glass vials. An internal standard ($^{13}C_{12}$ PCB 138) was added to the GC vial.

The carbon content of the soil was determined by a Laboratory Equipment Corporation (LECO) WR-12 Analyzer (St. Joseph, Michigan). The soil sample undergoes pyrolysis and the product of the reaction (CO_2) is measured using a gas chromatograph equipped with a thermal conductivity detector.

4.3.4 – Quality Control

Small-scale Field

Sand surrogate (PCB-209) recoveries averaged 71.9 ± 27.2% (*n*=62). Matrix surrogate recoveries had an average of 65.7 ± 22.7% (*n*=556). Soil samples with surrogate recoveries between 70 and 120% were utilized in the analysis and matrix recoveries for those samples (*n*=232) averaged 86.1 ± 10.9%. Congener recoveries varied greatly, especially for BDE-209, which is the most challenging chemical to analyze for. Sand samples that were spiked with known amounts of PBDEs resulted

in recoveries (*n*=57) from 82.3 to 91.2% for all congeners except BDE-209, which had an average recovery of 69.4%. Matrix spike recoveries varied even more, possibly due to the similar concentration spiked in the soil and contamination already present in the soil sample. Matrix congener recoveries ranged from (*n*=50) 65.0 to 83.7% for all congeners with average recovery of 91.5% for BDE-209. Matrix congener duplicates (*n*=54) ranged between 0.99 to 11.7% of difference.

Large-scale Fields

Soil samples with surrogate recoveries below 60% were eliminated and the average final soil matrix surrogate recovery (*n*=153) was 68.1 ± 5.14%, while matrix BDE-28, BDE-47, DBE-99, BDE-100, BDE-154, BDE-153, BDE-183 congener recoveries ranged from 61.5 to 69.2% and BDE-209 matrix recovery averaged 88.6% (*n*=9). Sand congener recoveries varied from 75.9 to 79.8% for all congeners while BDE-209 recovery was 51.8% (*n*=9). Lab duplicates were run and they were (*n*=8) within 2.77% each other with the exception of BDE-209 which had a %difference of 13%. Field duplicate samples were collected and analyzed and resulted in congener %differences of 11.1 ± 6.39% for all congeners.

4.4 – Results and Discussion

4.4.1 – Small-scale Field

General Trend

Biosolids that were applied to the small-scale field were sampled before application to identify the concentration of PBDEs being applied to the field. Average (*n*=6) concentrations were 1500 ± 231 and 371 ± 17.1 µg/kg d.w. for BDE-209 and BDE-47 + BDE-99 respectively. These values are in agreement with results of biosolids from the same WWTP in a six year study (Section 3.4.1) and also with levels found in other parts of the world (Clarke and Smith, 2011).

The 0.24-ha field which received one single biosolids application was monitored over three consecutive years to establish a relationship between biosolids application and PBDEs soil concentrations. Concentrations for day of application, 2 weeks, and two months after application could not be calculated due to inappropriate surrogate recoveries. Congeners BDE-209 and sum of BDE-47 and BDE-99 contributed to 82.3 ± 8.52 and 8.62 ± 4.76 respectively of the total concentration in soil samples (*n*=156) (Appendix H). Soil concentrations exhibited some variability throughout the field as expected in heterogeneous soil and spotty biosolids application. The nature of biosolids application is particularly important for hydrophobic compounds, as they do not have the tendency to move in the soil once incorporated into soil pores.

Control soil taken from outside the field throughout the 3-yr sampling period had PBDE concentrations below the detection limit for all congeners with the exception of BDE-209, which had an average concentration of 10.7 ± 2.94 µg/kg d.w. Background levels inside the field were also below detection limit for all BDEs, except for BDE-209 and the average concentration was 6.87 ± 0.77 µg/kg d.w., which is in agreement with controls outside the field. PBDE concentrations varied throughout the 3-yr of sample collection (Appendix I; Figure 4.6); they increased after biosolids application and reached the maximum concentration one year after application. Maximum average (*n*=38) was 43.7 ± 42.7 and 6.05 ± 7.15 µg/kg d.w. for BDE-209 and sum of BDE-47 and BDE-99 respectively. After the maximum concentration was reached, levels declined but background concentrations were never reached.

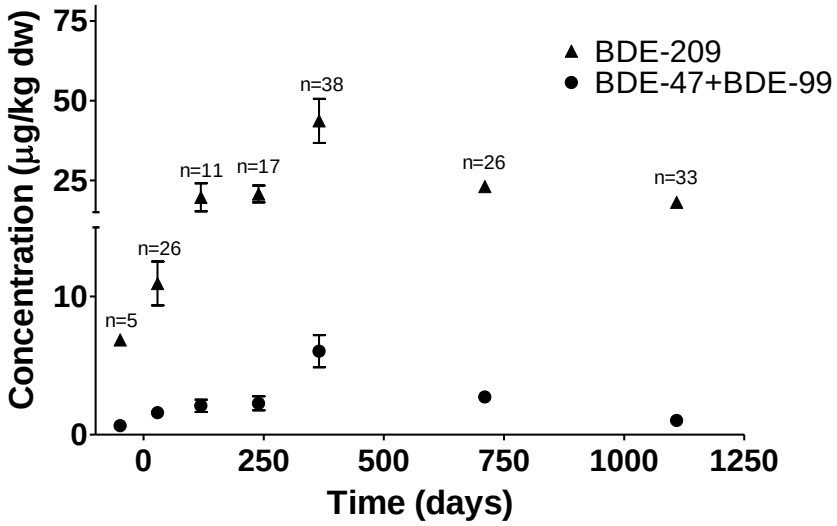


Figure 4.6 – Average concentrations (with standard error) for the entire field at different sample collection times for the experimental plot.

The temporal trend of PBDE concentrations in the small-scale experimental field is illustrated in Figure 4.7. Sample locations were recorded by a field GPS instrument and samples were taken at the same locations throughout the 3-yr. experiment. After sample analysis for PBDEs, concentrations were plotted in a satellite map. Visualization of the concentrations on a map increases the ability to detect spatial variability as well as temporal variability. In Figure 4.7, each yellow dot inside the field is a sampling location. The size of the yellow dot represents the total PBDE concentration on that location at the sampling time; a larger circle represents a larger concentration. Concentrations vary widely even in one sampling period, which is expected due to soil heterogeneity and spotty biosolids application (Lozano *et al.*, 2012). One can visualize that it takes about up to one year for the PBDEs to become incorporated into the soil; maximum soil concentrations are reached within one year and that while concentrations decrease after one year, significant levels of PBDEs are still present after 3 years.

Figure 4.7 – PBDE total concentration in each sampling location for each of the sampling periods.

The increase in PBDE concentration during the first year is likely due to degradation of the biosolids and the release of PBDEs onto the soil (Lozano *et al.*, 2012). Biosolids are applied in large chunks thrown on top of the field and even with tilling and planting of the field, the biosolids pieces are not completely assimilated by the soil. It takes several wetting and drying cycles of the biosolids applied-soil to incorporate the biosolids. The biosolids chunks need to break down and become part of the soil, and while that is happening, concentrations of PBDEs in the soil increase. When biosolids are completely incorporated to the soil, concentrations will be highest, however, they may never reach a theoretical high concentration, as during the incorporation process, loss and dissipation of organic pollutants is concurrently taking place.

Management Effects

Before application, the field was divided into eight columns that were alternately tilled (10 cm deep) and left untilled to investigate possible field management influence on PBDEs concentrations (Figure 4.1 B). Although not statistically different (t test, $p<0.05$), average concentrations for the untilled columns were higher than tilled columns concentrations for all collection time points (Figure 4.8), except for samples collected before application of biosolids, representing background concentrations. These results suggest that handling of the field can influence contaminants soil distribution. A no till soil may hold the contaminants on the

topsoil, while a tilled soil may facilitate the distribution of the chemicals further down the soil column, resulting in the "dilution effect" observed in Figure 4.8 as the collected samples were down to 7.6 cm.

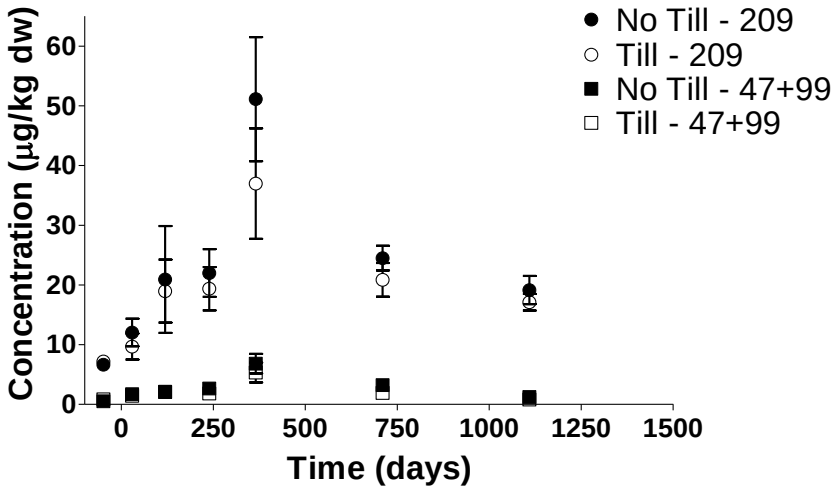


Figure 4.8 – Average PBDE concentrations (with standard deviation) for each sampling time for field areas that were tilled and areas left untilled.

Residence Time

Utilizing the average concentration of PBDEs in biosolids that were applied in the experimental field ([PBDE]), the application rate of biosolids, the area of the field, the soil volume of the field calculated utilizing an incorporation depth of 7.6 cm, and a soil density of 1.3 g/cm^3, a predicted concentration was calculated (Equation 4.1) for BDE-209 and also for BDE-47 + BDE-99. Average PBDE concentration in the applied biosolids was approximated to 370 and 1500 µg/kg d.w. for BDE-47 +BDE-99 and BDE-209, respectively.

$$C_{pred.}(\mu g / kg d.w.) = \frac{[PBDE] \times Application Rate \times Area}{SoilVolume \times SoilDensity} \quad (4.1)$$

The predicted concentration for the experimental field was 26.9 and 6.65 µg/kg d.w. for BDE-209 and BDE-47 + BDE-99, respectively. Because the predicted concentration assumes no dissipation and no movement of PBDE to another media or soil layer, it would be the maximum concentration possible in the field given the biosolids was the sole source of PBDEs to the soil. Compared to the maximum concentration measured in the field one year after biosolids application, the BDE-47 + BDE-99 concentrations are very similar, with the measured concentration at 6.05 µg/kg d.w. However, the maximum measured concentration for BDE-209 was 43.7 µg/kg d.w., which is roughly 50% higher than the calculated maximum concentration for BDE-209. This discrepancy between the predicted and measured concentrations for BDE-209 could be due to variability in the soil data, as the standard deviation of the measured maximum concentration in space was about the same value as the average itself.

This has been observed in another study that analyzed a field that received biosolids applications over 33 yr. PBDE concentrations in the field were higher than estimated by the biosolids applications (Xia *et al.*, 2010) and the authors qualitatively discussed that there is currently an underestimation of the persistence of these

chemicals.

One objective of this study was to calculate the topsoil residence time of selected PBDE congeners in biosolids-applied soil. This study (Figure 4.6) and another study (Lozano *et al.*, 2012) suggest that it can take up to one year for the biosolids, and thus the PBDEs as well, to be fully incorporated into the soil. In this study, we assumed that during the first year, both soil incorporation and soil dissipation are taking place simultaneously, and that one year after application all the biosolids has been incorporated and thus there is no additional release of PBDEs from biosolids. We then used the data after 1 year to determine a topsoil residence time, expressed as the half-residence time: $\tau_{0.5}$. As other studies that assess the fate of persistent organic pollutants in soils determine persistence by determining the chemicals half-life; we wanted to provide our residence time in a format that could be compared with that of other chemicals that may undergo transformations. Similarly, we used a pseudo-first order dissipation model to calculate residence time (Equation 4.2) (Jackson and Eduljee, 1994; Lozano *et al.*, 2012).

$$\tau_{0.5} = \frac{-t\ln(2)}{\ln\left(\frac{C_{soil(t)}}{C_{soil(t_o)}}\right)} \qquad (4.2)$$

The residence time ($\tau_{0.5}$) is calculated using the time in which dilution and

transport to other environmental media along with other possible dissipation processes (such as photodegradation) occurred (t), the top soil concentration at a time t ($C_{soil(t)}$), and the concentration at the beginning of the considered period ($C_{soil(t_0)}$). Average concentrations were skewed to a high level due to a few high concentration points inside the field (generating a relatively high standard deviation) that could not be removed as outliers using Grubbs' statistical outlier test, therefore median concentration were more appropriate and more field representative (Figure 4.9). To determine top soil residence times, median concentrations were used.

As Figure 4.9 illustrates, median soil concentrations for BDE-209 and BDE-47 + BDE-99 increased during the first year, reaching a maximum median 27.9 and 2.4 µg/kg d.w., respectively. After the first year, concentrations decreased. It is believed that the two processes of biosolids incorporation to the soil and soil dissipation by transferring to other environmental media are occurring simultaneously with the first being more relevant during the first year and the second being more predominant after the first year until the end of the collection period.

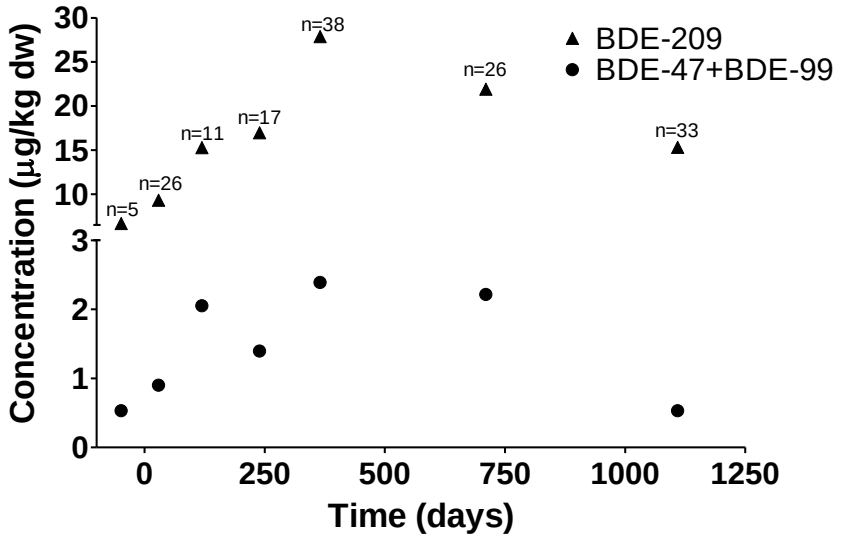


Figure 4.9 – Median BDE concentrations for the entire field during sample collection period.

Residence time calculated for BDE-209 was 861 d or 2.36 yr., while for the sum of BDE-47 and BDE-99, 342 d or 0.94 yr. However, these residence times may be specific for this field due to management, soil type, and nature of the application. PBDEs are not expected to undergo degradation in topsoil, as it is an aerobic environment and PBDEs undergo anaerobic degradation. No degradation was observed for BDE-47 and BDE-99 in one study with an urban soil from Canada (Wong *et al.*, 2012) that was carried out for 360 days. Reductive debromination has been observed for PBDEs during a 42-d experiment (Lee and He, 2010), however the study was carried out under anaerobic conditions and anaerobic microbes were responsible for this degradation, which occurred in laboratory experiments incubated with soil and sediment bacteria collected both in China and the US.

Also, the volatility of BDE-47 and BDE-99, among other congeners, was investigated, and it was concluded that during the 360-d experiment, the volatility of BDE-47 starting to decrease imediately after BDE introduction to the soil and continued to decrease until the end of the experiment. The authors associated the decrease in volatilization to the formation of soil-bound residues and sequestration of chemicals into the micropores of the soil (Wong *et al.*, 2012). Although no quantification was reported in the later study, their results suggest that loss of PBDEs by volatilization may be small. Moreover, studies have shown that selected PBDE congeners adsorb strongly to soil and may adsorb irreversably, making them unavailable for degradation (Liu *et al.*, 2012; Olshansky *et al.*, 2011).

Therefore, while PBDEs degradation in soil is minimum, the short residence time found in the controlled plot experiment compared to a previous estimate (Andrade *et al.*, 2010) is likely due to movement of PBDEs further down into the soil column (mixing of the soil with tilling and planting throughout the years) and also possible transport of PBDEs from soil to other environmental media. Furthermore, soil type and soil properties and site-specific properties may influence residence time more than previously expected.

4.4.2 – Large-scale Fields

General Trend

A total of 26 fields from 10 Virginia farms that were sampled in 2006 (Andrade *et al.*, 2010) were re-sampled in 2009. Fields were classified in three categories: multiple, single, and zero biosolids applications. Consistent with previous results, BDE-209, BDE-47, and BDE-99 were the major components of the total PBDE concentration in all the soils (Figure 4.10; Appendix J). For the major congeners, the profile is similar for all soils and for biosolids, suggesting the biosolids are the major source of these BDEs to soil.

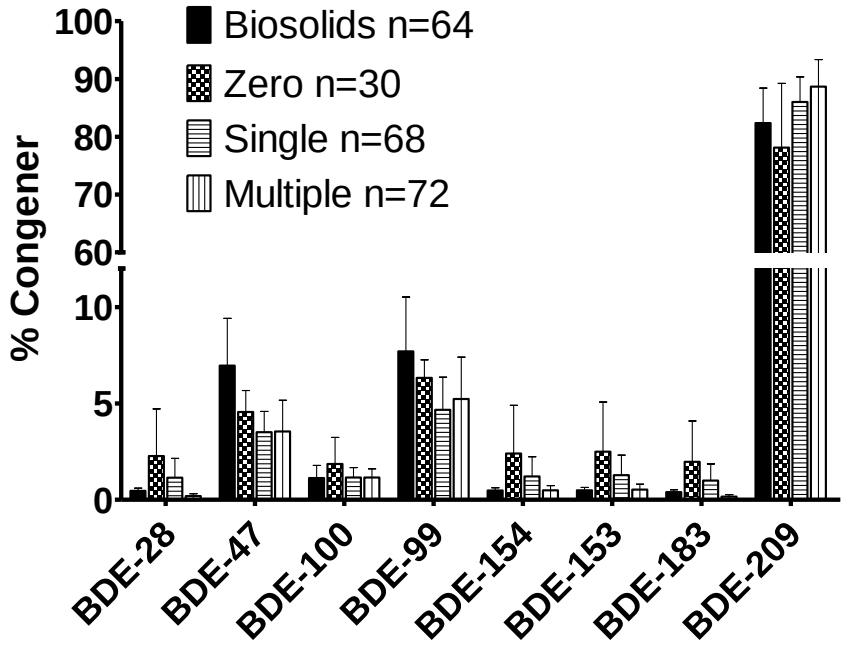


Figure 4.10 – Congener contribution (with standard deviation) to total soil PBDE concentration in biosolids (*n*=64), fields with no biosolids application (*n*=30), fields with a single biosolids application (*n*=68), and fields with multiple biosolids applications (*n*=72).

Total average PBDE concentrations for fields with zero applications (*n*=30), single application (*n*=68), and multiple applications (*n*=72) were 25.7 ± 21.0, 44.5 ± 45.4, and 163 ± 127 µg/kg d.w., respectively (Figure 4.11). These results are in agreement with other studies that conclude that the application of biosolids containing PBDEs increases soil PBDE concentrations and this increase is dependent on the number of applications the field receives, indicating accumulation of PBDEs in soils (Andrade *et al.*, 2010; Eljarrat *et al.*, 2008; Xia *et al.*, 2010). BDE-209 concentrations in fields that never received biosolids application were 21.5 ± 18.7 µg/kg d.w. (*n*=30); in fields with a single biosolids application was 39.2 ± 42.1 µg/kg d.w. (*n*=68); and in fields with multiple biosolids applications was 145 ± 117 µg/kg d.w. (*n*=72). Concentrations of all other target congeners can be found in Appendix K, L, and M.

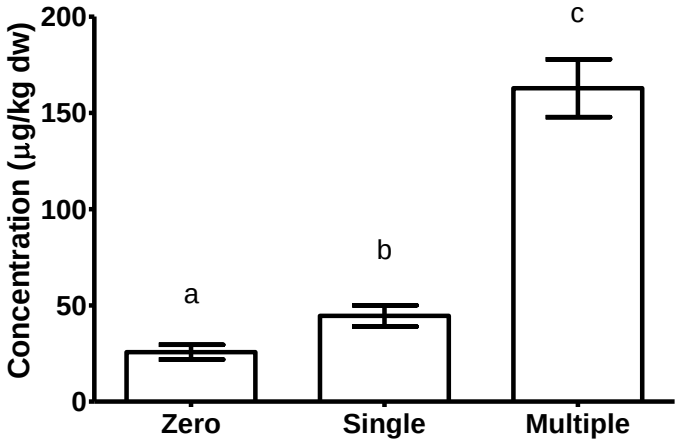


Figure 4.11 – Total PBDE concentration for fields with zero (*n*=30), single (*n*=68), and multiple biosolids applications (*n*=72). Letters on top of bars (a, b, c) represent averages are significantly different (t test, $p>0.05$).

Geospatial Analysis

All sampling locations were recorded using a field GPS instrument and concentrations were added to the location points and plotted in a satellite map to analyze discrepancies with records and to detect any field details. Typical PBDE concentrations can be observed for fields with multiple biosolids applications (red circles), single biosolids application (yellow circles), and for fields that have never received biosolids applications (green circles) (Figure 4.12); sizes of circles are proportional to concentrations. The concentrations in the satellite map are not only helpful to observe concentrations differences between fields, but also to identify within-field variability.

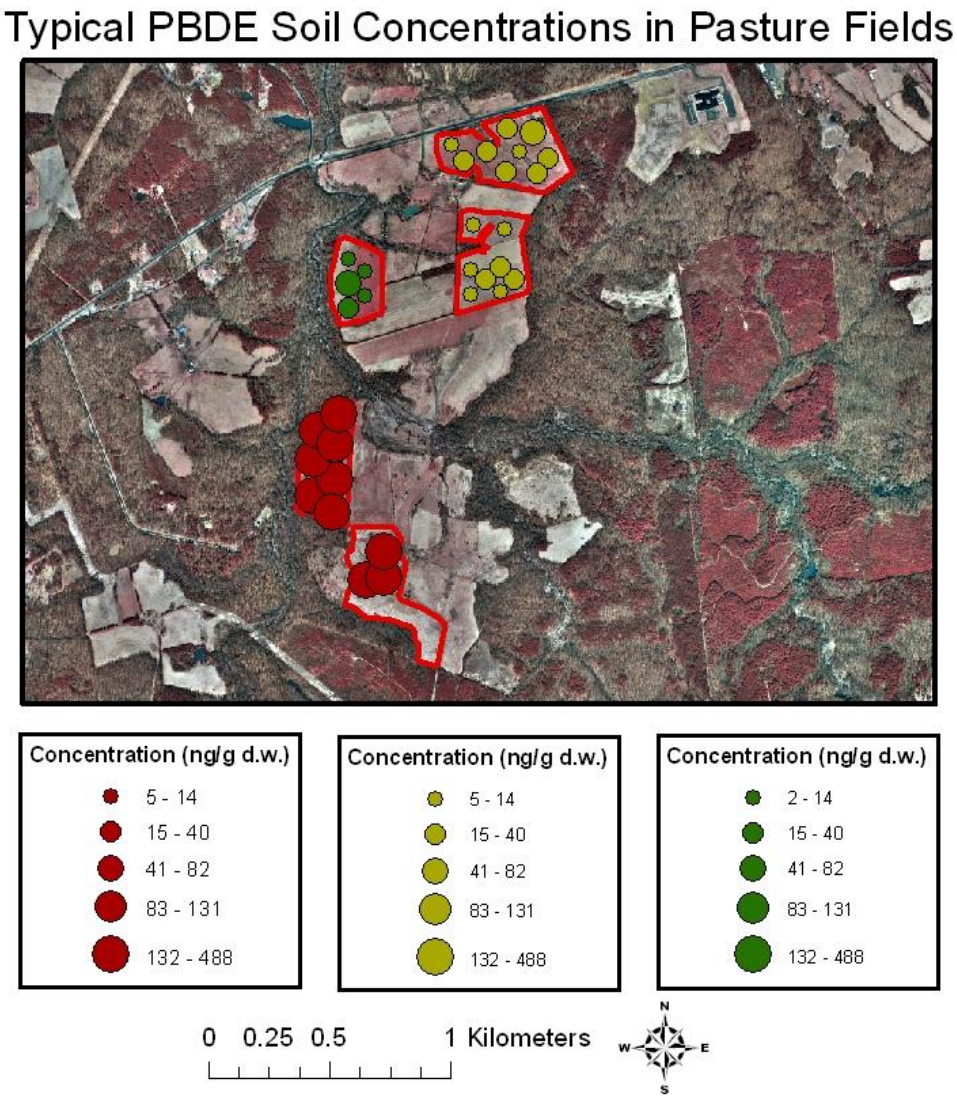


Figure 4.12 – Typical concentrations found in 2009 in large-scale fields with multiple (red circles), single (yellow circles), and zero (green circles) biosolids applications. Size of colored circles are proportional to concentrations.

Differences between fields that in 2006 had not received biosolids application but had received one single application in 2009 were easily observed with data and the aid of satellite imagery (Figure 4.13).

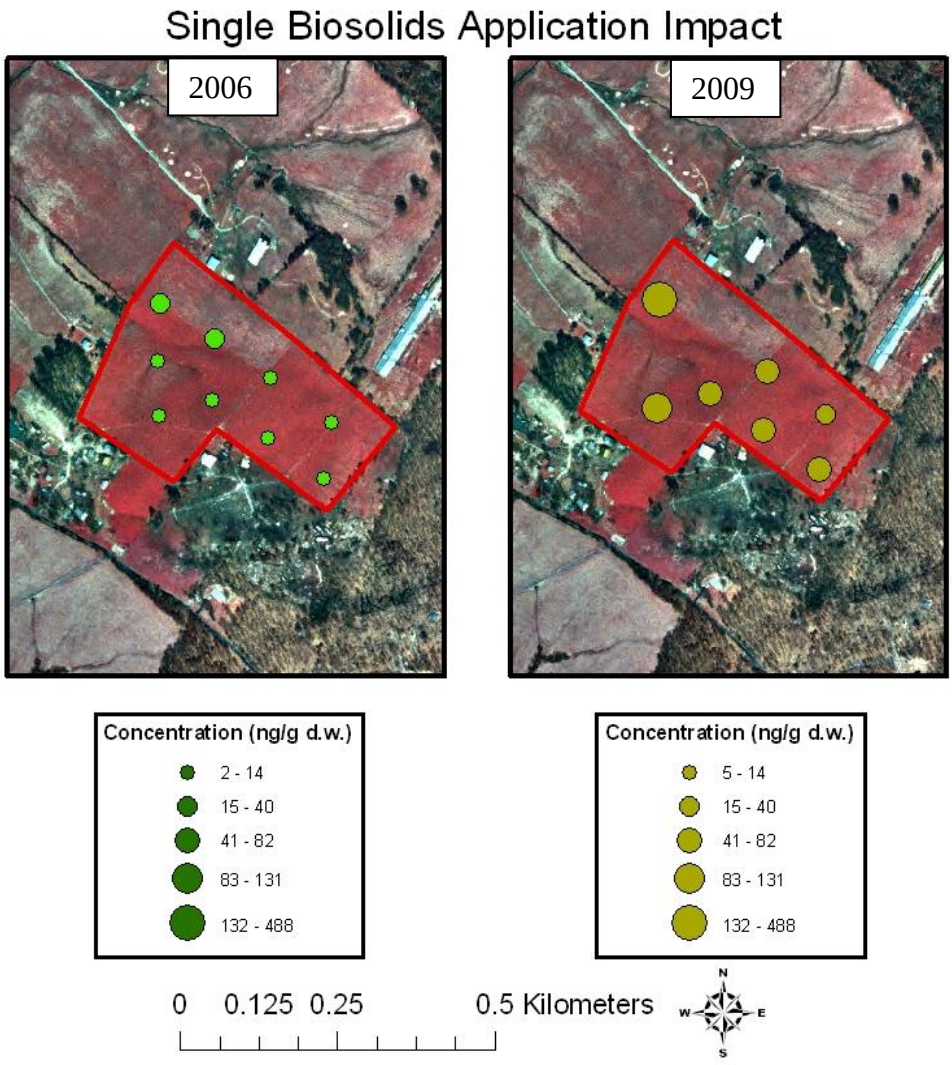


Figure 4.13 – Increase in concentration of PBDEs in a field that had not received any biosolids application in 2006 (left) and had received a single application by the 2009 sampling (right).

Another advantage of the spatial analysis tool is the detection of field characteristics that influence PBDE concentrations (Figure 4.14). Along with notes takes in the field during soil sampling, the field geography seen in the satellite

imagery combined with the sample locations and concentration information can provide insight on concentration discrepancy within a field. For example, in Figure 4.14, the field on the left had lower average PBDE concentration than the field on the right. However, it is clear from field notes and the satellite image that there is a creek running through the middle of the field and the samples taken along the creek are located within the buffer zone of the field and therefore did not receive biosolids application. Once the discrepancy was detected, concentrations were adjusted and the field concentration is compatible with fields that received a single biosolids application.

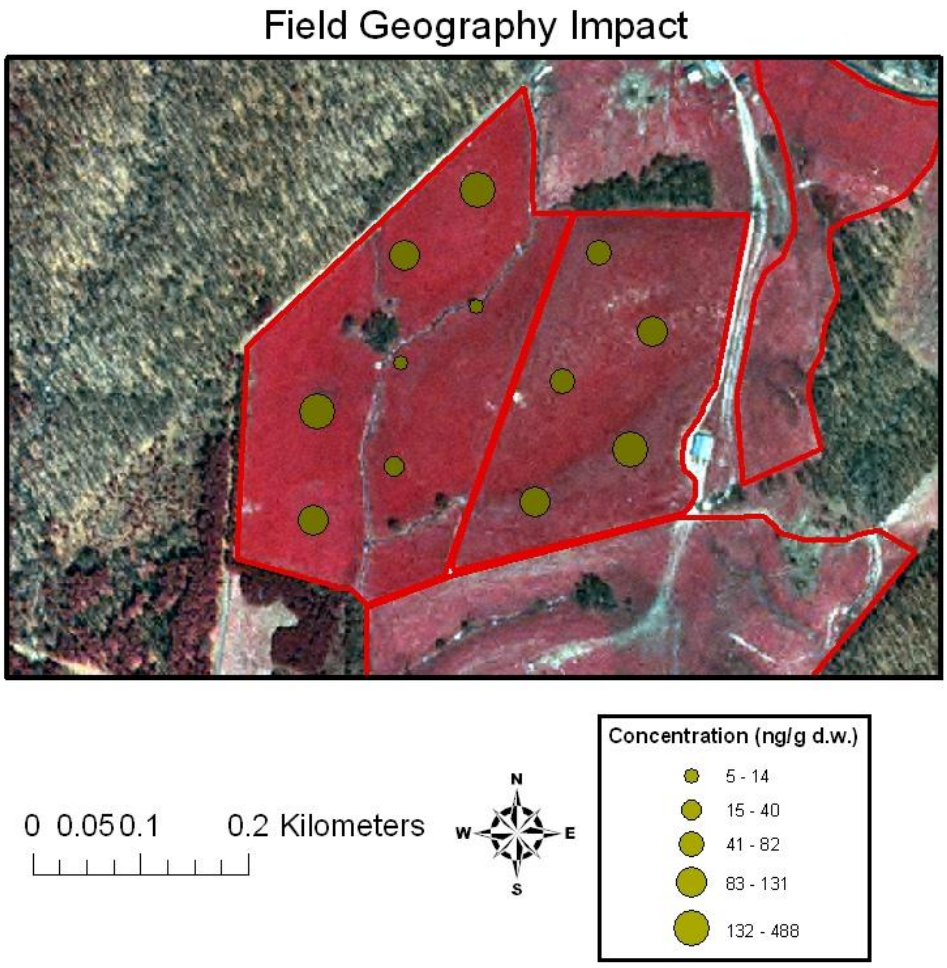


Figure 4.14 – Impact of the field geography on the concentration of PBDEs.

As expected from results from the small-scale experimental plot, large-scale fields that have different management with regards to crops and tilling should have different PBDE concentrations as well. Fields MA2 and MA10 received along the years similar application rates of biosolids (Appendix G) and PBDE concentrations reflect that on those two fields as concentrations are very similar (Figure 4.15). Field MA3 received slightly higher biosolids application; however, concentrations of PBDEs are

higher than expected by the difference in application rate when compared to neighboring fields MA2 and MA10. It is important to note then, that row crops (mostly corn and soybeans) were planted in fields MA2 and MA10, with full tillage of the fields during the sampling years while field MA3 was utilized for pasture. Concentrations of PBDEs are lower in fields that are tilled and planted, which could be due to the enhanced dissipation processes that follow tillage, such as mixing into lower soil layers and also volatilization and even photodegradation of newly light-exposed residues.

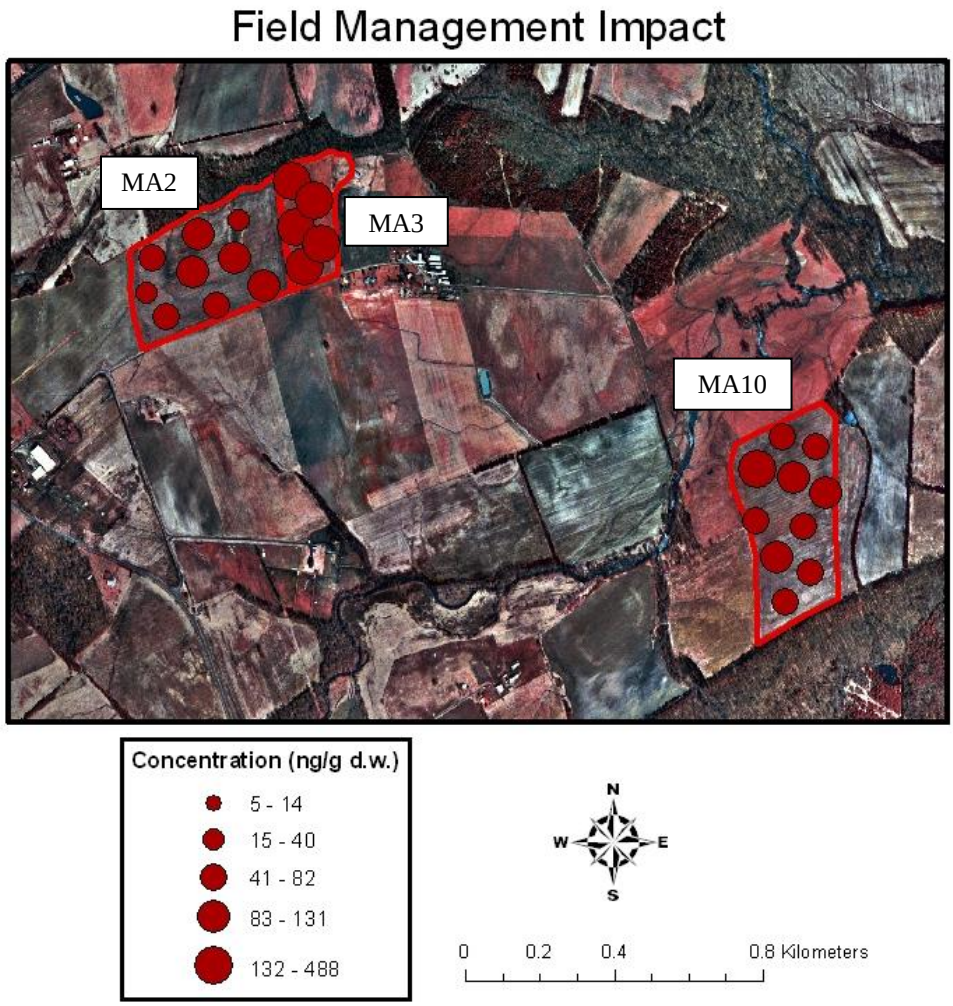


Figure 4.15 – Differences in PBDE concentrations between fields with multiple biosolids concentrations that are planted with row crops and tilled and fields that are used for pasture.

Topsoil Residence Time

We utilized Equation 4.1 using the average PBDE concentration in biosolids found in the 6-yr. biosolids collection of 250 and 1800 µg/kg d.w. for BDE-47 +BDE-99 and BDE-209, respectively to calculated predicted concentrations of PBDEs in fields that received biosolids applications. Predicted concentrations for

2009 were compared to the values measured in the soil samples from each field. Predictions assume no dissipation and no movement of PBDE to another media or soil layer, therefore, they would be the maximum given the biosolids was the sole source of PBDEs to the soil. For the sum of BDE-47 and BDE-99, the predicted concentration of 63.6% of the analyzed fields was higher than the measured concentration in 2009. This indicates some type of loss of PBDEs over the years; therefore the top soil residence time can be calculated. For BDE-209, only 31.8% of the fields had lower concentrations in 2009 than predicted, while the others were higher. This could be an indication that there were other sources of BDE-209 to the fields, possibly atmospheric deposit. This could also mean that the dissipation and transport of BDE-209 are lower than for the lower brominated congeners. Likely, a combination of factors (could also include soil type and heterogeneity) contributes to the fact that the majority of the predicted concentrations that assume no loss of BDE-209 are higher than the measured concentrations in 2009.

Employing the measured 2009 concentration (C_{2009}), the calculated predicted concentration ($C_{pred.}$), the time elapsed between the first biosolids application rate and the date of sample collection (t), the top soil residence time of BDE-47 + BDE-209 was calculated ($\tau_{0.5}$) (Equations 4.3 and 4.4).

$$C_{2009} = C_{pred.} e^{-\left(\frac{\ln 2}{\tau_{0.5}}\right)t} \qquad (4.3)$$

$$\tau_{0.5} = \frac{-t \ln 2}{\ln\left(\frac{C_{2009}}{C_{pred.}}\right)} \quad (4.4)$$

The top soil residence time for the sum of BDE-47 and BDE-99 was 16 yr. To our knowledge, there is no other report of field topsoil residence time calculated from measured concentrations available. Other study (Andrade *et al.*, 2010) estimated PBDEs soil residence from the calculated half-lives of PCBs and compared to field data. Residence time estimation was 12.7 yr., which is 11.5% different from the 16-yr estimation from the present study.

Dissipation and Transport Residence Time

Utilizing Equation 4.2, Equation 4.3, and the calculated residence time of 16 yr., we calculated, for each field and each biosolids application in that field, a predicted soil concentration that takes into consideration the dissipation and transport of these chemicals. For example, field MG1B received a total of five biosolids applications. The first biosolids application was in October of 1995 at an application rate of 5.3 dry tons/acre. Using this information and Equation 4.1, it is possible to calculate a predicted PBDE concentration in the soil after the application. Calculations for BDE-47 + BDE-99:

$$C_{pred.}(\mu g / kgd.w.) = \frac{[PBDE] \times Applicatio\,nRate \times Area}{SoilVolume \times SoilDensity}$$

$$= \frac{250 \times 5.3 \times 12.1}{3731282559 \times 1.3} = 3.31 \mu g / kgd.w.$$

The date of the first application was October of 1995 and the date of the second biosolids application was August of 1999. Between those dates, 1400 days elapsed. Employing the calculated 16-yr. residence time or 5800 d., Equation 4.3 gives the PBDE concentration predicted just before the second biosolids application:

$$C'_{pred.} = C_{pred.} e^{-\left(\frac{\ln 2}{t_{0.5}}\right)t} = 3.31 e^{-\left(\frac{\ln 2}{5800}\right)1400} = 2.80 \mu g / kgd.w.$$

The remaining concentration present in the soil after the first period of dissipation was then added to the next concentration calculated from the following biosolids application. This assumes that the entire amount of PBDEs that was added to the soil from biosolids application was available for dissipation processes, independent of application date. This assumption needs to be noted, as it represents a scenario where residues do not bind permanently to the soil, being completely available for transport

and dissipation. However, pollutants do bind with soil and remain unavailable, but because the amount of residues that irreversibly bind to soil cannot be calculated, we assume here the entire pool of chemical is available. The equations were then applied again to each of the five biosolids applications the field received. At the final application, PBDEs are allowed dissipation until the sample collection date and the final residual PBDE concentration is the sum of all residual concentrations over the years (Table 4.3).

Table 4.3 – Example calculation of predicted BDE-47 + BDE-99 for field MG1B. * indicates the 2009 sampling date.

Field MG1B				
Application Date	**Application Rate (dry ton/acre)**	**Days Elapsed**	**Predicted Concentration Added (µg/kg d.w.)**	**Residual Concentration (µg/kg d.w.)**
10/01/1995	5.3	1400	3.31	2.80
08/01/1999	3.38	1035	2.11	4.33
06/01/2002	4.07	1126	2.54	6.00
07/01/2005	2.27	1188	1.42	6.44
10/01/2008	1.85	237	1.15	7.38
05/26/2009*		**Total Concentration**		**7.38**

The total predicted concentration for all fields was calculated similarly to the example for BDE-47 + BDE99. Predicted concentrations were also calculated for BDE-209 utilizing the 16-yr. residence time calculated for BDE-47 + BDE-99. These predicted concentrations were then compared to the measured concentrations in the 2009 soil samples of the target fields. The ratio of the predicted and observed

concentrations was calculated for each of the fields and the relationship of the ratio to the organic carbon content of the soil was evaluated (Figure 4.16). For BDE-209 ($p>0.05$, $R^2 = 0.16$) and BDE-47 + BDE-99 ($p<0.05$, $R^2 = 0.30$), the ratio of predicted concentration to the measured concentration decreases as the organic carbon content of the soil increases. As organic carbon content of the soil increases, the PBDEs, being highly hydrophobic chemicals, will bind more strongly to the soil, becoming less available to any process of dissipation. Therefore, in the low end of the organic carbon content of the analyzed soils, predicted concentrations of BDEs were higher than measured, indicating that chemicals present in those soils were more prone to dissipation due to a lower organic carbon content.

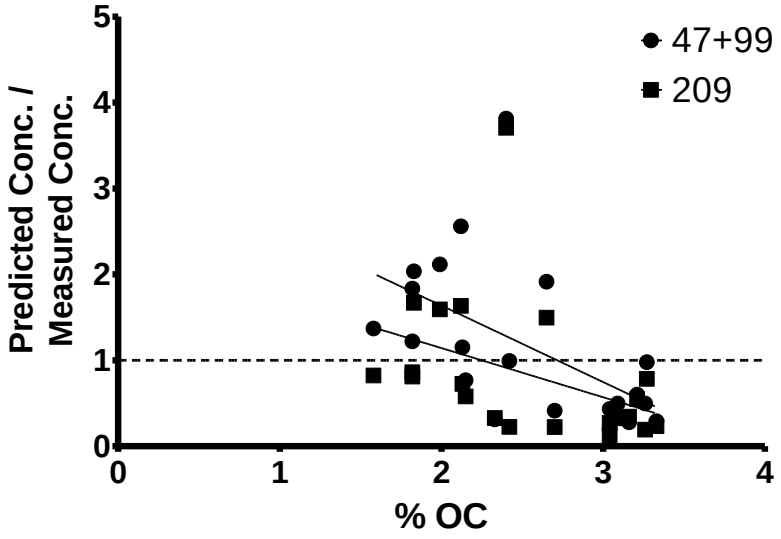


Figure 4.16 – Relationship between the ratios of predicted PBDE concentrations to measured concentrations in 2009 and the organic carbon content of the soils. Ratios decrease with increasing organic carbon content for BDE-209 ($p>0.05$, $R^2 = 0.16$) and for the sum of BDE-47 and BDE-99 ($p<0.05$, $R^2 = 0.30$). Dashed line represents equal predicted and measured concentrations.

The ratio of predicted concentration to the measured concentration is equal to one if concentrations are equal (dashed line in Figure 4.16), which indicates that the residence time utilized in the mathematical equations is a good estimate of these compounds soil residence time. If predicted concentrations are higher than measured concentrations, the ratios values will be above one, and this indicates that additional disappearance of PBDEs occurred in those soils, and the residence time was overestimated. In contrast, if predicted concentrations are lower than measured concentrations, ratio values will be below one, indicating that residence time was underestimated and chemicals remain in top soils more than predicted. Figure 4.16 shows that ratio values for BDE-209 are generally below the one-line, indicating that BDE-209 is more persistent than estimated by the 16-yr residence time. On the contrary, BDE-47 + BDE-99 ratios fall generally above the one-line, indicating that these congeners may disappear from soil faster than expected.

The difference between BDE-209 and the lower brominated congeners BDE-47 and BDE-99 was expected. The fully-brominated congener is much heavier, bigger, and more hydrophobic than the lower brominated congeners, therefore it is expected to be less available for transformations, transport, and dissipation in the environment.

Analysis of Experimental Field and Single Application Large-scale Fields

Large-scale fields that received a single biosolids application can be compared to the experimental field. There are a few differences between the fields that need to be

noted before the comparison can be made. The experimental field was being managed on a daily basis, being planted during the summer and winter with cover crops, harvested, and tilled, while single application large-scale fields were pasture fields. Also, all large-scale fields were in the same geographical region (State of Virginia neighboring counties), while the experimental plot was located in Maryland. Soil type and environmental conditions, such as precipitation, were not the same.

Applying the same equations used for calculations on the large-scale fields and the 16-yr. residence time on the experimental field results, we can compare the ratios (Figure 4.17). For BDE-209, the predicted concentration is very similar to the measured concentration in 2009; therefore the ratio is 1.21. However, for BDE-47 + BDE-99, the ratio is 5.71, which is high for single application fields (OC for the experimental field is 1.63 ± 0.45 (n=22). This result shows that in the experimental field, the dissipation and transport of the BDE-47 + BDE-99 was higher than predicted by the 16-yr residence time. This could be due to the fact that the field was tilled annually and also that the field was planted. BDE-47 and BDE-99 could be more susceptible to these managing practices as they are not as hydrophobic as the BDE-209, therefore would be more available when changes are made to the field.

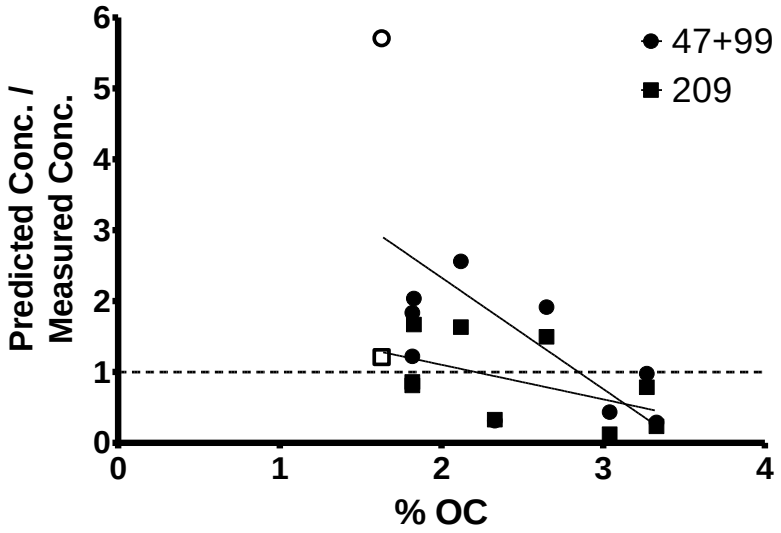


Figure 4.17 – Relationship between the ratios of predicted PBDE concentrations to measured concentrations in 2009 and the organic carbon content of the soils. Relationships are shown for large-scale fields that received a single biosolids application and the experimental field (open symbols). Ratios decrease with increasing organic carbon content for BDE-209 ($p>0.05$, $R^2 = 0.30$) and for the sum of BDE-47 and BDE-99 ($p<0.05$, $R^2 = 0.40$). Dashed line represents equal predicted and measured concentrations.

Analysis of Large-scale Fields 2006 versus 2009 Datasets

Six fields that were sampled in 2006 as zero-application had received one biosolids application by the second sampling and three of the fields that had received a single application in 2006 were, in 2009, moved to the multiple applications category (Table 4.4). All of the fields presented higher total PBDE concentrations in 2009, with the exception of fields SI2 and SI4, both of which did not receive biosolids application between the 2006 and the 2009 sampling events. However, other fields that did not receive biosolids application between the samplings had a higher concentration of PBDEs.

Concentrations in 2009 are higher than 2006 concentrations for BDE-209 in all fields, with the exception of field SI6, which had levels below detection limit in both sampling years (Table 4.4). The disproportional increase of BDE-209 is likely due to the improvement on the analysis methodology over the period. While the extraction and clean-up methodology remained unchanged, the GC-MS analysis improved significantly as important instrumental and analytical parameters were optimized. BDE-209 is particularly difficult to detect in GC-MS analysis due mostly to its molecular size and its high hydrophobicity. Some aspects of the analytical analysis were changed to optimize detection for this chemical because it is considered to be the most important congener of PBDEs as it is the major contributor to the only commercial formulation still in production.

Table 4.4 – Biosolids accumulated application rates (sum of all application rates up to the last application) with the year of last application in parentheses, BDE-209 concentration, and BDE-47+BDE-99 concentration of the large-scale fields that were sampled in 2006 and in 2009. NA represents that the field did not receive an application.

Field Code	Accumulated Application Rate (dry ton/acre)		BDE-209 Concentration (µg/kg d.w.)		BDE-47+BDE-99 Concentration (µg/kg d.w.)	
	2006	2009	2006	2009	2006	2009
MA2	14.1 (2002)	17.2 (2007)	3.0	66.2	7.6	5.5
MA3	16.0 (2005)	21.9 (2006)	49.4	240.8	67.9	44.9
MA10	13.8 (1999)	18.6 (2006)	6.1	78.0	7.0	6.8
MD1	30.0 (2005)	30.0 (NA)	78.7	255.7	48.4	23.2
MD2	26.7 (2001)	26.7 (NA)	38.5	323.3	17.4	10.1
MG2	16.4 (1998)	19.8 (2007)	13.7	103.4	16.7	13.1
MG1E	10.7 (2002)	13.9 (2008)	6.1	74.9	3.0	7.9
MG1B	15.0 (2005)	16.9 (2008)	25.3	154.2	30.5	26.5
MH6C	16.3 (2002)	20.1 (2006)	40.5	320.7	23.4	17.3
MH5C	18.2 (2002)	18.2 (NA)	30.4	236.9	16.2	17.7
MI5	3.8 (2004)	4.8 (2008)	3.0	11.4	0.8	1.2
MI11	4.7 (2004)	8.5 (2008)	3.0	6.7	0.8	0.5
MI12	10.0 (2005)	13.6 (2008)	7.7	14.4	6.0	2.0
SI2	9.8 (2005)	9.8 (NA)	22.9	24.9	10.4	2.7
SI4	10.1 (2005)	10.1 (NA)	18.7	23.0	10.9	2.6
SK2	5.9 (2005)	5.9 (NA)	6.9	96.0	11.0	10.7
SK1	5.8 (2005)	5.8 (NA)	3.0	67.1	7.5	9.8
SB5	0 (NA)	1.9 (2008)	3.0	10.7	0.5	1.2
SC11	0 (NA)	1.8 (2006)	3.0	8.2	0.5	0.5
SH10	0 (NA)	3.9 (2006)	3.0	9.6	0.5	0.8
SH15	0 (NA)	5.1 (2006)	3.0	25.1	0.9	2.3
SI6	0 (NA)	6.0 (2008)	3.0	3.0	0.5	0.9
SJ4	0 (NA)	3.9 (2006)	10.1	124.8	1.1	4.9

For BDE-47+BDE99, comparison between 2009 and 2006 levels vary and require further analysis. For the single application fields, there are two trends. First, fields

that received biosolids applications before the 2006 sampling showed a lower concentration in 2009 when compared to 2006, with the exception of field SK1, though the concentrations for this field were not very different. Field SK2, which is in the same farm as field SK1 also showed a concentration in 2009 similar to the 2006 concentration, showing those two fields retained lower brominated congeners more than other single application fields, which could be due to field management, soil characteristics, or due to the fact that biosolids were applied in 2005 and therefore during the 2006 sampling, PBDEs could have still being released from biosolids into the soil.

Second, fields that received an application between 2006 and 2009 samplings had increased concentrations of BDE-47 + BDE-99, with the exception of SC11, in which concentration remained constant. These two trends combined show that after biosolids application, soil PBDE concentrations increase, and after 3-4 years concentrations tend to decrease if no subsequent biosolids are applied.

For fields that received biosolids applications, of the three fields that did not receive an application between 2006 and 2009 samplings, two had lower concentrations in 2009 while one had higher concentrations. However, the fields with lower 2009 concentrations had a bigger difference in concentration (35.2 and 26.3% difference) than the one with higher 2009 concentration (4.48% difference). Of the 10 fields which received biosolids application between 2006 and 2009, only two fields (MG1E and MI5) showed higher concentrations in 2009. These two fields

received their last biosolids application in 2008, which could indicate that biosolids were still being incorporated into the soil during the 2009 sample collection, although three other fields that also received their last application in 2008 showed lower concentrations in 2009.

While these results indicate that BDE-47 + BDE-99 are available for soil dissipation and transport, down into the soil column and also to other environmental media, the complexity of interactions between biosolids, soils, and organic pollutants prevents a simple conclusion to be drawn. For example, in the multiple application fields, biosolids applications were from a variety of WWTPs, which certainly contained different concentrations of PBDEs at different time points. These WWTPs receive wastewater from different sources; therefore the composition of the biosolids and the organic pollutants in each one of them is different. While most of the fields were pasture, each farmer manages their fields with their own methods, which could impact biosolids incorporation and also availability of organic pollutants to dissipation processes. Though our experiments provide an estimate of an unique and important parameter, the top soil residence time, more research is needed to better understand the fate of these chemicals in soils that receive land application of biosolids.

4.5 – Conclusions

Results from the large-scale fields combined with results from the experimental small-scale field provide a better understanding of the fate of hydrophobic organic pollutants upon biosolids application to agricultural fields. Biosolids can take up to one year to completely incorporate into the soil matrix and biosolids-bound chemicals are released during this time. PBDEs profiles in soils that receive biosolids applications are similar to PBDEs profiles in biosolids and both reflect commercial formulations of this flame retardant. Estimated half-residence time was 16 yr. for BDE-47 and BDE-99, which is within 11.5% of the value that was estimated before based on polychlorinated biphenyls. Temporal and spatial variability were shown to be large in both studies, which suggests that a large number of samples may be needed to be representative of an agricultural field and care should be taken in generalizations with small sample sizes and limited soil characteristics. Comparison of the large-scale fields that were sampled in 2006 and 2009 provided insight in the complex nature of biosolids application to agricultural soil and also the interaction of organic pollutants with soil. Further research is necessary to better understand the fate of organic chemicals once they are introduced in the environment and cross the urban-agricultural border. This unique study offers important information that was previously unavailable to researchers interested in assessing the fate and environmental risks of PBDEs.

Chapter 5 – Utilizing thin-film solid-phase extraction to assess the effect of organic carbon amendments on the bioavailability of DDT and dieldrin to earthworms

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Natasha. A. Andrade, Tiziana Centofanti, Laura L. McConnell, Cathleen J. Hapeman, Alba Torrents, Anh Nguyen, W. Nelson Beyer, Rufus L. Chaney, Jeffery M. Novak, Marya O. Anderson, Keri B. Cantrell

5.1 – Abstract

Improved approaches are needed to rapidly and accurately assess the bioavailability of persistent, hydrophobic organic compounds in soils at contaminated sites. The performance of a thin-film solid-phase extraction (TF-SPE) assay using vials coated with ethylene vinyl acetate polymer was compared to an earthworm bioassay (*Lumbricus terrestris)*. Experiments utilized, as a control, contaminated soil from a former orchard that received routine DDT and dieldrin applications >40 years ago. The soil was amended with four different organic carbon materials at 5% by

weight to assess the change in pesticide bioavailability. In both assays, bioavailability of 4,4'-DDE, 4,4'-DDD, and dieldrin was higher than 4,4'-DDT in the control soil. Addition of organic carbon amendments significantly lowered bioavailability for all compounds except for 4,4'-DDT where bioavailability was significantly higher for three out of four amendments. Equilibrium concentrations of dieldrin and 4,4'-DDT + 4,4'-DDE in the polymer coating were strongly correlated with uptake by earthworms after 48 d exposure ($R^2 = 0.97$; $p < 0.001$) indicating TF-SPE provided an accurate simulation of uptake by *L. terrestris*. In a further test of the TF-SPE method, estimated bioavailability of dieldrin and DDX residues in the orchard soil was compared with a soil that was spiked with the same compounds and aged for 90 days in the laboratory. Differences in residue bioavailablity in the two soils were observed using TF-SPE. Dieldrin and DDX were only 18% and 11% less bioavailable, respectively, in the orchard soil relative to the spiked soil despite >40 years of aging. Results show that TF-SPE will be a useful tool in examining the potential risks associated with contaminated soils and to test the effectiveness of remediation efforts.

5.2 – Introduction

In assessing potential ecosystem exposure to hydrophobic pollutants, conservative estimates assume that 100% of soil-borne chemicals are available; however,

bioavailability measurements are more appropriate than total concentration (Alexander, 2000; Di Toro *et al.*, 1991). Bioavailability is frequently used to establish clean-up goals in historically-contaminated sites (Ehlers and Luthy, 2003). Although a variety of methods have been developed to assess bioavailability, no one method has been established as the most appropriate, therefore the acquisition of data for risk assessments remains difficult (Reichenberg and Mayer, 2006). Hydrophobic contaminant bioavailability is typically obtained by measuring contaminant concentrations in organisms dwelling within the contaminated medium (Lanno *et al.*, 2004) and by using a chemical assay to extract the bioavailable portion from the contaminated medium (Mäempää *et al.*, 2011; Tang *et al.*, 1999). The latter usually involves mild solvent extractions, Tenax®-aided desorption, cyclodextrin extraction, and solid-phase micro extractions (Hunter *et al.*, 2011). To ensure that this chemical extraction is representative of bioavailability, measurements are then compared to organism concentrations.

Some methods estimate bioavailability using fugacity (Mäempää *et al.*, 2011; Reichenberg and Mayer, 2006; Wilcockson and Gobas, 2001) or the tendency of a molecule to escape from a physical phase (Mackay, 1979). Fugacity (f_s, Pa) of a chemical in soil is calculated as the ratio between the soil concentration (C_s, mol/m^3) and the sorptive capacity (Z_s, mol m^{-3} Pa^{-1}) of the soil for a specific chemical ($f_s = C_s/Z_s$) (Golding *et al.*, 2008). Methods used to estimate bioavailability also rely on the equilibrium partitioning theory (Di Toro *et al.*, 1991), which assumes that given

sufficient time, equilibrium is reached between environmental phases. Equilibrium is controlled by differences in fugacities of each environmental phase, and if two or more phases are present, contaminants move between phases until fugacities in all phases are equal.

We evaluated the suitability of thin-film solid-phase extraction (TF-SPE) to assess the bioavailability of soil-bound pesticides to earthworms, which to our knowledge has never been evaluated before. TF-SPE assumes that the fugacity of a chemical in the soil (f_s) is equal to the fugacity of the chemical in the ethylene vinyl acetate (EVA) (f_{EVA}) (Equation 5.1).

$$f_s = f_{EVA} = \frac{C_S}{Z_S} = \frac{C_{EVA}}{Z_{EVA}} \quad (5.1)$$

Although useful in some instances, calculating fugacity may not be necessary as the measured concentration in the EVA film can be used as a surrogate for fugacity (Meloche *et al.*, 2009). For example, in comparing two soils: 1 and 2, $C_{EVA,1}/C_{s,1}$ equals $Z_{EVA}/Z_{s,1}$ and $C_{EVA,2}/C_{s,2}$ equals $Z_{EVA}/Z_{s,2}$ and since Z_{EVA} is the same, the ratio of EVA concentration and soil concentration is inversely proportional to the sorptive capacity of the soil. Consequently, a higher ratio indicates a lower sorptive capacity or a higher bioavailability in the specific soil.

Soil was obtained from a former orchard that received multiple applications of DDT and dieldrin prior to the 1970s. The soil contained total DDT-related compound concentrations of 2-10 ppm, and dieldrin concentrations of 1-2 ppm, providing an

excellent opportunity to test the performance of the TF-SPE method with aged pesticide residues. The first objective of the present study was to compare the TF-SPE method (Meloche *et al.*, 2009) to an earthworm (*Lumbricus terrestris*) bioassay to predict bioavailability of dieldrin, 4,4'-DDT, 4,4-DDE, and 4,4'-DDD in soil. The second objective was to test, utilizing TF-SPE and bioassay, the effect of four types of organic amendments on the bioavailability of contaminants to earthworms. Thirdly, TF-SPE was used to assess differences in the bioavailability of aged dieldrin, 4,4'-DDT and 4,4'-DDE residues (>40 yr) with the bioavailability of the same compounds which have been laboratory- aged for three months in an uncontaminated soil. Results are expected to further the development of rapid screening approaches for contaminated sites and to provide further information on the bioavailability of aged DDT and dieldrin residues in soils.

5.3 – Materials and Methods

5.3.1 – Soil collection and organic carbon amendments

A surface soil sample (15-cm depth) of approximately 2 m^2 was collected in March 2011 from an abandoned orchard in Beltsville, Maryland, USA that had received routine DDT and dieldrin insecticide applications prior to the 1970s. Details

of collection and processing are provided in supplementary materials (SM5.1). Briefly, the control soil sample was sieved (4 mm), well mixed, and stored at room temperature for 67 days prior to use. The soil series consisted of a gradation between Sassafras and Croom sandy loams and had an organic carbon (OC) content of 2.43 ± 0.07% (*n*=14).

Aliquots of the orchard soil sample (denoted as Control in the text and figures) were mixed with four organic amendments at a 5% rate by mass: dairy manure compost, aged four months; dairy manure compost, aged two years; Orgro®, a biosolids compost; and pine biochar pyrolyzed at 500°C. The OC content of the mixed amended soils was: 4-mo. Compost (Soil 1), 3.34 ± 0.23% (*n*=10); 2-yr. Compost (Soil 2), 3.31 ± 0.22% (*n*=9); Biosolids Compost (Soil 3), 3.70 ± 0.46% (*n*=10); and Biochar (Soil 4), 6.69 ± 0.83% (*n*=10).

5.3.2 – Earthworm exposure

Earthworms (*Lumbricus terrestris*) (Wholesale Bait, Hamilton, OH USA) were introduced onto the pots containing control and amended soils. Details of collection and processing are provided in supplementary materials (SM5.2) and pictures can be found in Appendix N. Briefly, 12 earthworms were added to each of the 4-L polyethylene pots for each treatment. Mesh was put around the pots and on the bottom drain holes to prevent worms from escaping. Pots were held in a temperature-

and light-controlled chamber at 15°/10°C day/night and 16-hr days. Earthworms were exposed to the contaminated soil for 48 days, after which they were removed from the soil, washed with deionized water, weighed, and stored non-depurated at -20°C until processing. Survivability was good and averaged 98% in the control 77% in amended soils. Earthworm samples were freeze-dried, weighed, and ground prior to extraction.

5.3.3 – Thin-film solid-phase extraction

TF-SPE experiments were carried out in parallel with earthworm exposure experiments, utilizing the same soil mixtures; coated vials were kept in the same temperature-controlled chamber. The polymer film and coated vials were prepared as described previously (Meloche *et al.*, 2009). In brief, the inside surface of 20-mL glass vials (Fisher Scientific, Fair Lawn, NJ, USA) were coated with a thin film of polymer using 250 µL of a 6.21-g/L solution of EVA (ELVAX® 240 resin, DuPont, Wilmington, DE, USA) containing 28% vinyl acetate (density = 0.951 g/cm^3) and 5 µL of a 2% solution of dichlorodimethylsilane (CAS# 75-78-5, Chemtrec, Falls Church, VA, USA). The 0.55-µm thick polymer film was created by allowing the dichloromethane to evaporate from the vial. Coated vials were heated to 40˚C for one hour prior to use.

For control and each amended soil, 26 coated vials were prepared with soil and

four remained empty as blanks. Thirty grams of the soil (unamended or amended) were added to each coated vial; deionized water was added to achieve 33% moisture content. Duplicate vials were capped and incubated in the growth chamber for 30 min, one, two, four, eight hours (h), one, two, four, eight, 15, 25, 35, and 45 days (d) and extracted; blanks were extracted at eight d and 45 d (Appendix O). Vials were sacrificed at different times to observe uptake of contaminants by the polymer film.

After each incubation period, soil and water were removed from the vials, which were rinsed with approximately 10 mL of deionized water until all soil residues were removed and only the polymer film remained. Vials were centrifuged for four minutes at 3000 rpm, allowing the remaining water in the vials to accumulate in the bottom. Water was removed using a syringe, 500 µL hexane was added to extract pesticide residues from the polymer, and vials were transferred to a roller mixer and allowed to rotate for five minutes at 60 rpm. The extract was transferred to a 1-mL glass vial, and the extraction was repeated. The final volume in the analysis vial was adjusted to 1 mL and 40 µL of a 500 ppm ^{13}C-labeled 4,4'-DDT solution was added as an internal standard.

5.3.4 – Assessment of long term and short term aging on availability

To evaluate bioavailability differences between pesticide residues naturally aged in the field with the same compounds in a laboratory-aged soil, a sample from a

nearby field with undetectable amounts of contaminants was spiked with 1570 µg of dieldrin; 5120 µg of 4,4'-DDT; 3210 µg of 4,4'-DDE; and 1420 µg of 4,4'- DDD to achieve similar concentrations to those found in the control (orchard) soil. The spiked soil was mixed for 15 hours and aged in a closed container for three months at room temperature to allow the analytes to be absorbed into the soil matrix. The spiked soil was transferred to coated vials and incubated at same conditions as control soil for 15 and 30 min, one, two, four, and eight h, one, two, four, eight, 15, 20, 30, and 40 d, and then processed as above. Details of soil sample collection and preparation are provided in supplementary materials (SM5.3).

5.3.5 – Residue analysis

All soil, earthworm, and polymer extracts were analyzed for 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*endo*-1,4-*exo*-5,8-dimethanonaphthalene (dieldrin, CAS#60-57-1); 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (4,4'-DDT, CAS#50-29-3); 1,1-dichloro-2,2-di(4-chlorophenyl) ethylene (4,4'-DDE, CAS#72-55-9), and 1,1-dichloro-2,2-di(4-chlorophenyl)ethane (4,4'-DDD, CAS#72-54-8) using an Agilent 6890 gas chromatograph (GC) coupled with an Agilent 5973 mass spectrometer (MS) in electron impact (EI) ionization mode (Appendix P). Details of extraction and analysis parameters are provided in supplemental materials (SM5.4).

The limit of quantitation for each of the analytes was 0.8 mg/kg based on a 2-g sample of either soil or earthworms; instrument detection limit was 13 µg/µL for each compound in polymer extracts. In soil, average sand spike recovery of analytes was 94.9 ± 3.6% (*n*=12), extraction surrogate recoveries averaged 91.8 ± 9.2% (*n*=35); duplicate samples resulted in an average percent difference of 7.0 ± 5.6% (*n*=105) for all analytes. In earthworms, average spike recovery was 82.4 ± 6.6% (*n*=12), extraction surrogate recoveries averaged 92.4 ± 16.6% (*n*=48), and duplicate samples resulted in an average percent difference of 12.0 ± 10.5% (*n*=96) for all compounds.

5.3.6 – Statistical analysis methods

Dunnet's test was used to evaluate differences in soil concentration and bioaccumulation factors (BAFs) for control and amended soils. Pearson's test was used to analyze correlation between BAFs and partition coefficients. All tests were performed using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA, USA).

5.4 – Results and discussion

5.4.1 – Hydrophobic contaminants in soil

Concentrations in control soil before earthworms were introduced were 4.76 ± 0.20, 3.44 ± 0.18, 0.90 ± 0.01, and 1.73 ± 0.06 μg/g (d.w.) for 4,4'-DDT, 4,4'-DDE, 4,4'-DDD, and dieldrin, respectively. Results were in the same range as results reported by Gaw *et al.* (2012), and one order of magnitude higher than White *et al.* (2007) results. Although contaminant concentration in amendments were below detection limit, concentration in amended soils were different ($p<0.05$) from the control (Figure 5.1 and Table S5.1). However, identical exposure concentrations are not required to measure bioavailability, and all amended soils were within 1-2 μg/g of the control concentrations.

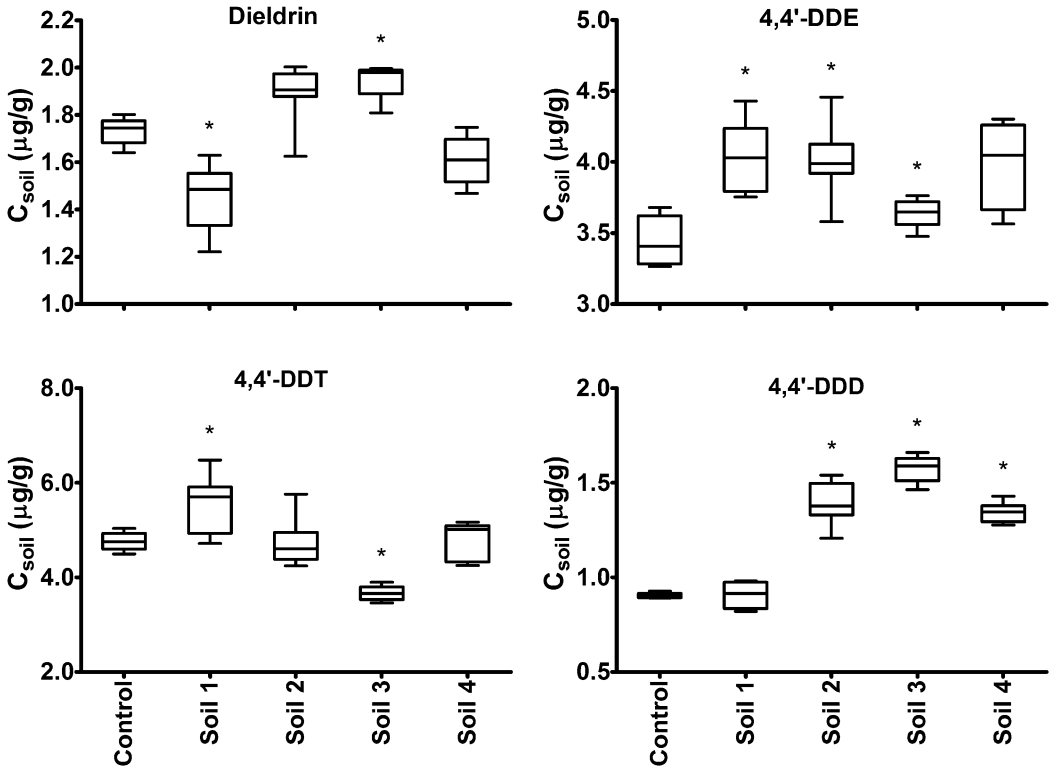

Figure 5.1 – Measured concentrations of dieldrin, 4,4'-DDT, 4,4'-DDE, and 4,4'-DDD (µg/g d.w.) in the control soil (*n*=5), Soil 1 (*n*=8), Soil 2 (*n*=7), Soil 3 (*n*=5), and for Soil 4 (*n*=9). Samples were collected the day earthworms were inserted in the pots. The line inside the box represents the mean, while whiskers represent minimum and maximum values. A * above the box indicates that the average concentration was different from the control ($p<0.05$).

5.4.2 – Bioaccumulation in earthworms

Bioaccumulation factors (BAFs), defined as the ratio of earthworm concentration (Table S5.2) to soil concentration, were calculated for control and amended soils on a dry weight and a lipid normalized basis (Figure 5.2A, 5.2B). Dry weight BAFs in the control soil were 1.52 ± 0.22, 4.69 ± 0.54, 4.86 ± 0.46, and 2.65 ± 0.22 (g dry worm/g dry soil) for 4,4'-DDT, 4,4'-DDE, 4,4'-DDD , and dieldrin, respectively. BAFs of

4,4'-DDE and 4,4'-DDD were significantly higher ($p<0.05$) than 4,4'-DDT despite the similarity in log K_{ow} values (6.39, 6.93, 6.33, and for 4,4'-DDT, 4,4'-DDE, and 4,4'-DDD, respectively) (Shen and Wania, 2005) (Figure 5.2A). Although dieldrin is less hydrophobic (log K_{ow} = 5.48), its BAF was also larger than 4,4'-DDT. Higher bioaccumulation of 4,4'-DDE and 4,4'-DDD when compared to 4,4'-DDT has also been observed for mussels living in DDT contaminated sediments (Tomaszewski *et al.*, 2008). BAFs measured in the current work were comparable to previous studies where earthworms were exposed to aged DDT residues (Gaw *et al.*, 2012; Kelsey and White, 2005).

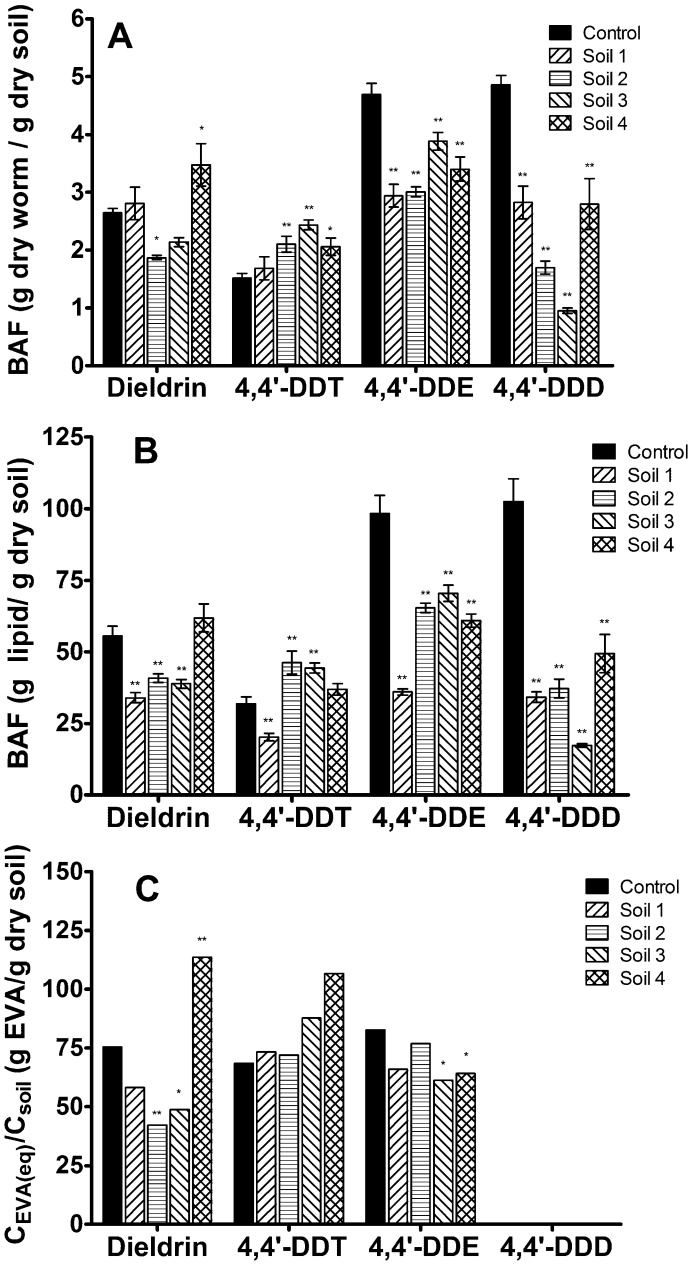

Figure 5.2 – (A) Bioaccumulation factors (BAFs) (n=8) of dieldrin, 4,4'-DDT, 4,4'-DDE, and 4,4'-DDD for control and amended soils on a dry weight basis and (B) on a lipid normalized basis. (C) Ratios (n=2) of dieldrin, 4,4'-DDT, 4,4'-DDE polymer equilibrium concentration and soil concentration for control and amended soils. 4,4'-DDD was below detection limits detected in the polymer. Symbol over bars represents statistically different averages when compared to control soil: ** (p<0.01) and * (p<0.05). Whiskers on bars represent standard error.

For amended soils, dry weight BAFs for 4,4'-DDE and 4,4'-DDD, were 17 to 37% and 42 to 80% lower ($p<0.05$), respectively, than the control soil, suggesting that organic carbon addition reduced bioavailability to earthworms (Figure 5.2A). For dieldrin, in comparison to the control, only Soil 2 produced a statistically smaller BAF, whereas Soil 4 produced a larger BAF ($p<0.05$). However, for 4,4'-DDT, BAFs of Soils 2, 3, and 4 were larger ($p<0.05$) than in control soil, indicating an increase in bioavailability. Organic amendments have been shown to increase sorption and reduce bioavailabity of herbicides and polycyclic aromatic hydrocarbons (Gomez-Eyles *et al.*, 2011; Spokas *et al.*, 2009); therefore, increased bioavailability in our study was unexpected. Tomaszewski *et al.* (2008) treated contaminated sediments with activated carbon and observed a decreased 4,4'-DDE and 4,4'-DDD bioavailability while 4,4'-DDT bioavailability was slightly larger in comparison to control sediment, thereby supporting our findings.

Examination of lipid-normalized BAF values revealed trends similar to dry weight values. However the reductions in BAF values in amended soils relative to the control were more pronounced for 4,4'-DDE and 4,4'-DDD (Figure 5.2B). For dieldrin, all amended soils were lower than the control except for soil 4. For 4,4'-DDT, only soil 1 had a statistically lower BAF value than the control with 2, 3, and 4 being either equal to or higher.

Dry weight BAF results for the four compounds did not correlate with hydrophobicity (log K_{ow}) ($p>0.05$), suggesting that other factors also influenced the

BAFs. In assessing the bioaccumulation of polychlorinated biphenyls (PCBs) in mussels, Jonker *et al.*, (2004) showed that bioaccumulation decreased with increasing PCB planarity when sediments were amended with coal and charcoal. Bucheli and Gustafsson (2001) reported that the relatively planar compound, 4,4'-DDE, exhibited a higher partition coefficient into soot-contaminated sediments than 4,4'-DDT, indicating that 4,4'-DDT would be more bioavailable. Of the four analytes here, 4,4'-DDE is the most planar and was strongly sorbed by the organic carbon amendments (Figure 5.2A). Nonetheless, 4,4'-DDD was the most strongly sorbed despite its non-planar structure. These results illustrate the complexity of interactions between aged soil residues, organic carbon amendments and the bioaccumulation of hydrophobic compounds by organisms.

5.4.3 – Comparison of TF-SPE with earthworm bioavailability

TF-SPE was utilized to estimate the bioavailability of hydrophobic organic compounds using subsamples of the control and amended soils in polymer-coated vials. Time-dependent EVA concentrations obtained from the TF-SPE experiment were fitted with mathematical models (Figure 5.3; Table S5.3) and resulted in polymer phase equilibrium concentrations, which were utilized for further analysis. One-phase (Equation 5.2) and two-phase (Equation 5.3) nonlinear models were tested on measured polymer concentration, C_{EVA}(µg/mL), versus incubation time, t(h), and

the best fit was chosen by comparing the R^2 values obtained (Meloche *et al.*, 2009).

$$C_{EVA} = C_{EVA(eq)}\left(1 - e^{-kt}\right) \tag{5.2}$$

$$C_{EVA} = C_{eq(\,fast)}\left(1 - e^{-k_{fast}t}\right) + C_{eq(slow)}\left(1 - e^{-k_{slow}t}\right) \tag{5.3}$$

The two-phase model was the best fit for dieldrin (R^2=0.95-0.98), for 4,4'-DDE (R^2=0.98-1.00), and for 66% of the 4,4'-DDT datasets (R^2=0.95-0.99). The one-phase model was used for 34% of the datasets for 4,4'-DDT (R^2=0.89-0.96). For these datasets, equilibrium had not been reached at the end of the experiment and a two-phase transfer could not be distinguished. Polymer concentrations of 4,4'-DDD were below analytical detection limits and could not confidently be used to calculate equilibrium concentrations.

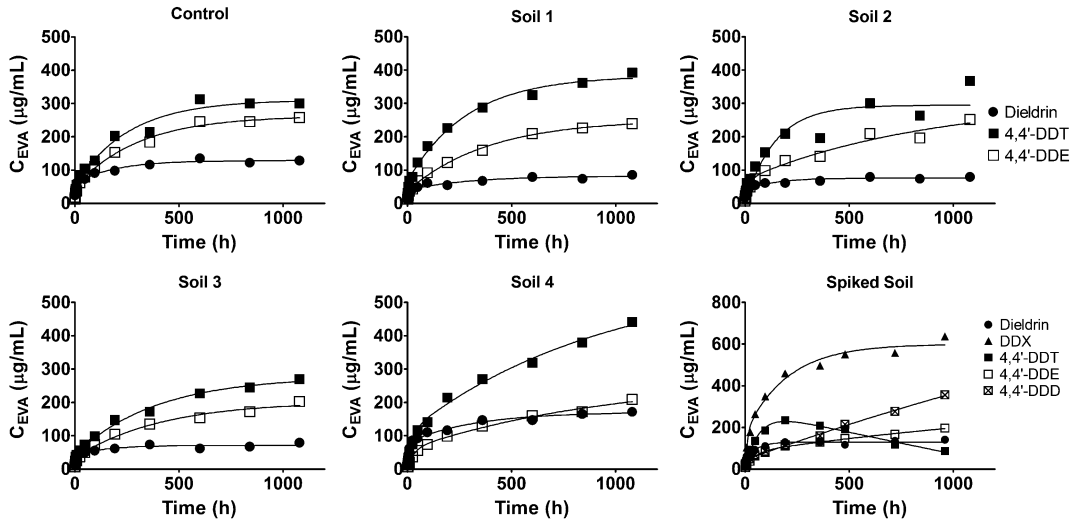

Figure 5.3 – Dieldrin, 4,4'-DDT, and 4,4'-DDE polymer concentrations C_{EVA}(µg/mL) versus incubation time *t*(h) for control and amended soils. Dieldrin, DDX, 4,4'-DDT, 4,4'-DDE, and 4,4'-DDD C_{EVA}(µg/mL) versus incubation time *t*(h) for the spiked soil. Lines are the model fits from either two-phase or one-phase models (Equation 5.2, Equation 5.3).

Model-calculated $C_{EVA(eq)}$ were normalized to soil concentration to examine the effect of organic carbon amendments on the uptake of DDT and dieldrin by the polymer, or simulated bioavailability. For dieldrin, model results predicted that addition of organic amendments significantly reduced bioavailability (p<0.01) relative to the control, except for biochar-amended soil (soil 4), which showed no change in bioavailability (Figure 5.2C), agreeing with our BAFs in earthworms (Figure 5.2A). Wang *et al.* (2012) reported that a biochar with 566 m^2g^-1 of surface area was more effective in reducing bioavailability of the pesticide chlorantraniprole residue in soils than a biochar with 27 m^2g^{-1} of surface area. The surface area of the pine biochar used in the current study was 10 times lower at 2.77 m^2g^{-1} and was likely

less efficient in adsorbing residues. Simulated bioavailability in amended soils were lower than control soil for 4,4'-DDE but higher for 4,4'-DDT, which was also observed in BAFs measured in earthworms.

The relationship between the equilibrium polymer concentration and the earthworm concentration was evaluated (Figure 5.4A). Linear regression of the log of the concentrations was unsatisfactory; R^2 was equal to 0.53 and 0.61 for the lipid and dry weight normalized data, respectively. Thus, the TF-SPE method was unable to predict earthworm concentrations for all chemicals effectively. $C_{EVA(eq)}/C_{soil}$ values for 4,4'-DDT were higher than earthworm concentrations (Figure 5.2), which suggests that some degradation of 4,4-DDT to presumably 4,4-DDE or 4,4'-DDD took place in the earthworm assay (Davis and French, 1969).

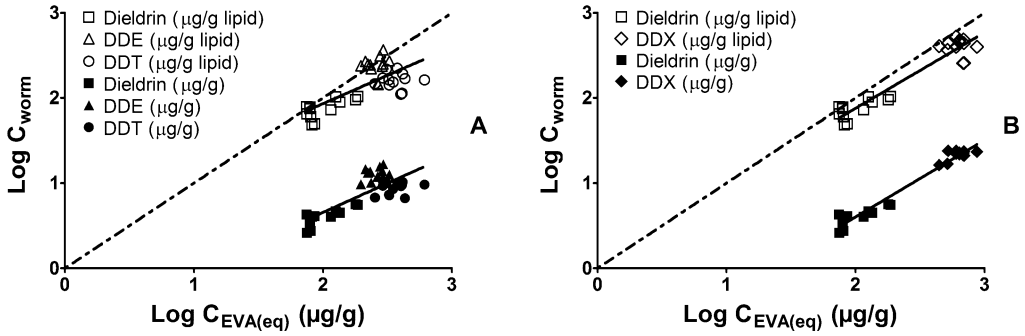

Figure 5.4 – Relationship between the log of equilibrium polymer concentration log $C_{EVA(eq)}$(μg/g) and the log of *Lumbricus terrestris* earthworm concentration on a lipid basis log C_{worm} (μg/g lipid) (white symbols) and on a dry weight basis log C_{worm} (μg/g) (black symbols) for (**A**) dieldrin, DDE, and DDT and for (**B**) dieldrin and DDX $\left(\sum DDT, DDE\right)$ in control and amended soils. Dashed line is 1:1. Solids lines are the linear fit of the datasets: **A** – R^2 = 0.53 for lipid-based and R^2 = 0.61 for dry weight based; **B** – R^2 = 0.92 for lipid based and R^2 = 0.97 for dry-weight based.

To address this finding, the linear regression of the log of the concentrations were recalculated based on the combined concentrations of 4,4'-DDT and 4,4'-DDE (DDX) (Figure 5.4B). Correlation of the log concentrations was examined using concentrations based on dry weight ($\log C_{worm}$ (µg/g) = 0.90 $\log C_{EVA(eq)}$(µg/g) – 1.20 (R^2 = 0.97; $p < 0.001$)). Results suggest that the polymer film was 22 times more efficient in accumulating residues from soil than *L. terrestris* earthworms. Similar results were observed for organochlorines in clams (*Macoma balthica*) (Meloche *et al.*, 2009), where polymer was 7.5 times more sorptive than the lipids present in clams. However, a near 1:1 relationship was found when the concentrations were normalized on a lipid basis ($\log C_{worm}$ (µg/g lipid) = 0.89 $\log C_{EVA(eq)}$(µg/g) – 0.10 (R^2 = 0.92; $p < 0.001$)). This indicates that the equilibrium concentration in the polymer was similar to the concentration in the earthworms, and suggests that the polymer and the *L. terrestris* lipid content have proportional sorptive capacities.

5.4.4 – Historically versus artificially-aged soil residues

Organic pollutants in soils are expected to become less bioavailable available with time (Alexander, 2000 and references therein). The TF-SPE method was tested for sensitivity to differences in historical field-aged versus laboratory-aged residues. Polymer-coated vials were prepared with spiked soil that had been aged for three months in the laboratory, and uptake by the polymer was measured as in the earlier

experiment. Results were compared with those from the historically contaminated control soil. The C_{EVA} uptake curves in the spiked soil indicated that the residues were much more available to undergo degradation during the course of the experiment as compared to the field-aged soil (Figure 5.3). For spike soil, polymer concentrations of 4,4'-DDT increased until approximately day 8, followed by a decline and an increase in the concentrations of 4,4'-DDD and 4,4'-DDE. Degradation of 4,4'-DDT to 4,4'-DDE or to 4,4'-DDD could have occurred *via* elimination or reductive dechlorination, respectively, inside the polymer coated vial (Brooks, 1974; Plimmer *et al.*, 1968).

Calculation of $C_{EVA(eq)}$ values for the DDT compounds individually in the spiked soil was not possible because concentration of these compounds continued to change throughout the experiment. Therefore, the models (Equation 5.3) were applied using DDX $\left(\sum DDT, DDE, DDD\right)$ concentrations. $C_{EVA(eq)}/C_{soil}$ results for field-aged residues and the spiked residues were 75.5±3.8 and 110±0, respectively, for dieldrin and 68.0±2.7 and 85.4±4.5, respectively, for DDX. Results equate to approximately 18% and 11% lower bioavailability of dieldrin and DDX, respectively, in the orchard soil compared to the spiked soil. However, ratio averages for DDX were not statistically different between treatments. From this experiment, it appears that the dieldrin and DDT residues that have been present in the orchard soil for more than 40 years are only moderately less bioavailable than a soil that has aged for only 3

months.

5.5 – Conclusions

Because bioassays can be costly and time consuming, a chemical based methodology can be useful to assess the effectiveness of possible remediation actions. The present study illustrates that the TF-SPE methodology can be used to assess the bioavailability of soil-bound hydrophobic organic chemicals to earthworms. Measured soil-polymer equilibrium concentration ratios for DDT+DDE and dieldrin correlated strongly with measured earthworm bioaccumulation factors using the same soils. Our results demonstrate that the polymer coating was similar in sorptive capacity to lipids found in *L.terrestris*. While further experiments with additional earthworm species may be needed, results indicate this approach may be used as a screening tool to detect differences in bioavailability of hydrophobic compounds in soil.

Our results also indicate that bioavailability predictions based on a single chemical property like log K_{ow} or planarity should be used with caution. Neither property was useful in predicting the trend observed for 4,4'-DDT, as this compound became more bioavailable when organic carbon was added to the control soil. Comparison of pesticide bioavailability in the orchard soil with a laboratory spiked soil suggests that aged residues were largely available for uptake by earthworms.

Acknowledgements

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Disclaimer

Mention of specific products is for identification and does not imply endorsement by the US Department of Agriculture or the US Geological Survey to the exclusion of other suitable products or suppliers.

5.6 – Supplementary material

SM5.1 – Soil collection and organic carbon amendments

Inside the orchard field, a high concentration, 2-m^2 area was chosen to be sampled for the study. The area was chosen because tests had shown it to contain DDT concentrations that would enable a study without the possibility of samples with concentrations below detection limit. The surface bulk soil sample (15-cm depth) was collected with shovels. The soil was partially dried and sieved to <4mm to remove stones and debris. The bulk soil sample was mixed using cross mixing on a clean plastic tarp.

Bulk soil was then split into five sub-samples, one was used as the control and four sub-samples were mixed with four organic carbon amendments at a 5% rate by mass. Soil was mixed with amendments using the same tarp mixing method used to homogenize the bulk soil sample. Control and amended soils were wetted to 27 ± 1.0% moisture and stored inside buckets at room temperature. Amended soils were mixed once again 2 weeks after initial mixing. At 67 days after initial mixing, control and amended soils were transferred to pots and placed in a growth chamber with a controlled temperature and light schedule. Four pots for control and four pots for each treatment were randomized in the growth chamber. Five soil samples from each treatment were collected for assessment of pesticide concentrations and organic carbon content.

SM5.2 – Soil and earthworm sample processing and extraction

Soil samples (2-g aliquot) were ground and mixed with Hydromatrix (Agilent Technologies, Wilmington, DE, USA) to achieve sample dryness and transferred to a 22-ml stainless-steel extraction cell. Any remaining space was filled with clean sand. An extraction surrogate (10 ng of $^{13}C_{12}$ 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane ($^{13}C_{12}$-4,4'-DDT, CAS# 104215-84-1) (Cambridge Isotope Laboratories, Inc, Andover, MA USA) was added to each sample. Samples were extracted by accelerated solvent extraction (ASE) (Model ASE 200, Dionex Corporation, Sunnyvale, CA, USA) using the following parameters: preheat time: 5 minutes,

temperature: 120ºC, pressure: 2000 psi, static time: 10 min, flush %: 60%, purge time: 200 sec, cycles: 2, solvents: acetone (20%) (HPLC grade, Fisher Scientific, Fair Lawn, NJ, USA) and hexane (80%) (HPLC grade, Fisher Scientific, Fair Lawn, NJ, USA). Extracts were dried with 5 g of anhydrous sodium sulfate (Na_2SO_4) (Fisher Scientific, Fair Lawn, NJ, USA) then reduced to dryness. Extracts were reconstituted in 2 mL of hexane and 20 ng of pentachloronitrobenzene (CAS# 82-68-8) (Chem Service, West Chester, PA, USA) was added as an internal standard.

Earthworm samples were freeze dried, hand cut and ground using a mortar and pestle. Aliquots (0.5 to 2.0 g) were weighed and transferred to a 22-ml ASE cell. Any remaining space in the cell was filled with clean sand. Earthworms were extracted using ASE with the same parameters used for soil extraction, and the same recovery surrogate and internal standards were used. Extracts were concentrated to 4 mL and a 1-mL aliquot was used for gravimetric determination of lipid content. The remaining 3-mL extract was evaporated to dryness. A 5-mL volume of acetonitrile was added to the vial and the extract was vortex mixed for 15 min. The acetonitrile was and transferred to a clean vial and the process was repeated. This procedure was used to selectively transfer the DDT and dieldrin analytes without retaining the interfering lipid material. The clean extract was again evaporated to dryness and reconstituted with 2 mL hexane. Soil and earthworms were extracted in batches of approximately 10 samples extracted in duplicate including sand blanks, and sand spikes. The polymer film extracted between 0.02 to 0.63% of the total dieldrin and

between 0.01 to 0.46% of the total DDT in the soil. Therefore this process did not alter the original concentration in the soil.

SM5.3 – Assessment of long term and short term aging on availability

A field near the contaminated orchard, containing soil of the same series, was sampled with a manual auger. Soil sample was air dried and sieved to <2mm to remove rocks and debris. One kg of the uncontaminated soil was spiked with 1570 µg of dieldrin; 5120 µg of 4,4'-DDT; 3210 µg of 4,4'-DDE; and 1420 µg of 4,4'-DDD. The known amount of contaminants were dissolved in 50 mL of hexane, transferred to a spray bottle, and sprayed onto the soil, which was inside a wide-mouth glass jar and was being turned by hand during application of spikes. The spiked soil was mixed for 15 hours in a dry soil y-shape mixer and stored in a closed container for three months at room temperature. Before using the soil in bioavailability study, sub-samples were collected from the soil to check for homogeneity in pesticide concentration. Results showed all analyte concentrations varied by approximately ≤8.0% (n=3).

SM5.3 – Residue analysis methods

Extracts were analyzed by Agilent 6890 gas chromatograph (GC) coupled with an Agilent 5975 mass spectrometer (MS) in electron impact (EI) ionization mode using selected-ion monitoring. The GC inlet was operated in splitless mode using a 1- µL

injection volume. The inlet was set to 210°C, inlet pressure 75.9 kPa, and purge flow 30 mL/min for 1 min. The capillary column was a DB-5-MS with a length of 30 m, diameter of 0.25 mm, and film thickness of 0.25 µm (J&W Scientific, Folsom, CA, USA). The carrier gas used was helium with a constant flow of 1.2 mL/min. The oven temperature program was as follows: 70°C for 1 min, 20°C/min to 210°C, hold 1 min, 5°C/min to 280°C, hold 1 min, 20°C/min to 300°C, and hold 10 min. The GC-MS interface was kept at a temperature of 280°C. The source temperature was 230 °C and the quadrupole temperature was 150 °C. Sample concentrations were quantified using the internal standard method with a seven point calibration curve.

Table S5.1 – Concentrations of dieldrin, 4,4'-DDT, 4,4'-DDE, and 4,4'-DDD in control soil and amended soils just before earthworms were introduced. Data are presented as mean ± standard deviation. The value of *n* is between 5 and 9.

Treatment	Soil (µg/g d.w.)[a]			
	Dieldrin	4,4'-DDT	4,4'-DDE	4,4'-DDD
Control	1.73 ± 0.1	4.76 ± 0.2	3.44 ± 0.2	0.90 ± 0.1
Soil 1	1.45 ± 0.1	5.55 ± 0.6	4.04 ± 0.2	0.91 ± 0.1
Soil 2	1.88 ± 0.1	4.77 ± 0.5	4.01 ± 0.3	1.39 ± 0.1
Soil 3	1.95 ± 0.1	3.66 ± 0.2	3.64 ± 0.1	1.57 ± 0.1
Soil 4	1.61 ± 0.1	4.81 ± 0.4	3.99 ± 0.3	1.34 ± 0.1

[a] d.w. = dry weight

Table S5.2 – Concentrations of dieldrin, 4,4'-DDT, 4,4'-DDE, and 4,4'-DDD in earthworms. Data are presented as mean ± standard deviation (*n*=8).

Treatment	Earthworms (µg/g d.w.)[a]			
	Dieldrin	4,4'-DDT	4,4'-DDE	4,4'-DDD
Control	4.58 ± 0.4	7.23 ± 1.1	16.2 ± 1.9	4.39 ± 0.4
Soil 1	4.08 ± 1.2	9.34 ± 3.1	11.9 ± 2.3	2.56 ± 0.7
Soil 2	3.52 ± 0.2	10.1 ± 1.9	12.1 ± 1.0	2.36 ± 0.5
Soil 3	4.17 ± 0.4	8.93 ± 0.9	14.1 ± 1.6	1.50 ± 0.2
Soil 4	5.60 ± 1.7	9.91 ± 2.0	13.6 ± 2.4	3.75 ± 1.7

[a] d.w. = dry weight

Table S5.3 – Parameters of mathematical models fit to polymer coated vial concentration data.

	Dieldrin	4,4'-DDE	4,4'-DDT	
	Two phase	Two phase	One phase	Two phase
Samples fitted (%)	100	100	33	66
R^2	0.95-0.98	0.98-1.00	0.89-0.96	0.95-0.99
$C_{eq(fast)}$ (µg/mL)	28.5-79.2	25.6-71.0	NA	40.4-115
$C_{eq(slow)}$ (µg/mL)	28.7-109	159-239	NA	193-474
$k_{(fast)}$ (10^{-2} h^{-1})	18.3-87.6	3.43-74.1	NA	2.1-98.2
$k_{(slow)}$ (10^{-2} h^{-1})	0.07-0.95	0.10-0.34	NA	0.04-0.40
$C_{EVA(eq)}$ (µg/mL)	NA	NA	295-394	NA
k (10^{-2} h^{-1})	NA	NA	0.40-0.70	NA

Chapter 6 – Utilizing Polymer-coated Vials to Illustrate the Fugacity and Bioavailability of Chlorinated Pesticide Residues in Contaminated Soils

This chapter has been accepted for publication to the Journal of Chemical Education and is pending minor revisions.

Natasha A. Andrade, Laura L. McConnell, Alba Torrents, Cathleen J. Hapeman

6.1 – Abstract

Fugacity and bioavailability can be used to facilitate students' understanding of potential environmental risks associated with toxic chemicals and therefore should be incorporated in environmental chemistry/science laboratories. While the concept of concentration is easy to grasp, fugacity and bioavailability can be challenging topics to communicate effectively in the timeframe of an academic laboratory course setting. In the experiment reported here, students observed the partitioning of chemical residues over time from soil into an artificial biological matrix. The two compounds utilized here as an example are commonly found in historically-contaminated

agricultural sites: 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT) and 1,1-bis-(4-chlorophenyl)-2,2-dichloroethane (DDE). A known quantity of the compounds was spiked and mixed into soil, which was then placed in replicate glass vials coated with a thin layer of polymer to mimic contact between soil and an organism. The polymer was then extracted and analyzed by standard gas chromatography-mass spectrometry (GC-MS). The fugacity gradient across the soil-polymer interface and the hydrophobic nature of the compounds drive the residues into the polymer, illustrating the concept of fugacity. A number of variations of this experiment, such as the comparison of different soils or the use of different contaminants could also be utilized for more advanced laboratory courses.

6.2 – Introduction

A fundamental aspect of environmental chemistry courses is the fate and distribution of organic pollutants in the environment (Domènech *et al.*, 2006; Casey and Pittman, 2005). Instructional laboratory experiments that are relevant to real world environmental problems while demonstrating key environmental chemistry concepts can provide substantial reinforcement of important lecture topics and increase the interaction between students and teachers. A well-designed laboratory experiment can inspire students to develop their own knowledge-gaining abilities and increase their skills in critical thinking (Zoller, 2005). A survey of science students

revealed that students do not usually learn from laboratory experiments that do not work and that laboratory experiments often do not relate to tangible issues (Phelps and Lee, 2003). The experiment reported here is designed to challenge and engage students by making use of an environmental problem that is relevant to scientists and regulators around the world: the bioavailability of persistent, toxic and bioaccumulative organic pollutants in contaminated soils. This experiment demonstrated the frequently misunderstood concept of fugacity which is used to estimate bioavailability. The experiment utilized a chemical assay method to simulate the bioavailability of 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT) and 1,1-bis-(4-chlorophenyl)-2,2-dichloroethane (DDE) (Figure 6.1) to earthworms living in a historically-contaminated agricultural site.

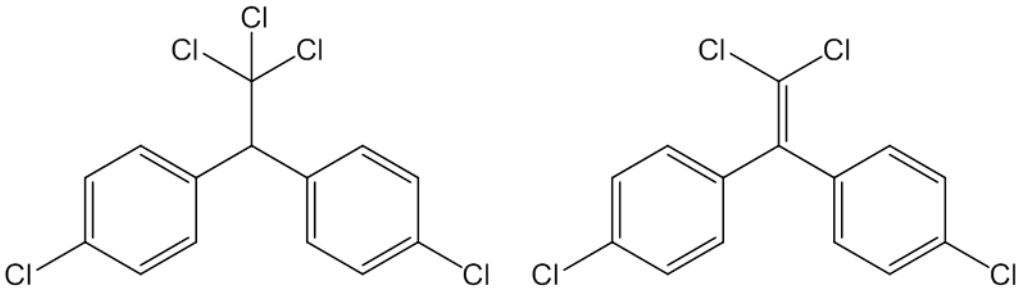


Figure 6.1 – Structure of 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT) and1,1-bis-(4-chlorophenyl)-2,2-dichloroethane (DDE).

6.3 – Rationale

Fugacity is defined as the tendency of a substance to leave an environmental phase (Mackay, 1979; Lewis, 1908); the word has its origins in the Latin word *fugere*, meaning to flee (Schwarzenbach *et al.*, 2003). In a multi-compartment environment, which in the present example consists of air, water, soil, and earthworms, an organic contaminant is distributed in each of the compartments depending on the chemical nature of the compartment itself, the chemical and physical properties of the organic contaminant, and environmental factors such as temperature. This distribution can be calculated in terms of concentrations which are proportional to the fugacity of the organic contamination in each of the compartments (Domènech *et al.*, 2006).

Fugacity and bioavailability rather than concentrations should be used to assess potential risk of toxic chemicals (Alexander, 2000; Reichenberg and Mayer, 2006; Di Toro *et al.*, 1991). Fugacity (*f* in Pa) of a contaminant in a particular environmental compartment can be expressed as the ratio of the contaminant concentration (*C* in mol L^{-1}) to its fugacity capacity (*Z* in mol L^{-1} Pa^{-1}) in that compartment (Equation 6.1).

$$f = \frac{C}{Z} \qquad (6.1)$$

The concept of fugacity can be difficult to acquire, especially when more than two phases are present, but it is demonstrated in this experiment utilizing a simple

method. A contaminant moves from a compartment of higher fugacity to a compartment of lower fugacity until the fugacity values are equal. The fugacity of the contaminants DDT and DDE in each of the phases fluctuates with time until equilibrium is reached and all the fugacities are similar. The concentrations, however, are different because they depend on the fugacity capacities (*Z*) of each chemical in each phase, which are different (Domènech *et al.*, 2006).

In this experiment, soil and water were added to vials where the inside walls were coated with a polymer which simulated the earthworms' cellular membrane. Although some air remained in the vial headspace (less than 2.5% of total vial volume), the air phase was assumed to be negligible, resulting in a three-phase system. The difference in fugacities between the three phases drives the mass transfer of the organic contamination from the soil, into the water, and into the most favorable phase, the polymer film. Utilizing polymer-coated vials to examine bioavailable residues was originally developed by Wilcockson and Gobas (2001), and later the methodology was modified to be used with sediments (Meloche *et al.*, 2009). The methodology utilized here was optimized for use with soil and based on the methodology by Meloche *et al.* (2009).

Fugacity models are usually used in environmental chemistry and environmental engineering classes to demonstrate the concept of fugacity (Domènech *et al.*, 2006), but these models involve calculations that are associated with a variety of assumptions thus introducing error. This hands-on experiment measures fugacity,

and therefore can be more effective when assessing risk (Wilcockson and Gobas, 2001). Another teaching laboratory protocol that measures fugacity was not found, making this experiment unique and easily incorporated into teaching laboratories. This experiment was used during one semester by senior undergraduate students (total of 12 students performed the experiment at this level) taking an Environmental Engineering Science course which included two lecture hours and four laboratory hours per week and in the following semester by graduate students (total of 6 students at this level) enrolled in an elective Environmental Behavior of Organic Pollutants course with a laboratory component to teach laboratory techniques and demonstrate lecture concepts.

6.4 – Procedure

Prior to conducting the experiment, students were instructed on the concept of fugacity and bioavailability as well their linkage in estimating environmental risk. A dialog about organic pollutants, especially DDT and its metabolites, was included to acquaint students to the topic and engage their curiosity on the effects of anthropogenic activities on the environment. Additional information on DDT and related environmental issues is available at several websites (EPA[a], NPIC). A pre-laboratory homework and discussion further engaged the students motivating them to ask more questions. The topic evolved to other organic pollutants and to possible

solutions for the various environmental problems that regulators and policy-makers encounter.

Questions utilized for the pre-laboratory homework were: define fugacity and bioavailability, write the fugacity-concentration equations for the two most important phases in the experiment, determine the partition constants needed, and calculate the amount of water to add to the soil to achieve 33% moisture content. The amount of water needed and the equations are necessary to conduct the experiment and carrying out the data analysis, respectively. A discussion of the pre-laboratory answers was beneficial and provided a link between the experiment and the environmental problem posed.

In the lab, students coated 20 mL glass vials with ethylene vinyl acetate (EVA) solution previously prepared by the instructor (Figures 6.2A, 6.2B). Students placed the coated vials into an oven set to 40^{o}C for one hour. Discussion ensued as to what makes a good surrogate for living organisms and the difficulty in conducting tests with living organisms. These questions emphasized the importance of the methodology utilized, as experiments with living organisms in a teaching laboratory would be more difficult to implement. When the vials were cool, the students added 30 g of the soil prepared by the instructor to each of the vials and added the mass of water they calculated from their homework to reach the desired moisture content. The vials were kept at room temperature.

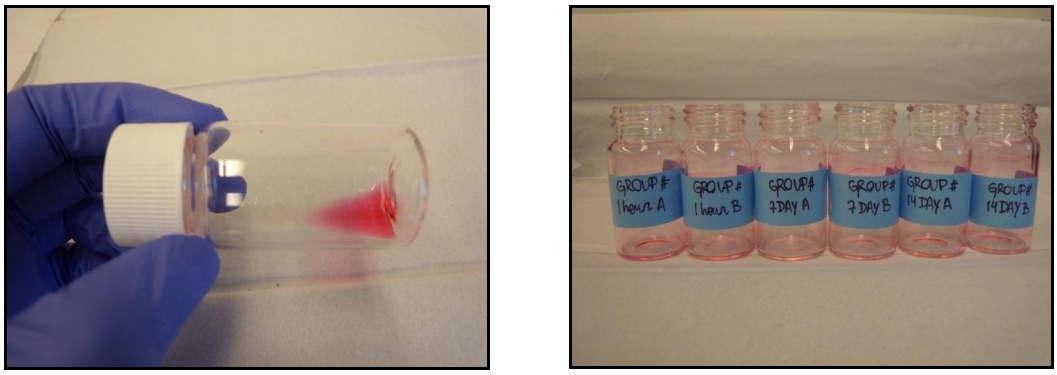


Figure 6.2 – A) Polymer solution inside the glass vial before the solvent has evaporated to form the film. The red color is due to the addition of a red dye to ensure the coating was homogeneous. B) Polymer-coated 20-mL glass vials labeled with the groups' numbers and incubation times.

After a 1-hour incubation time, students removed the soil from two vials and rinsed the vials with distilled water to remove all soil particles. This is a critical step because the film can easily be removed with the soil; students unintentionally removed approximately 6% of the films from the walls. Adding extra water to the vials during the soil removal can minimize this issue. Vials were centrifuged at 5000 rpm for 5 minutes and the remaining water removed. Hexane (500 µL) was added to each vial to extract the pesticides. Contact between the polymer coating and the solvent was accomplished using a roller at 60 rpm for 5 minutes. If a roller is not available, students can shake solvent inside the vial for 5 minutes, but in either method, care should be taken to avoid solvent loss from the cap. The extract was transferred to a labeled 2-ml GC vial, and the polymer was extracted a second time. Duplicate vials were extracted at 7 and 14 days for comparison with the 1-hour samples. All sample extracts were analyzed by GC-MS using US EPA method

8270D (EPA[b]).

6.5 – Results and Discussion

Students observed an increase in contaminant concentration in the polymer film over time, and the generated data was used in calculations related to fugacity concepts. The portion of the chemicals that partitioned from the soil into the polymer film was associated with the bioavailable portion of the contaminants. The concept of bioavailability was addressed with both undergraduate and graduate students, but can be left out, depending on the students' level and the instructor needs. The experiment was completed in a 4-hour laboratory session and in 20 minutes each during the two following laboratory sessions for two final extractions. The experiment also exposed students to proper handling of solvents and common analytical techniques, such as the use of syringes, centrifuges, analytical balances, and pipettes, and the quantitative analysis of contaminants using GC-MS.

Concentration data from the polymer extraction C_{EVA} (µg/mL) were plotted versus time *t* (hrs) and fitted to an exponential mathematical model (Equation 6.2; Figure 6.3). Students were able to calculate the equilibrium film concentration, $C_{EVA\text{-}MAX}$ (µg/mL), and the rate constant, *k* (hrs^{-1}), using non-linear regression.

$$C_{EVA}(\mu g / mL) = C_{EVA-MAX}\left(1 - e^{-kt}\right) \qquad (6.2)$$

The maximum (equilibrium) concentration, $C_{EVA\text{-}MAX}$ (µg/mL), was defined as the concentration of pollutant in the polymer film at the point which the contaminants' fugacities in the soil, water, and EVA film were equal. Students also calculated the ratio of the equilibrium concentration in the EVA film and the bulk soil concentration. Bulk soil concentration is obtained by spiking an uncontaminated soil with a known amount of pollutants. Also, during equilibrium time, the mass of pollutants that partition from the soil to the polymer film were 0.4 ± 0.01% (n=30) of the total mass, which indicates that the bulk soil concentration can be considered unchanged throughout the experiment. They compared the results for the two chemicals and determined that a higher ratio represented a higher potential for bioconcentration into organisms.

The goals of the experiment were to provide hands-on experimentation with fugacity to facilitate the understanding of the concept. Students wrote reports of the experiment and demonstrated that the concepts were well-understood. In-class discussions also provided insight and helped to determine that the experiment was successful in teaching fugacity and bioavailability. Possible modifications and adaptations of this experiment, which have not been performed in class, are addressed in Supporting Information.

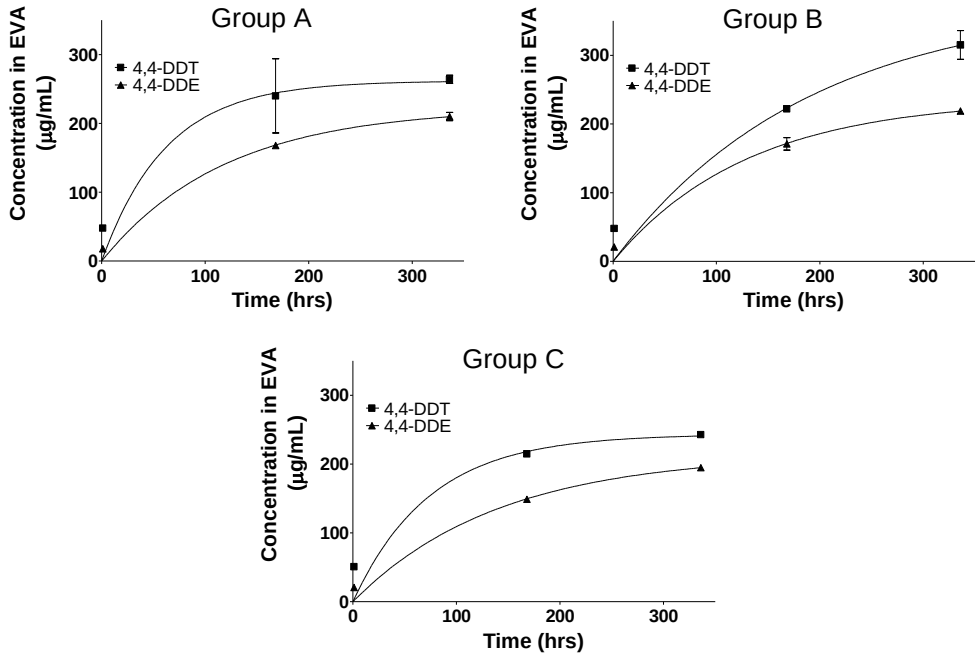


Figure 6.3 – **C**oncentration of DDT and DDE in EVA film (µg/mL) versus time (h) for three graduate student groups A, B, and C. Duplicate values were within at least 5% of each other; bars around points represent standard error and missing error bars represent equal duplicates. Lines represent a one-phase exponential fit based on Equation 6.2 (Equation parameters in Supporting Information).

6.6 – Conclusion

The experiment described here introduced students to the concepts of fugacity and bioavailability using a hands-on experiment that generated questions about important current environmental issues. It provided an excellent opportunity for students to become familiar with the laboratory protocols and techniques for quantitative analysis as well as graphical analysis of data.

Hazards

The hazards associated with this experiment include hexane, DDT, DDE, and EVA toxicity, along with the handling of laboratory glassware. Hexane toxicity is considered low, although inhalation should be prevented as it may cause nausea. DDT and DDE toxicity is generally linked to endocrine disruption, although effects are mostly seen in long-term exposure. EVA can cause mild skin irritation, which can be prevented by use of gloves. Work should be performed in a fume hood and eye protection, gloves, and laboratory coats must be worn at all times. If the instructor prefers, other representative, less toxic chemicals could be used as surrogates for toxic chemicals.

Acknowledgments

This work was partially supported by District of Columbia Water and Sewer Authority. We also acknowledge Marya Anderson for her assistance in the laboratory experiments and the University of Maryland students enrolled in ENCE 411 and ENCE 655 for their comments and help in making this experiment more comprehensive.

6.7 – Lab Documentation

6.7.1 – Student Handout

Extracting DDT and DDE from soil samples

1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT) was a commonly used pesticide in the US until it was banned in 1972. It was first introduced and used with great success in the second half of World War II to control mosquitoes that transmitted malaria and typhus among civilians and troops, and it has been credited for the control of malaria around the world. In 1962, Silent Spring (Carson, 2002) was published questioning its widespread use. Even though the chemical is no longer used, various contaminated agricultural sites exist with high levels of DDT and 1,1-bis-(4-chlorophenyl)-2,2-dichloroethane (DDE), one of its metabolites. DDT is part of a large family of anthropogenic, hydrophobic chemicals that were voluntarily and involuntarily introduced to the environment. Due to their hydrophobicity and long half-life (USDHHHS, 1994) (2 to 15 years in soil) they tend to remain in the soil environment for many years, contaminating earthworms, and subsequently accumulating in birds that feed on contaminated earthworms. Contaminated birds can produce eggs with a thin and soft shell (Hickey & Anderson, 1968).

While DDT is one compound of concern, many other organic compounds behave similarly. Many persistent organic pollutants (POPs) (EPA[c]) cause apprehension and

extensive research is being done to assess the risks associated with such chemicals. In the United States, release of some chemicals is regulated in terms of total maximum daily loads (TMDLs) allowed into the environment. Other regulations are associated with contaminant release and exposure from contaminated sites, such as the Comprehensive Environmental Response, Compensation, and Liability Act (EPA[d]) (CERCLA) and the Resource Conservation and Recovery Act (EPA[e]) (RCRA). Globally, there is great concern over the accumulation of POPs in all environmental compartments and their movement between compartments.

Chemicals accumulated in the soil can move though cell membranes and accumulate in the lipids or tissue of organisms. This process occurs due to the different fugacities of organic contaminant in the soil and the organisms. An understanding of their accumulation to earthworms and other soil organisms is needed to fully assess their potential environmental risk. To conduct direct experiments with earthworms is laborious and time-consuming. Therefore the use of ethylene vinyl acetate (EVA) to mimic the transfer of the contaminant to the earthworm is appropriate and provides the ability to calculate the amount of chemical that will be transferred from a contaminated soil to EVA, illustrating the fugacity and bioavailability concepts.

Answer the following questions before starting any experimental work:

1. What is the definition of fugacity?
2. What is the definition of bioavailability of organic compounds in the environment?
3. When equilibrium is reached, fugacity of the soil phase is equal to the fugacity of the EVA phase. Write the fugacity-concentration equation needed to perform calculations and determine the chemical partition coefficients needed.
4. Given that the soil you will use is at 20% of moisture content and that you will use 30 grams of soil in each vial, what is the amount of water you need to add to this soil to achieve 33% moisture content?

6.7.2 – Experimental Work

Vial Preparation

1. Safety considerations. All work with solvents must be conducted in the hood. Dispose of the solvents in a proper manner. Wear a lab coat, gloves and safety glasses at all times.
2. Rinse a 250 µL syringe with hexane 5 times. Inject 250 µL of the 6,210 mg/L EVA solution prepared by the instructor into a clean, dry 20 mL vial. Cap the vial.
3. Rinse a 10 µL Drummond pipette with hexane. Pipette 5 µL of the silane solution prepared by the instructor into the same vial.

4. Rotate the vial slowly making sure that the solution coats the vial uniformly. Slowly remove the cap and keep rotating the vial until all solvent has evaporated and a uniform film is formed on the internal wall of the vial.
5. Prepare five more vials the same way. It may be advised to prepare two extra vials in case any vial is broken during the experiment.
6. Place the vials into an oven at 40ºC for 60 minutes.
7. Label the vials with the following: Group # - 1 hour A, Group # - 1 hour B, Group # - 7 days Group # - 7 days B, Group # - 14 days Group # - 14 days B.
8. Add 30 g of the contaminated soil prepared by the instructor into the vial. Tap the vial on the lab bench if needed to insert all the soil.
9. Add the amount of water you calculated that is needed to reach 33% moisture content to the vial, then cap the vial and shake it gently for 5 seconds or until the mixture is homogeneous.

Film Extraction

10. After necessary incubation time (which will be 1 hour for two vials, 7 days for another two vials, and 14 days for the last two vials), remove the soil from each vial with vigorous shakes or with the help of a spatula, taking care not to destroy the film that was formed on the internal walls of the vials. Be careful as this step is critical, if you lose the film, the experiment must be repeated.
11. Rinse each of the vials with approximately 10 mL of DI water three times until

there are no soil particles left in the vial.

12. Transfer the vials to the centrifuge and spin them for 5 minutes at 5000rpm. Make sure that the caps are on but loose. This step is important, as if the caps are too tight, the vials may burst inside the centrifuge.
13. Remove the vials from the centrifuge and, with a syringe, remove the water that has collected in the bottom of each vial.
14. With a clean 500 µL syringe, transfer 0.5 mL of hexane to each vial. Place the vials on the roller and roll them for 5 minutes at 60 rpm. If no roller is available, shake the vial by hand for 5 minutes, making sure the cap is tight to prevent solvent loss.
15. With a syringe, remove the hexane from each vial and transfer it to a labeled GC vial.
16. Repeat steps 14 and 15, obtaining a final volume of approximately 1 mL in the GC vial.
17. Add additional hexane to reach the 1 mL mark on the side of the vial using a pipette.
18. Steps 10 through 17 will be performed one hour after samples were prepared (labeled Group # - 1 hour A and B), 7 days after they were prepared (labeled Group # - 7 days A and B), and 14 days after they were prepared (labeled Group # - 14 days A and B).
19. At the end of the experiment, each of the samples will be analyzed by GC-MS

using EPA method 8270D (EPA[b]).

Analyzing your results

1. GC-MS analysis of the hexane extracts will results in a concentration of DDT and DDE in units of µg/mL hexane. Calculate the concentration of the two compounds in the polymer (C_{EVA}) on both a volume and a mass basis.

Variables list:

a) V_{EVA} (mL) is the volume of EVA polymer added to each vial
b) V (mL) is the volume of EVA solution added to each vial; V = 0.25 mL
c) D_{EVA} (mg/L) is the density of EVA polymer; D_{EVA} = 931500 mg/L
d) [EVA] (mg/L) is the concentration of EVA polymer in the EVA solution; [EVA] = 6210 mg/L
e) C_{EVA} is the concentration of the chemical in the EVA polymer calculated by volume (µg/mL) or by mass (µg/g)
f) C_{HEX} (µg/mL) is the concentration of the chemical in the hexane solvent
g) M_{EVA} (g) is the mass of EVA polymer added to each vial

2. Prepare a plot of C_{EVA} (µg/mL) versus time of incubation.

3. Fit your data using non-linear regression to a one-phase exponential model and calculate the estimated equilibrium concentration, $C_{EVA\text{-}MAX}$.

$$C_{EVA}(\mu g / mL) = C_{EVA-MAX}\left(1 - e^{-kt}\right)$$

Calculate the ratio of the equilibrium concentration ($C_{EVA\text{-}MAX}$ in µg/g) to the soil bulk concentration (provided by the instructor) for each of the chemicals and explain your observations using the fugacity concept and the differences between chemicals.

Hazards

The hazards associated with this experiment include hexane, DDT, DDE, and EVA toxicity, along with the handling of laboratory glassware. Hexane toxicity is considered low, although inhalation should be prevented as it may cause nausea. DDT and DDE toxicity is generally linked to endocrine disruption, although effects are mostly seen in long-term exposure. EVA can cause mild skin irritation, which can be prevented by use of gloves. Work should be performed in a fume hood and eye protection, gloves, and laboratory coats must be worn at all times. If the instructor prefers, other representative, less toxic chemicals could be used as surrogates for toxic chemicals.

6.7.3 – Instructor Handout

Background information

This laboratory experiment was inspired by a project carried out in the authors' laboratory to examine the bioavailability of pesticides in aged, contaminated soil. Fugacity is defined as the tendency of a substance to leave an environmental phase (Mackay & Paterson, 1982) and the word has its origins in the Latin word *fugere*, meaning to flee (Schwarzenbach *et al.*, 2003). A contaminant moves from a compartment of higher fugacity to a compartment of lower fugacity until the fugacity values in those compartments are equal. In a multi-compartment environment, such as the one simulated in this experiment which consists of air, water, soil, and earthworms, an organic contaminant is distributed in each of the compartments depending on the chemical nature of the compartment itself, the chemical and physical properties of the organic contaminant, and environmental factors such as temperature. At equilibrium, the concentrations in each of the compartments will be different, but the fugacities will be the same.

Bioavailability, which is another concept addressed in this experiment, is directly related to fugacity. It can be defined as the portion of a chemical that is available to partake in biological reactions, and in this experiment, is the portion of the chemical that is able to move from the soil to the water to the EVA film. An expansion of this experiment and more thorough discussion is possible. Expansions possibilities

include the comparison of this methodology to concentrations found in earthworms, which lead to method calibration and confirmation (Wilcockson & Gobas, 2001; (Meloche *et al.*, 2009). Also, discussion of specific chemical properties and the resulting different behavior in the environment and the relationship between what is observed in the laboratory setting with what is observed in the environment usually leads to more interest from students.

Questions from the students were mostly related to toxic effects of other chemicals and the pesticides utilized in the experiment and the risk of other pesticides currently utilized. There were also questions about US Environmental Protection Agency (US EPA) programs and what is currently being done to assess environmental risk of organic pollutants. Therefore, it is advisable to read the material suggested in the manuscript and this document and consult information available on the web, such as the US EPA pesticides program (EPA[f]).

Answers to the questions in the material for students

Pre-lab:

1. What is the definition of fugacity?

"Fugacity is a thermodynamic quantity related to chemical potential or activity that characterizes the escaping tendency from a phase. At equilibrium, fugacities [at different phases], which have units of pressure, are equal" (Mackay, 1979).

2. What is the definition of bioavailability of organic compounds in the environment? Bioavailability of an organic compound in any environmental phase is the degree at which this compound is available to take part in chemical and biological reactions. Only part of the total concentration of an organic compound is available, depending of the physical and chemicals characteristics of both the compound and the environmental phase in which the compound is located.

3. When equilibrium is reached, fugacity of the soil phase is equal to the fugacity of the EVA phase. Write the fugacity-concentration equation needed to perform calculations and determine the chemical partition coefficients needed.

$$f_{EVA} = f_{soil} = \frac{C_{EVA} \times R \times T}{K_{EVA-OCTANOL} \times K_{OCTANOL-AIR}}$$

f_{EVA} is the fugacity of the EVA phase, f_{soil} is the fugacity of the soil phase, C_{EVA} is the concentration of the chemical in the EVA, R is the gas constant (8.314 $J.mol^{-1}.K^{-1}$), T is the temperature (Kelvin), $K_{EVA\text{-}OCTANOL}$ is the EVA film-octanol partition coefficient, and $K_{OCTANOL\text{-}AIR}$ is the octanol-air partition coefficient. Even though the calculation using above equation could be useful, it may not be necessary, as we can use the concentration in the EVA film as a surrogate for fugacity as the partition coefficients are the same. Therefore, a higher concentration in the EVA film

represents a higher fugacity of that chemical.

4. Given that the soil you will use is at 20% of moisture content and that you will use 30 grams of soil in each vial, what is the amount of water you need to add to this soil to achieve 33% moisture content?

$$Water amount = \frac{soil amount \times moisture content}{100\%} = \frac{30g \times 20\%}{100\%} = 6g$$

If the water amount in the soil is 6 g, this means 24 g of dry soil is present in each vial. To achieve 33% moisture content, we need to have 2 parts of soil to one part of water, which in our case amounts to 12 g (12 mL) of water to 24 g of soil. Since we know that 6 g (6 mL) of water is already in the soil, we need to add 6 g (6 mL) of water to the already moist soil to achieve 33% moisture content.

Post-lab:

1. GC-MS analysis of the hexane extracts will results in a concentration of DDT and DDE in units of μg/mL hexane. Calculate the concentration of the two compounds in the polymer (C_{EVA}) on both a volume and a mass basis.

Variables list:

a) V_{EVA} (mL) is the volume of EVA polymer added to each vial

b) V (mL) is the volume of EVA solution added to each vial; V = 0.25 mL

c) D_{EVA} (mg/L) is the density of EVA polymer; D_{EVA} = 931500 mg/L

d) [EVA] (mg/L) is the concentration of EVA polymer in the EVA solution; [EVA] = 6210 mg/L

e) C_{EVA} is the concentration of the chemical in the EVA polymer calculated by volume (µg/mL) or by mass (µg/g)

f) C_{HEX} (µg/mL) is the concentration of the chemical in the hexane solvent

g) M_{EVA} (g) is the mass of EVA polymer added to each vial

To calculate concentrations in the EVA we need to use the following equations:

$$V_{EVA}(mL) = \frac{V(mL) \times D_{EVA}(mg/L)}{[EVA](mg/L)}$$

$$C_{EVA}(\mu g/mL) = \frac{C_{HEX}(\mu g/mL)}{V_{EVA}(mL)}$$

$$M_{EVA}(g) = V(mL) \times D_{EVA}(g/mL)$$

$$C_{EVA}(\mu g/g) = \frac{C_{HEX}(\mu g/mL)}{M_{EVA}(g)}$$

2. Prepare a plot of C_{EVA} (µg/mL) versus time of incubation.

Each student plot will look different depending on the results from each of the vials, but representative student data is shown below:

Equations:

Group A, DDT: $C_{EVA}(\mu g/mL) = 261.9\left(1 - e^{-0.01605 \cdot t}\right)$

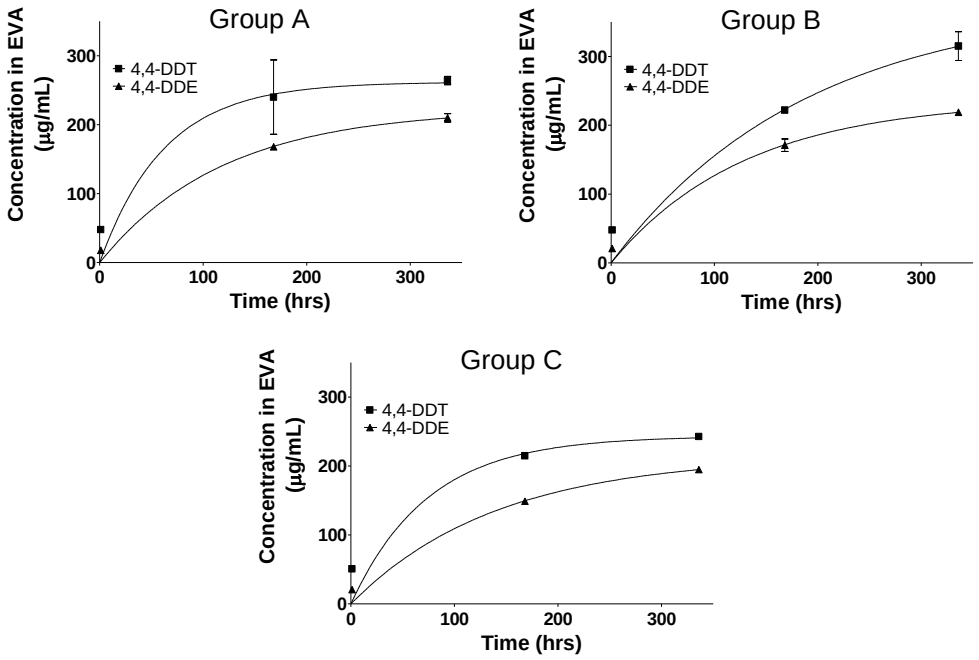
Group B, DDT: $C_{EVA}(\mu g/mL) = 378.4\left(1 - e^{-0.00530 \cdot t}\right)$

Group C, DDT: $C_{EVA}(\mu g/mL) = 243.4\left(1 - e^{-0.01349 \cdot t}\right)$

Group A, DDE: $C_{EVA}(\mu g/mL) = 223.0\left(1 - e^{-0.00839 \cdot t}\right)$

Group B, DDE: $C_{EVA}(\mu g/mL) = 236.6\left(1 - e^{-0.00769 \cdot t}\right)$

Group C, DDE: $C_{EVA}(\mu g/mL) = 214.3\left(1 - e^{-0.00712 \cdot t}\right)$



3. Fit your data to a one-phase exponential model and calculate the estimated equilibrium concentration using non-linear regression. The model equation for one-phase exponential association is:

$$C_{EVA}(\mu g / mL) = C_{EVA-MAX}\left(1 - e^{-kt}\right)$$

The equilibrium concentration is the maximum concentration ($C_{EVA\text{-}MAX}$) observed in the EVA film. In the model utilized, the equilibrium concentration is obtained when time reaches infinity.

4. Calculate the ratio of the equilibrium concentration ($C_{EVA\text{-}MAX}$ in µg/g) to the soil bulk concentration (provided by the instructor) for each of the chemicals and explain your observations using the fugacity concept and the differences between chemicals.

A higher ratio of maximum concentration in the EVA film to the bulk soil concentration implies a lower soil sorptive capacity and a higher fugacity of the chemical at a given concentration. The difference can be linked to octanol-water partition coefficients which differ from one chemical to another. The higher the octanol-water partition coefficient of a chemical, the higher the tendency of this chemical to remain adsorbed into the soil organic matter; therefore, the fugacity will

be lower as well as the bioavailability of the chemical.

For graduate student only: Besides the octanol-water partition coefficients, the compartment sizes and soil pore adsorption can be addressed with this question. In the case of this experiment, the size of the polymer compartment is considerably smaller than the size of the soil compartment; therefore hydrophobic chemicals will tend to remain in the soil. Also, chemicals that are deep into soil pores will likely remain in the pores even if the available compartment is also hydrophobic.

Possible Modifications/Adaptations of Experiment

Modifications or adaptations for this experiment can provide a richer demonstration of the primary concepts. Different soils of high and low organic matter can be used to illustrate differences in fugacity in dissimilar materials and potential differences in bioavailability. Additional contaminants with a wider range of octanol-water partition coefficients can be added. For example, three congeners of polychlorinated biphenyls, one tri-chlorinated, one penta-chlorinated, and one highly chlorinated congener, can be used to demonstrate differences in fugacity of the compounds in the same matrix. Students can also be challenged to spike a soil and allow it to age during the semester to assess the effects of soil-aging on the contaminant bioavailability.

DDT and DDE Spike solution preparation

1. Weigh 5.0 mg of DDT and 5.0 mg of DDE.
2. Transfer the chemicals to a 50 mL volumetric flask.
3. Add hexane to the 50 mL mark and mix well.
4. Secure spike solution.

Soil samples – preparation

1. Collect non-contaminated soil from a site.
2. Air-dry the soil and sieve soil to 2 mm.
3. Using a squeeze bottle, mix the spike solution into 1.0 kg of the soil ensuring that the solution does not coat only one spot on the soil surface.
4. Clean a dry soil mixer before use with water and acetone. Mix spiked soil using the clean dry soil mixer for 12 hours to achieve homogeneity. Soil can also be mixed by hand.
5. Transfer soil to jar.
6. Add enough water to dry soil until 20% moisture content is reached.
7. Mix spiked and moist soil using the clean dry soil mixer for 12 hours to achieve homogeneity.

EVA Film preparation

1. Weigh 10 mg of red dye SudanIV and transfer it to a 100 mL volumetric flask.

2. Weigh 0.6210 g of ELVAX240 or other EVA polymer.
3. Add approximately 75 mL of dichloromethane (DCM), a stir bar, and the pellets of ELVAX240 to the volumetric flask.
4. Place the volumetric flask on a heated stir plate (Corning Hot Plate Stirrer PC-351) with the following settings: 3rd bar of stirring and lowest heating set.
5. Add more DCM until all ELVAX240 pellets have dissolved.
6. Remove volumetric flask from the stir plate, remove the stir bar from the volumetric flask, and allow cooling. Add DCM to bring solution to volume.
7. Transfer the solution to labeled vials for storage.

Silane solution preparation

1. Transfer a 2 mL of dichlorodimethylsilane into a 100 mL volumetric flask.
2. Add approximately 75 mL of hexane.
3. Mix well by shaking; bring solution to volume with hexane.
4. Transfer the solution to labeled vials for storage.

Lab reagents and equipment needs

Reagents:

- Uncontaminated soil.
- Distilled water.

- ELVAX240 from DuPont®. Alternatively another EVA polymer may be used.
- Compounds listed under CAS numbers below.

Equipment:

- Analytical balance.
- 2 mm sieve.
- Soil mixer or tumbler.
- Volumetric flasks (100 mL and 50 mL).
- Heated stir plate.
- Weighing dishes/paper.
- Spatula.
- Drummond pipette (10 µL).
- 20 mL glass vials.
- Hamilton syringes (500 µL and 250 µL).
- Volumetric pipette (10 mL).
- Oven
- Centrifuge that will fit 20 mL vials.
- Vial roller mixer.
- GC auto-sampler vials and caps.
- GC-MS.

CAS numbers

- DCM: 75-09-2
- Hexane:2493-44-9
- 4,4-DDT:50-29-3
- 4,4-DDE:72-55-9
- Dichlorodimethylsilane:75-78-5
- EVA: 24937-78-8

Appendices

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 BDE-209	493, 495, 497
13C12 PCB-138	338, 372
PCB-209	464, 482

Appendix B – Moisture content in biosolids samples from Mid-Atlantic WWTP.

Sample	Moisture Content (%)
Limed 7/20/2005	62.9
Limed 9/19/2005	67.9
Limed 1/5/2006	66.8
Limed 03/06/2006	70.6
Limed 05/25/2006	69.7
Limed 07/25/2006	67.1
Limed 09/28/2006	68.1
Limed 11/28/2006	68.1
Limed 01/29/2007	70.9
Limed 03/30/2007	74.6
Limed 05/30/2007	67.3
Limed 08/10/2007	64.4
Limed 10/02/2007	60.7

Limed 12/03/2007	64.0
Limed 03/13/2008	73.4
Limed 06/05/2008	68.2
Limed 08/06/2008	71.1
Limed 10/30/2008	67.1
Limed 12/16/2008	65.5
Limed 02/26/2009	64.3
Limed 05/20/2009	69.1
Limed 08/28/2009	62.2
Limed 01/11/2010	65.3
Limed 03/16/2010	66.1
Limed 06/01/2010	66.3
Limed 08/05/2010	67.2
Limed 10/07/2010	58.7
Limed 10/12/2010	70.6
Limed 12/07/2010	67.8
Limed 02/09/2011	67.2
Limed 04/06/2011	70.2

Appendix C – Concentrations (µg/kg d.w.) of PBDE congeners in biosolids samples.

BDL – Below detection limit. BQL – Below quantitation limit.

Conc (µg/kg d.w.)	**BDE 28**	**BDE 47**	**BDE 100**	**BDE 99**	**BDE 154**	**BDE 153**	**BDE 183**	**BDE 209**
Limed A 7/20/05	BDL	137.36	25.49	149.84	BDL	BDL	BDL	1081.09
Limed B 7/20/05	BDL	190.73	35.79	209.43	BQL	BQL	BDL	2061.29
Limed A 9/19/05	BDL	247.11	46.40	273.95	BQL	BQL	BDL	1259.07
Limed B 9/19/05	BDL	252.09	48.81	289.49	BQL	BQL	BDL	1114.38
Limed A 1/5/06	BDL	140.56	25.95	162.47	BDL	BDL	BDL	1413.51

Limed B 1/5/06	BDL	157.59	27.93	191.42	BDL	BQL	BDL	1652.29
Limed A 3/6/06	BDL	146.22	27.52	164.32	BDL	BDL	BDL	1274.77
Limed B 3/6/06	BDL	152.09	30.66	168.83	BDL	BDL	BDL	1293.66
Limed A 5/25/06	BDL	165.91	30.15	185.09	BDL	BQL	BDL	563.40
Limed B 5/25/06	BDL	141.42	27.09	160.75	BDL	BDL	BDL	910.08
Limed A 7/25/06	BDL	147.55	30.46	169.92	BDL	BDL	BDL	1283.02
Limed B 7/25/06	BDL	148.52	28.53	177.44	BDL	BQL	BDL	1193.65
Limed A 9/28/06	BDL	134.79	27.76	158.34	BDL	BDL	BDL	1588.28
Limed B 9/28/06	BDL	123.31	24.57	137.17	BDL	BDL	BDL	1279.58
Limed A 11/28/06	BDL	144.55	28.69	162.43	BDL	BDL	BDL	2552.31
Limed B 11/28/06	BDL	144.42	27.76	165.42	BDL	BDL	BDL	1805.79
Limed A 1/29/07	BDL	117.35	24.79	131.49	BDL	BDL	BDL	1032.41
Limed B 1/29/07	BDL	96.85	BQL	106.21	BDL	BDL	BDL	890.09
Limed A 3/30/07	BDL	113.88	22.22	126.06	BDL	BDL	BDL	2328.61
Limed B 3/30/07	BDL	112.96	22.09	129.45	BDL	BDL	BDL	2171.56
Limed A 5/30/07	BDL	116.70	22.79	129.19	BDL	BDL	BDL	2012.13
Limed B 5/30/07	BDL	114.93	22.54	126.94	BDL	BDL	BDL	1684.31
Limed A 8/10/07	BDL	117.31	24.79	124.59	BDL	BDL	BDL	1623.03
Limed B 8/10/07	BDL	137.17	27.76	146.43	BDL	BDL	BDL	2226.53
Limed A 10/02/07	BDL	153.92	32.01	180.65	BDL	BQL	BDL	2087.68
Limed B 10/02/07	BDL	172.35	33.53	188.17	BDL	BQL	BDL	2574.47

Limed A 12/03/07	BDL	112.77	23.12	125.14	BDL	BDL	BDL	1327.01
Limed B 12/03/07	BDL	123.81	26.29	140.74	BDL	BDL	BDL	1579.61
Limed A 3/13/08	BDL	113.51	23.36	125.46	BDL	BDL	BDL	1092.63
Limed B 3/13/08	BDL	123.30	26.22	137.06	BDL	BDL	BDL	1499.01
Limed A 6/05/08	BDL	92.82	20.79	111.73	BDL	BDL	BDL	1240.09
Limed B 6/05/08	BDL	96.42	BQL	105.97	BDL	BDL	BDL	2070.64
Limed A 8/06/08	BDL	131.26	26.11	139.35	BDL	BDL	BDL	1430.04
Limed B 8/06/08	BDL	135.09	27.36	146.32	BDL	BDL	BDL	1241.88
Limed A 10/30/08	BDL	59.32	BQL	64.58	BDL	BDL	BDL	680.73
Limed B 10/30/08	BDL	68.73	BQL	76.37	BDL	BDL	BDL	877.49
Limed A 12/16/08	BDL	90.95	BQL	94.94	BDL	BDL	BDL	1392.81
Limed B 12/16/08	BDL	101.35	20.69	106.48	BDL	BDL	BDL	1417.29
Limed A 2/26/09	BDL	99.70	BQL	106.14	BDL	BDL	BDL	1161.72
Limed B 2/26/09	BDL	93.02	BQL	102.31	BDL	BDL	BDL	909.04
Limed A 5/20/09	BDL	87.07	BQL	94.80	BDL	BDL	BDL	689.67
Limed B 5/20/09	BDL	89.79	BQL	99.43	BDL	BDL	BDL	1104.11
Limed A 8/28/09	BDL	95.23	BQL	104.98	BDL	BDL	BDL	1359.27
Limed B 8/28/09	BDL	87.96	BQL	98.52	BDL	BDL	BDL	1428.48
Limed A 1/11/10	BDL	113.17	21.55	116.22	BDL	BDL	BDL	1212.53
Limed B 1/11/10	BDL	104.82	21.74	109.67	BDL	BDL	BDL	1498.44
Limed A 3/16/10	BDL	96.66	BQL	104.13	BDL	BDL	BDL	1363.42

Limed B 3/16/10	BDL	98.13	BQL	105.73	BDL	BDL	BDL	1466.69
Limed A 6/1/10	BDL	132.13	26.17	140.10	BDL	BDL	BDL	2206.43
Limed B 6/1/10	BDL	128.37	25.70	135.72	BDL	BDL	BDL	2346.24
Limed A 8/5/10	BDL	98.86	BQL	107.89	BDL	BDL	BDL	2354.06
Limed B 8/5/10	BDL	114.12	21.47	125.56	BDL	BDL	BDL	2904.92
Limed A 10/12/10	BDL	147.64	31.03	161.36	BDL	BQL	BDL	1846.99
Limed B 10/12/10	BDL	130.72	26.28	141.97	BDL	BDL	BDL	1459.83
Limed A 12/7/10	BDL	112.92	22.77	121.91	BDL	BDL	BDL	1354.02
Limed B 12/7/10	BDL	94.19	BQL	99.78	BDL	BDL	BDL	1184.33
Limed A 2/9/11	BDL	64.53	BQL	68.33	BDL	BDL	BDL	1025.04
Limed B 2/9/11	BDL	62.50	BQL	67.39	BDL	BDL	BDL	1034.91
Limed A 4/6/11	BDL	104.41	21.02	117.91	BDL	BDL	BDL	1301.20
Limed B 4/6/11	BDL	97.29	23.61	107.18	BDL	BDL	BDL	1205.75
Limed A 6/6/11	BDL	89.10	20.42	94.12	BDL	BDL	BDL	1313.46
Limed B 6/6/11	BDL	92.70	BQL	97.10	BDL	BDL	BDL	1647.73

Appendix D – Contribution of each PBDE congener to the total concentration of PBDEs in biosolids samples. Congeners that were BDL and BQL received the value of one half of the method detection limit for the particular congener.

% Cong	**BDE 28**	**BDE 47**	**BDE 100**	**BDE 99**	**BDE 154**	**BDE 153**	**BDE 183**	**BDE 209**
Limed A 7/20/05	0.54	9.64	1.79	10.52	0.56	0.58	0.47	75.90

Limed B 7/20/05	0.30	7.55	1.42	8.29	0.32	0.33	0.26	81.55
Limed A 9/19/05	0.41	13.31	2.50	14.75	0.43	0.44	0.36	67.80
Limed B 9/19/05	0.44	14.53	2.81	16.68	0.46	0.47	0.38	64.22
Limed A 1/5/06	0.43	7.93	1.46	9.16	0.45	0.46	0.37	79.72
Limed B 1/5/06	0.37	7.65	1.36	9.29	0.39	0.40	0.32	80.22
Limed A 3/6/06	0.47	8.90	1.67	10.00	0.48	0.50	0.40	77.57
Limed B 3/6/06	0.46	9.08	1.83	10.08	0.48	0.49	0.40	77.20
Limed A 5/25/06	0.79	17.02	3.09	18.98	0.82	0.84	0.68	57.78
Limed B 5/25/06	0.60	11.14	2.13	12.66	0.63	0.65	0.52	71.67
Limed A 7/25/06	0.46	8.88	1.83	10.23	0.48	0.50	0.40	77.22
Limed B 7/25/06	0.49	9.41	1.81	11.24	0.50	0.52	0.42	75.61
Limed A 9/28/06	0.40	6.95	1.43	8.16	0.41	0.42	0.34	81.88
Limed B 9/28/06	0.48	7.73	1.54	8.60	0.50	0.52	0.42	80.22
Limed A 11/28/06	0.26	4.95	0.98	5.57	0.27	0.28	0.23	87.45
Limed B 11/28/06	0.35	6.64	1.28	7.61	0.37	0.38	0.31	83.07
Limed A 1/29/07	0.57	8.78	1.85	9.84	0.60	0.62	0.50	77.24
Limed B 1/29/07	0.68	8.58	0.45	9.41	0.71	0.73	0.59	78.86
Limed A 3/30/07	0.29	4.34	0.85	4.81	0.30	0.31	0.25	88.84
Limed B 3/30/07	0.31	4.58	0.90	5.25	0.32	0.33	0.27	88.04
Limed A 5/30/07	0.33	5.05	0.99	5.59	0.34	0.36	0.29	87.06
Limed B 5/30/07	0.39	5.81	1.14	6.41	0.40	0.42	0.34	85.10

Limed A 8/10/07	0.40	6.11	1.29	6.49	0.41	0.43	0.35	84.52
Limed B 8/10/07	0.30	5.34	1.08	5.70	0.31	0.32	0.26	86.69
Limed A 10/02/07	0.31	6.19	1.29	7.27	0.32	0.33	0.27	84.02
Limed B 10/02/07	0.26	5.75	1.12	6.27	0.27	0.27	0.22	85.84
Limed A 12/03/07	0.47	6.97	1.43	7.73	0.49	0.51	0.41	81.99
Limed B 12/03/07	0.40	6.51	1.38	7.40	0.42	0.43	0.35	83.10
Limed A 3/13/08	0.55	8.19	1.69	9.06	0.58	0.59	0.48	78.86
Limed B 3/13/08	0.42	6.79	1.44	7.55	0.44	0.45	0.37	82.54
Limed A 6/05/08	0.51	6.20	1.39	7.47	0.53	0.55	0.44	82.90
Limed B 6/05/08	0.33	4.18	0.22	4.59	0.35	0.36	0.29	89.69
Limed A 8/06/08	0.44	7.47	1.49	7.93	0.45	0.47	0.38	81.38
Limed B 8/06/08	0.48	8.54	1.73	9.25	0.50	0.52	0.42	78.54
Limed A 10/30/08	0.91	7.06	0.61	7.69	0.95	0.98	0.79	81.02
Limed B 10/30/08	0.72	6.50	0.48	7.22	0.75	0.78	0.63	82.92
Limed A 12/16/08	0.47	5.63	0.32	5.88	0.49	0.51	0.41	86.28
Limed B 12/16/08	0.46	6.05	1.23	6.35	0.48	0.49	0.40	84.55
Limed A 2/26/09	0.55	7.11	0.36	7.56	0.57	0.59	0.47	82.79
Limed B 2/26/09	0.67	8.16	0.45	8.98	0.70	0.72	0.58	79.74
Limed A 5/20/09	0.84	9.60	0.56	10.45	0.88	0.91	0.73	76.03
Limed B 5/20/09	0.58	6.76	0.38	7.48	0.60	0.62	0.50	83.08
Limed A 8/28/09	0.48	5.97	0.32	6.58	0.50	0.52	0.42	85.22

Limed B 8/28/09	0.46	5.33	0.31	5.97	0.48	0.50	0.40	86.55
Limed A 1/11/10	0.51	7.57	1.44	7.78	0.53	0.55	0.44	81.16
Limed B 1/11/10	0.43	5.94	1.23	6.21	0.45	0.47	0.38	84.89
Limed A 3/16/10	0.48	6.04	0.32	6.51	0.50	0.51	0.42	85.22
Limed B 3/16/10	0.45	5.75	0.30	6.20	0.47	0.48	0.39	85.96
Limed A 6/1/10	0.30	5.21	1.03	5.53	0.31	0.32	0.26	87.03
Limed B 6/1/10	0.29	4.81	0.96	5.09	0.30	0.31	0.25	87.99
Limed A 8/5/10	0.30	3.81	0.20	4.16	0.31	0.32	0.26	90.67
Limed B 8/5/10	0.24	3.57	0.67	3.93	0.25	0.26	0.21	90.88
Limed A 10/12/10	0.35	6.66	1.40	7.28	0.36	0.37	0.30	83.29
Limed B 10/12/10	0.43	7.31	1.47	7.93	0.45	0.46	0.37	81.59
Limed A 12/7/10	0.47	6.88	1.39	7.42	0.49	0.50	0.40	82.46
Limed B 12/7/10	0.54	6.66	0.36	7.06	0.56	0.58	0.47	83.76
Limed A 2/9/11	0.64	5.41	0.43	5.73	0.67	0.69	0.56	85.88
Limed B 2/9/11	0.64	5.21	0.42	5.61	0.66	0.69	0.55	86.21
Limed A 4/6/11	0.49	6.63	1.33	7.49	0.51	0.52	0.42	82.61
Limed B 4/6/11	0.52	6.64	1.61	7.32	0.54	0.56	0.45	82.34
Limed A 6/6/11	0.50	5.76	1.32	6.08	0.51	0.53	0.43	84.87
Limed B 6/6/11	0.41	4.95	0.27	5.18	0.43	0.44	0.35	87.97

Appendix E – Average influent flow to the wastewater treatment plant in million gallons per day (mgd). Flows were averaged for the two days before the collection day, and also for the three day period including the collection day.

Biosolids Collection Date	Average Flow (mgd, 2-d before collection)	Average Flow (mgd, 2-d before collection and collection day)
11/28/2006	296.9	296.9
1/29/2007	285.1	291.3
3/30/2007	302.8	305.5
5/30/2007	293.4	295.1
8/10/2007	293.6	289.6
10/2/2007	266.7	268.2
12/3/2007	265.2	274.6
3/13/2008	287.3	287.1
6/5/2008	418.1	416.4
8/6/2008	277.9	278.2
10/30/2008	275.2	268.5
12/16/2008	285.1	294.3
2/26/2009	266.1	265.9
5/20/2009	298.7	299.2
8/28/2009	273.5	282.8
1/11/2010	309.1	311.4
3/16/2010	466.2	436.1
6/1/2010	300.2	297.3
8/5/2010	320.4	310.9
10/12/2010	274.0	273.9
12/7/2010	236.4	241.6
2/9/2011	282.8	280.4
4/6/2011	318.0	303.4
6/6/2011	253.0	253.5

Appendix F – Fields, soil characteristics, samples collected.

Field Code	Area (acre)	Soil type	Carbon %	# of Samples	Collection Date
MA2	27.7	loam	1.58	9	5/26/2009
MA3	9.6	loam	3.04	5	5/26/2009
MA10	30.1	loam	2.13	10	5/26/2009
MD1	27.7	loam	3.09	5	5/25/2009
MD2	15.5	loam	2.42	7	5/25/2009
MG2	22.3	silt loam	3.21	7	5/26/2009
MG1E	16.7	silt loam	2.15	5	5/26/2009
MG1B	12.1	silt loam	3.16	5	5/26/2009
MH6C	21.4	loam	3.26	7	5/25/2009
MH5C	34.7	loam	2.70	5	5/25/2009
MI5	16.7	loam	1.99	5	5/26/2009
MI11	16.2	loam	1.77	5	5/26/2009
MI12	14.6	loam	2.40	5	5/26/2009
SI2	9.2	loam	2.65	5	5/26/2009
SI4	24.6	loam	1.83	8	5/26/2009
SK2	14.5	loam	3.33	5	5/26/2009
SK1	18.1	loam	2.33	7	5/26/2009
SB5	11.6	silt loam	3.27	6	5/26/2009
SC11	31.2	loam	1.82	9	5/26/2009
SH10	24.2	loam	2.12	8	5/25/2009
SH15	26.1	loam	1.82	9	5/25/2009
SI6	15.6	loam	3.13	6	5/26/2009
SJ4	24.2	loam	3.04	9	5/26/2009
ZC8	27.7	loam	2.58	9	5/26/2009
ZH12	15.7	loam	1.83	5	5/25/2009
ZK3	31.7	loam	2.89	10	5/26/2009
ZK5		loam		6	5/26/2009

Appendix G – Fields crop information and biosolids applications information.

Field Code	Crops	App. Rate (dry ton/acre)	Year of Application
MA2	row crop	17.17	05/95, 05/98, 10/02, 05/07
MA3	hay	21.91	10/94, 04/99, 10/05, 07/06
MA10	row crop	18.64	10/94, 08/99, 11/06
MD1	pasture	30.02	08/93, 08/97, 09/01, 11/05
MD2	hay	26.72	08/93, 08/97, 09/01
MG2	pasture	19.78	08/94, 11/98, 11/07
MG1E	pasture	13.85	10/95, 08/99, 06/02, 10/08
MG1B	hay	16.87	10/95, 08/99, 06/02, 07/05, 10/08
MH6C	hay and pasture	20.122	12/96, 03/01, 04/02, 09/06
MH5C	hay and pasture	18.194	12/96, 02/01, 02/02
MI5	pasture	4.76	11/04, 06/08
MI11	hay	8.51	06/04, 06/08
MI12	hay	13.61	08/05, 06/08
SI2	hay	9.8	08/05
SI4	hay	10.1	08/05
SK2	pasture	5.9	08/05
SK1	pasture	5.8	08/05
SB5	hay	1.94	08/08
SC11	hay	1.8	04/06
SH10	hay and pasture	3.9	10/06
SH15	hay and pasture	5.1	10/06
SI6	pasture	5.99	06/08
SJ4	hay	3.9	07/06
ZC8	hay	NA	NA
ZH12	hay and pasture	NA	NA
ZK3	unused	NA	NA
ZK5	unsued	NA	NA

Appendix H – Congener contribution to the concentration of soils samples collected at the experimental plot for three years.

% Cong	BDE 28	BDE 47	BDE 100	BDE 99	BDE 154	BDE 153	BDE 183	BDE 209	47+99
4.5 5/30/06	2.86	2.80	1.90	2.50	2.97	3.07	2.48	81.43	5.30
5.1 5/30/06	3.43	3.35	2.28	3.00	3.56	3.68	2.97	77.72	6.36
5.3 5/30/06	3.36	3.29	2.23	2.94	3.49	3.61	2.91	78.17	6.23
6.2 5/30/06	3.32	3.24	2.21	10.12	3.45	3.56	2.87	71.23	13.36
7.2 5/30/06	3.33	3.26	2.21	2.91	3.46	3.57	2.88	78.38	6.17
1.1 8/16/06	5.60	5.48	3.73	9.78	5.82	6.02	4.85	58.71	15.26
1.3 8/16/06	1.46	7.53	1.99	9.23	1.52	1.57	1.27	75.43	16.76
1.4 8/16/06	2.86	2.80	1.90	2.50	2.97	3.07	2.48	81.42	5.30
1.5 8/16/06	0.72	5.60	1.32	6.40	0.75	0.78	0.63	83.80	11.99
2.1 8/16/06	5.89	5.76	3.92	5.16	6.12	6.33	5.10	61.72	10.92
2.2 8/16/06	1.41	4.75	0.94	5.73	1.47	1.52	1.22	82.97	10.47
2.4 8/16/06	3.22	3.15	2.14	6.65	3.34	3.45	2.79	75.27	9.79
2.5 8/16/06	5.89	5.76	3.92	5.16	6.12	6.33	5.10	61.72	10.92
3.1 8/16/06	3.19	6.42	2.13	9.32	3.32	3.43	2.77	69.43	15.74
3.3 8/16/06	2.22	7.81	1.48	9.07	2.31	2.38	1.92	72.82	16.88
3.4 8/16/06	2.59	2.53	1.72	2.27	2.69	2.78	2.24	83.18	4.80
3.5 8/16/06	3.17	3.10	2.11	2.77	3.29	3.40	2.74	79.42	5.87
4.1 8/16/06	5.56	5.44	3.70	10.45	5.78	5.97	4.82	58.27	15.89

4.2 8/16/06	5.89	5.76	3.92	5.16	6.12	6.33	5.10	61.72	10.92
4.4 8/16/06	5.89	5.76	3.92	5.16	6.12	6.33	5.10	61.72	10.92
4.5 8/16/06	0.89	6.78	1.61	8.04	0.92	0.95	0.77	80.04	14.82
5.1 8/16/06	5.89	5.76	3.92	5.16	6.12	6.33	5.10	61.72	10.92
5.2 8/16/06	1.48	1.45	0.99	3.17	1.54	1.59	1.29	88.48	4.63
5.3 8/16/06	0.92	8.40	1.87	9.59	0.96	0.99	0.80	76.48	17.99
5.4 8/16/06	1.64	3.48	1.09	4.32	1.70	1.76	1.42	84.58	7.81
5.5 8/16/06	1.64	1.61	1.09	3.59	1.71	1.77	1.42	87.17	5.20
6.2 8/16/06	1.95	4.15	1.30	4.79	2.03	2.10	1.69	81.98	8.94
6.3 8/16/06	1.26	6.22	0.84	7.06	1.31	1.35	1.09	80.87	13.28
7.4 8/16/06	27.89	4.42	3.00	3.95	4.69	4.85	3.91	47.29	8.37
8.1 8/16/06	2.28	5.46	1.52	5.23	2.37	2.45	1.98	78.71	10.69
8.2 8/16/06	2.12	2.08	1.41	5.25	2.21	2.28	1.84	82.81	7.33
5.3 11/14/06	0.54	3.65	0.83	4.61	0.56	0.58	0.47	88.76	8.25
6.1 11/14/06	5.89	5.76	3.92	5.16	6.12	6.33	5.10	61.72	10.92
6.4 11/14/06	1.23	4.09	0.82	4.97	1.28	1.32	1.06	85.23	9.06
7.3 11/14/06	3.53	3.46	2.35	3.09	3.67	3.79	3.06	77.05	6.55
7.4 11/14/06	1.75	3.68	1.16	4.72	1.82	1.88	1.52	83.48	8.40
7.5 11/14/06	1.42	4.80	0.95	5.91	1.48	1.53	1.23	82.70	10.71
8.1 11/14/06	0.54	4.50	1.01	5.29	0.56	0.58	0.46	87.08	9.79
8.2 11/14/06	1.04	3.53	0.69	4.26	1.08	1.12	0.90	87.37	7.79

8.3 11/14/06	1.84	6.13	1.23	7.02	1.91	1.98	1.60	78.30	13.14
8.4 11/14/06	2.10	2.05	1.40	4.00	2.18	2.25	1.82	84.19	6.06
8.5 11/14/06	1.57	3.96	1.05	4.99	1.64	1.69	1.36	83.73	8.95
1.1 3/14/07	1.30	7.73	1.76	8.42	0.68	0.71	0.57	78.84	16.15
1.5 3/14/07	2.59	2.54	1.73	4.69	2.70	2.78	2.25	80.73	7.23
4.2 3/14/07	1.79	6.10	1.19	7.19	1.86	1.93	1.55	78.37	13.29
4.4 3/14/07	2.82	2.76	1.88	2.47	2.93	3.03	2.44	81.66	5.23
5.1 3/14/07	1.00	7.47	1.89	8.38	1.04	1.08	0.87	78.28	15.85
5.2 3/14/07	1.65	5.42	1.10	6.36	1.71	1.77	1.43	80.57	11.78
6.4 3/14/07	1.46	3.18	0.97	3.90	1.52	1.57	1.26	86.15	7.08
7.1 3/14/07	1.97	1.93	1.31	1.73	2.05	2.12	1.71	87.17	3.66
7.2 3/14/07	0.53	5.17	1.33	5.79	0.55	1.17	0.46	85.01	10.96
7.3 3/14/07	2.02	1.97	1.34	1.77	2.10	2.17	1.75	86.88	3.74
7.4 3/14/07	1.07	3.43	1.48	3.85	1.11	1.15	0.92	87.00	7.28
7.5 3/14/07	1.13	1.11	0.75	0.99	1.18	1.22	0.98	92.63	2.10
8.1 3/14/07	1.58	1.54	1.05	2.82	1.64	1.70	1.37	88.30	4.36
8.2 3/14/07	1.43	1.40	0.95	3.26	1.49	1.54	1.24	88.67	4.66
8.3 3/14/07	0.66	4.49	1.29	4.98	0.69	0.71	0.57	86.61	9.48
8.4 3/14/07	0.75	5.24	1.60	5.75	0.77	0.80	0.65	84.45	10.98
8.5 3/14/07	1.92	1.87	1.27	1.68	1.99	2.06	1.66	87.55	3.55
1.1 7/18/07	0.21	4.54	0.96	5.26	0.50	0.67	0.37	87.49	9.80

1.2 7/18/07	1.29	1.26	0.86	1.13	1.34	1.39	1.12	91.60	2.40
1.3 7/18/07	0.16	3.49	0.72	3.98	0.38	0.54	0.28	90.46	7.47
1.4 7/18/07	0.56	3.33	0.98	4.01	0.58	0.60	0.48	89.45	7.34
1.5 7/18/07	0.24	2.92	0.69	3.43	0.25	0.55	0.21	91.72	6.34
2.1 7/18/07	0.37	1.72	0.59	2.05	0.38	0.40	0.32	94.16	3.77
2.2 7/18/07	0.48	1.78	0.67	2.09	0.50	0.51	0.41	93.56	3.87
2.3 7/18/07	0.15	4.32	0.87	5.10	0.46	0.62	0.32	88.15	9.43
2.4 7/18/07	1.34	1.31	0.89	2.87	1.39	1.44	1.16	89.61	4.17
2.5 7/18/07	1.25	1.22	0.83	2.79	1.30	1.34	1.08	90.18	4.01
3.2 7/18/07	0.80	2.63	0.53	3.15	0.83	0.86	0.70	90.49	5.78
3.3 7/18/07	1.63	1.60	1.09	1.43	1.69	1.75	1.41	89.40	3.02
3.4 7/18/07	0.59	2.04	0.78	2.52	0.61	0.63	0.51	92.32	4.56
3.5 7/18/07	0.87	1.91	0.58	2.50	0.90	0.93	0.75	91.57	4.41
4.1 7/18/07	0.54	11.65	2.41	13.01	1.25	1.68	0.47	68.99	24.66
4.2 7/18/07	0.81	10.56	2.35	11.39	0.84	1.81	0.70	71.54	21.95
4.3 7/18/07	2.74	2.68	1.82	5.35	2.85	2.94	2.37	79.25	8.03
4.4 7/18/07	6.16	3.39	1.02	4.09	1.60	1.65	1.33	80.76	7.48
4.5 7/18/07	1.99	7.55	2.18	8.59	1.11	1.14	0.92	76.52	16.15
5.1 7/18/07	0.44	8.42	1.82	10.01	1.02	1.38	0.38	76.52	18.43
5.2 7/18/07	2.78	2.72	1.85	2.43	2.89	2.98	2.41	81.95	5.15
5.3 7/18/07	35.75	2.75	0.94	3.53	1.47	1.51	1.22	52.83	6.28

5.4 7/18/07	1.73	7.39	1.52	8.86	0.86	1.15	0.61	77.87	16.25
6.1 7/18/07	6.93	4.92	2.55	8.33	2.02	2.08	1.68	71.48	13.25
6.2 7/18/07	0.68	7.40	1.75	8.36	0.70	1.47	0.59	79.06	15.76
6.3 7/18/07	1.48	1.45	0.99	3.10	1.54	1.59	1.29	88.56	4.55
6.4 7/18/07	1.55	5.50	1.52	6.46	0.77	0.79	0.64	82.78	11.95
6.5 7/18/07	2.90	4.40	1.97	5.30	1.51	1.56	1.26	81.11	9.70
7.1 7/18/07	1.80	7.04	2.55	8.13	1.87	1.93	1.56	75.13	15.17
7.2 7/18/07	0.67	8.55	1.90	9.41	0.70	0.72	0.58	77.46	17.96
7.3 7/18/07	0.25	9.62	1.80	11.16	0.85	1.22	0.50	74.59	20.79
7.4 7/18/07	0.34	9.03	1.78	10.53	0.96	1.28	0.64	75.43	19.56
7.5 7/18/07	2.69	2.63	1.79	2.35	2.79	2.88	2.33	82.54	4.98
8.1 7/18/07	3.05	2.98	2.03	2.67	3.17	3.27	2.64	80.20	5.65
8.2 7/18/07	2.28	2.23	1.52	2.00	2.37	2.45	1.98	85.17	4.23
8.3 7/18/07	1.08	7.78	2.09	8.66	1.12	1.16	0.94	77.18	16.44
8.4 7/18/07	0.18	8.55	1.58	10.05	0.73	1.00	0.42	77.50	18.60
8.5 7/18/07	1.11	4.21	1.65	4.91	1.16	1.20	0.96	84.81	9.11
1.3 6/27/08	1.60	3.28	1.07	4.16	1.66	1.72	1.39	85.13	7.44
1.4 6/27/08	1.00	3.48	1.39	4.43	1.04	1.08	0.87	86.71	7.91
1.5 6/27/08	1.10	5.31	1.81	7.22	1.14	1.18	0.95	81.31	12.53
3.1 6/27/08	0.69	6.95	1.75	7.82	0.72	0.74	0.60	80.72	14.77
3.3 6/27/08	0.92	5.73	1.76	6.60	0.95	0.99	0.80	82.25	12.33

3.4 6/27/08	1.10	4.95	1.74	5.82	1.14	1.18	0.95	83.11	10.77
4.2 6/27/08	1.66	4.82	1.10	6.01	1.72	1.78	1.43	81.48	10.83
4.3 6/27/08	30.68	4.93	1.39	5.72	0.72	0.74	0.60	55.22	10.65
4.4 6/27/08	0.98	5.19	1.69	6.18	1.02	1.05	0.85	83.04	11.37
4.5 6/27/08	0.80	3.16	1.25	4.00	0.83	0.85	0.69	88.42	7.16
5.1 6/27/08	1.48	1.45	0.98	3.51	1.54	1.59	1.28	88.17	4.96
5.2 6/27/08	3.94	4.21	0.96	5.07	1.50	1.55	1.25	81.51	9.28
5.3 6/27/08	0.81	6.35	1.69	7.20	0.84	0.87	0.70	81.53	13.55
5.4 6/27/08	0.73	5.33	1.50	5.96	0.76	0.79	0.64	84.29	11.29
5.5 6/27/08	0.80	2.21	0.54	2.68	0.84	0.86	0.70	91.37	4.89
6.3 6/27/08	1.61	3.60	1.07	4.48	1.68	1.73	1.40	84.42	8.08
6.4 6/27/08	1.18	1.15	0.78	2.86	1.22	1.26	1.02	90.53	4.01
6.5 6/27/08	0.70	1.45	0.47	1.93	0.73	0.75	0.61	93.37	3.38
7.1 6/27/08	1.32	4.56	1.75	5.36	1.37	1.41	1.14	83.09	9.92
7.2 6/27/08	1.88	5.42	1.25	6.50	1.95	2.02	1.63	79.36	11.92
7.3 6/27/08	0.72	4.84	1.35	5.48	0.74	0.77	0.62	85.49	10.31
7.4 6/27/08	0.58	7.24	1.69	8.04	0.60	1.29	0.50	80.04	15.29
7.5 6/27/08	1.32	5.62	1.97	6.70	1.38	1.42	1.15	80.44	12.32
8.2 6/27/08	2.22	2.17	1.48	3.98	2.30	2.38	1.92	83.55	6.15
8.3 6/27/08	1.52	3.89	1.01	5.32	1.58	1.63	1.31	83.74	9.21
8.4 6/27/08	1.94	1.90	1.29	1.70	2.02	2.09	1.69	87.36	3.61

1.1 7/31/09	0.71	4.87	1.04	5.43	0.74	0.77	0.62	85.82	10.30
1.2 7/31/09	1.61	1.57	1.07	3.27	1.67	1.73	1.39	87.69	4.84
1.3 7/31/09	2.31	2.26	1.54	2.02	2.40	2.48	2.00	84.98	4.28
1.4 7/31/09	2.07	2.03	1.38	1.81	2.15	2.22	1.79	86.54	3.84
1.5 7/31/09	2.44	2.39	1.63	2.14	2.54	2.62	2.12	84.12	4.53
2.1 7/31/09	1.10	3.25	0.73	3.94	1.14	1.18	0.95	87.73	7.18
2.3 7/31/09	2.56	2.50	1.70	2.24	2.66	2.74	2.21	83.39	4.74
3.1 7/31/09	1.66	4.09	1.10	5.11	1.72	1.78	1.44	83.09	9.21
3.2 7/31/09	1.79	1.75	1.19	3.41	1.86	1.92	1.55	86.53	5.16
3.3 7/31/09	0.84	4.50	0.56	5.13	0.87	0.90	0.73	86.49	9.62
3.4 7/31/09	1.78	1.74	1.18	4.02	1.85	1.91	1.54	85.98	5.76
3.5 7/31/09	2.36	2.31	1.57	2.07	2.45	2.53	2.05	84.66	4.38
4.1 7/31/09	1.19	5.00	0.79	5.69	1.24	1.28	1.04	83.76	10.69
4.2 7/31/09	2.38	2.33	1.59	2.09	2.48	2.56	2.07	84.50	4.42
4.3 7/31/09	0.99	0.97	0.66	0.87	1.03	1.06	0.86	93.56	1.84
4.5 7/31/09	1.13	1.10	0.75	0.99	1.17	1.21	0.98	92.67	2.09
5.1 7/31/09	0.76	2.85	0.50	3.40	0.79	0.81	0.66	90.24	6.24
5.2 7/31/09	0.87	0.85	0.58	0.76	0.90	0.93	0.75	94.34	1.61
5.3 7/31/09	0.72	2.18	0.48	2.65	0.75	0.78	0.63	91.82	4.82
6.1 7/31/09	2.14	2.10	1.43	1.88	2.23	2.30	1.86	86.07	3.97
6.2 7/31/09	1.67	1.64	1.11	1.46	1.74	1.79	1.45	89.14	3.10

6.3 7/31/09	1.83	1.79	1.22	1.60	1.90	1.96	1.58	88.12	3.39
6.4 7/31/09	1.69	1.65	1.12	1.48	1.75	1.81	1.46	89.04	3.13
6.5 7/31/09	0.95	2.32	0.63	2.86	0.99	1.02	0.83	90.39	5.18
7.2 7/31/09	2.10	2.06	1.40	1.84	2.19	2.26	1.82	86.33	3.90
7.3 7/31/09	1.37	1.34	0.91	3.25	1.42	1.47	1.18	89.06	4.59
7.4 7/31/09	1.61	1.58	1.07	1.41	1.67	1.73	1.40	89.53	2.99
7.5 7/31/09	2.05	2.00	1.36	1.79	2.13	2.20	1.77	86.69	3.80
8.1 7/31/09	1.88	1.84	1.25	1.64	1.95	2.02	1.63	87.79	3.48
8.2 7/31/09	1.64	1.61	1.09	1.44	1.71	1.76	1.42	89.32	3.05
8.3 7/31/09	1.42	1.39	0.95	1.25	1.48	1.53	1.23	90.75	2.64
8.4 7/31/09	1.63	1.59	1.08	1.43	1.69	1.75	1.41	89.42	3.02
8.5 7/31/09	1.81	1.77	1.20	1.58	1.88	1.94	1.57	88.25	3.35
Average	**2.39**	**3.92**	**1.48**	**4.69**	**1.81**	**1.91**	**1.50**	**82.28**	**8.62**
Standard Deviation	**4.32**	**2.22**	**0.75**	**2.64**	**1.28**	**1.30**	**1.07**	**8.52**	**4.76**
n	**156**	**156**	**156**	**156**	**156**	**156**	**156**	**156**	**156**

Appendix I – Soil PBDE concentration inside experimental plot for all sample collection period.

Conc (µg/kg)	**BDE 28**	**BDE 47**	**BDE 100**	**BDE 99**	**BDE 154**	**BDE 153**	**BDE 183**	**BDE 209**
4.5 5/30/06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	8.19
5.1 5/30/06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	6.52

5.3 5/30/06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	6.69
6.2 5/30/06	BDL	BDL	BDL	0.88	BDL	BDL	BDL	6.17
7.2 5/30/06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	6.77
1.1 8/16/06	BDL	BDL	BDL	0.50	BDL	BDL	BDL	BDL
1.3 8/16/06	BDL	1.48	0.39	1.81	BDL	BDL	BDL	14.83
1.4 8/16/06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	8.18
1.5 8/16/06	BDL	2.22	0.52	2.54	BDL	BDL	BDL	33.25
2.1 8/16/06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
2.2 8/16/06	BDL	0.97	BDL	1.17	BDL	BDL	BDL	16.90
2.4 8/16/06	BDL	BDL	BDL	0.59	BDL	BDL	BDL	6.73
2.5 8/16/06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
3.1 8/16/06	BDL	0.58	BDL	0.84	BDL	BDL	BDL	6.25
3.3 8/16/06	BDL	1.01	BDL	1.18	BDL	BDL	BDL	9.44
3.4 8/16/06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	9.24
3.5 8/16/06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	7.21
4.1 8/16/06	BDL	BDL	BDL	0.54	BDL	BDL	BDL	BDL
4.2 8/16/06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
4.4 8/16/06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
4.5 8/16/06	BDL	2.19	0.52	2.60	BDL	BDL	BDL	25.90
5.1 8/16/06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
5.2 8/16/06	BDL	BDL	BDL	0.62	BDL	BDL	BDL	17.14

5.3 8/16/06	BDL	2.62	0.58	2.99	BDL	BDL	BDL	23.86
5.4 8/16/06	BDL	0.61	BDL	0.76	BDL	BDL	BDL	14.83
5.5 8/16/06	BDL	BDL	BDL	0.63	BDL	BDL	BDL	15.24
6.2 8/16/06	BDL	0.61	BDL	0.70	BDL	BDL	BDL	12.06
6.3 8/16/06	BDL	1.42	BDL	1.61	BDL	BDL	BDL	18.45
7.4 8/16/06	1.78	BDL	BDL	BDL	BDL	BDL	BDL	BDL
8.1 8/16/06	BDL	0.69	BDL	0.66	BDL	BDL	BDL	9.91
8.2 8/16/06	BDL	BDL	BDL	0.71	BDL	BDL	BDL	11.21
5.3 11/14/06	BDL	1.93	0.44	2.44	BDL	BDL	BDL	46.95
6.1 11/14/06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
6.4 11/14/06	BDL	0.96	BDL	1.16	BDL	BDL	BDL	19.95
7.3 11/14/06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	6.27
7.4 11/14/06	BDL	0.60	BDL	0.78	BDL	BDL	BDL	13.72
7.5 11/14/06	BDL	0.97	BDL	1.20	BDL	BDL	BDL	16.73
8.1 11/14/06	BDL	2.41	0.54	2.83	BDL	BDL	BDL	46.69
8.2 11/14/06	BDL	0.98	BDL	1.18	BDL	BDL	BDL	24.11
8.3 11/14/06	BDL	0.96	BDL	1.09	BDL	BDL	BDL	12.22
8.4 11/14/06	BDL	BDL	BDL	0.55	BDL	BDL	BDL	11.53
8.5 11/14/06	BDL	0.72	BDL	0.91	BDL	BDL	BDL	15.28
1.1 3/14/07	0.57	3.38	0.77	3.68	BDL	BDL	BDL	34.49
1.5 3/14/07	BDL	BDL	BDL	0.52	BDL	BDL	BDL	8.95

4.2 3/14/07	BDL	0.98	BDL	1.15	BDL	BDL	BDL	12.56
4.4 3/14/07	BDL	BDL	BDL	BDL	BDL	BDL	BDL	8.32
5.1 3/14/07	BDL	2.14	0.54	2.40	BDL	BDL	BDL	22.45
5.2 3/14/07	BDL	0.95	BDL	1.11	BDL	BDL	BDL	14.07
6.4 3/14/07	BDL	0.63	BDL	0.77	BDL	BDL	BDL	16.98
7.1 3/14/07	BDL	BDL	BDL	BDL	BDL	BDL	BDL	12.69
7.2 3/14/07	BDL	2.80	0.72	3.13	BDL	0.63	BDL	46.00
7.3 3/14/07	BDL	BDL	BDL	BDL	BDL	BDL	BDL	12.37
7.4 3/14/07	BDL	0.92	0.40	1.04	BDL	BDL	BDL	23.43
7.5 3/14/07	BDL	BDL	BDL	BDL	BDL	BDL	BDL	23.48
8.1 3/14/07	BDL	BDL	BDL	0.51	BDL	BDL	BDL	16.08
8.2 3/14/07	BDL	BDL	BDL	0.65	BDL	BDL	BDL	17.76
8.3 3/14/07	BDL	1.96	0.56	2.17	BDL	BDL	BDL	37.70
8.4 3/14/07	BDL	2.02	0.62	2.22	BDL	BDL	BDL	32.58
8.5 3/14/07	BDL	BDL	BDL	BDL	BDL	BDL	BDL	13.13
1.1 7/18/07	BDL	6.20	1.31	7.18	0.69	0.92	0.50	119.42
1.2 7/18/07	BDL	BDL	BDL	BDL	BDL	BDL	BDL	20.38
1.3 7/18/07	BDL	6.42	1.32	7.33	0.70	0.99	0.52	166.64
1.4 7/18/07	BDL	1.71	0.51	2.06	BDL	BDL	BDL	46.03
1.5 7/18/07	BDL	3.49	0.82	4.10	BDL	0.66	BDL	109.70
2.1 7/18/07	BDL	1.34	0.46	1.59	BDL	BDL	BDL	73.08

2.2 7/18/07	BDL	1.07	0.40	1.25	BDL	BDL	BDL	56.15
2.3 7/18/07	BDL	8.17	1.65	9.64	0.87	1.18	0.60	166.60
2.4 7/18/07	BDL	BDL	BDL	0.62	BDL	BDL	BDL	19.25
2.5 7/18/07	BDL	BDL	BDL	0.64	BDL	BDL	BDL	20.74
3.2 7/18/07	BDL	0.94	BDL	1.13	BDL	BDL	BDL	32.38
3.3 7/18/07	BDL	BDL	BDL	BDL	BDL	BDL	BDL	15.76
3.4 7/18/07	BDL	0.99	0.38	1.23	BDL	BDL	BDL	45.11
3.5 7/18/07	BDL	0.63	BDL	0.83	BDL	BDL	BDL	30.37
4.1 7/18/07	BDL	6.20	1.28	6.91	0.67	0.89	BDL	36.67
4.2 7/18/07	BDL	3.75	0.83	4.04	BDL	0.64	BDL	25.38
4.3 7/18/07	BDL	BDL	BDL	0.56	BDL	BDL	BDL	8.32
4.4 7/18/07	1.15	0.63	BDL	0.76	BDL	BDL	BDL	15.09
4.5 7/18/07	0.54	2.04	0.59	2.32	BDL	BDL	BDL	20.66
5.1 7/18/07	BDL	5.48	1.18	6.52	0.67	0.90	BDL	49.81
5.2 7/18/07	BDL	BDL	BDL	BDL	BDL	BDL	BDL	8.48
5.3 7/18/07	7.29	0.56	BDL	0.72	BDL	BDL	BDL	10.77
5.4 7/18/07	1.41	6.02	1.24	7.22	0.70	0.94	0.50	63.42
6.1 7/18/07	1.03	0.73	0.38	1.23	BDL	BDL	BDL	10.60
6.2 7/18/07	BDL	3.14	0.74	3.55	BDL	0.62	BDL	33.58
6.3 7/18/07	BDL	BDL	BDL	0.60	BDL	BDL	BDL	17.16
6.4 7/18/07	0.60	2.14	0.59	2.51	BDL	BDL	BDL	32.22

6.5 7/18/07	0.57	0.87	0.39	1.05	BDL	BDL	BDL	16.06
7.1 7/18/07	BDL	1.13	0.41	1.30	BDL	BDL	BDL	12.02
7.2 7/18/07	BDL	3.66	0.81	4.03	BDL	BDL	BDL	33.17
7.3 7/18/07	BDL	10.95	2.05	12.71	0.97	1.38	0.57	84.89
7.4 7/18/07	BDL	7.53	1.49	8.78	0.80	1.07	0.54	62.92
7.5 7/18/07	BDL	BDL	BDL	BDL	BDL	BDL	BDL	8.83
8.1 7/18/07	BDL	BDL	BDL	BDL	BDL	BDL	BDL	7.57
8.2 7/18/07	BDL	BDL	BDL	BDL	BDL	BDL	BDL	10.73
8.3 7/18/07	BDL	2.07	0.56	2.30	BDL	BDL	BDL	20.55
8.4 7/18/07	BDL	14.02	2.59	16.47	1.19	1.65	0.68	127.04
8.5 7/18/07	BDL	1.09	0.43	1.27	BDL	BDL	BDL	21.89
1.3 6/27/08	BDL	0.59	BDL	0.75	BDL	BDL	BDL	15.28
1.4 6/27/08	BDL	1.00	0.40	1.27	BDL	BDL	BDL	24.81
1.5 6/27/08	BDL	1.39	0.47	1.89	BDL	BDL	BDL	21.34
3.1 6/27/08	BDL	2.88	0.73	3.24	BDL	BDL	BDL	33.48
3.3 6/27/08	BDL	1.79	0.55	2.06	BDL	BDL	BDL	25.74
3.4 6/27/08	BDL	1.29	0.45	1.52	BDL	BDL	BDL	21.70
4.2 6/27/08	BDL	0.84	BDL	1.04	BDL	BDL	BDL	14.14
4.3 6/27/08	12.78	2.05	0.58	2.38	BDL	BDL	BDL	23.01
4.4 6/27/08	BDL	1.52	0.50	1.81	BDL	BDL	BDL	24.34
4.5 6/27/08	BDL	1.14	0.45	1.44	BDL	BDL	BDL	31.94

5.1 6/27/08	BDL	BDL	BDL	0.68	BDL	BDL	BDL	17.13
5.2 6/27/08	0.78	0.84	BDL	1.01	BDL	BDL	BDL	16.21
5.3 6/27/08	BDL	2.25	0.60	2.55	BDL	BDL	BDL	28.88
5.4 6/27/08	BDL	2.09	0.59	2.34	BDL	BDL	BDL	33.04
5.5 6/27/08	BDL	0.79	BDL	0.96	BDL	BDL	BDL	32.66
6.3 6/27/08	BDL	0.64	BDL	0.80	BDL	BDL	BDL	15.03
6.4 6/27/08	BDL	BDL	BDL	0.70	BDL	BDL	BDL	22.14
6.5 6/27/08	BDL	0.60	BDL	0.79	BDL	BDL	BDL	38.33
7.1 6/27/08	BDL	1.00	0.38	1.17	BDL	BDL	BDL	18.14
7.2 6/27/08	BDL	0.83	BDL	0.99	BDL	BDL	BDL	12.15
7.3 6/27/08	BDL	1.94	0.54	2.20	BDL	BDL	BDL	34.33
7.4 6/27/08	BDL	3.59	0.84	3.99	BDL	0.64	BDL	39.66
7.5 6/27/08	BDL	1.22	0.43	1.45	BDL	BDL	BDL	17.45
8.2 6/27/08	BDL	BDL	BDL	0.52	BDL	BDL	BDL	10.83
8.3 6/27/08	BDL	0.74	BDL	1.01	BDL	BDL	BDL	15.87
8.4 6/27/08	BDL	BDL	BDL	BDL	BDL	BDL	BDL	12.91
1.1 7/31/09	BDL	1.96	0.42	2.18	BDL	BDL	BDL	34.51
1.2 7/31/09	BDL	BDL	BDL	0.58	BDL	BDL	BDL	15.68
1.3 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	10.57
1.4 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	12.01
1.5 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	9.90

2.1 7/31/09	BDL	0.85	BDL	1.03	BDL	BDL	BDL	23.02
2.3 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	9.38
3.1 7/31/09	BDL	0.71	BDL	0.89	BDL	BDL	BDL	14.41
3.2 7/31/09	BDL	BDL	BDL	0.55	BDL	BDL	BDL	13.91
3.3 7/31/09	BDL	1.54	BDL	1.76	BDL	BDL	BDL	29.70
3.4 7/31/09	BDL	BDL	BDL	0.65	BDL	BDL	BDL	13.89
3.5 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	10.31
4.1 7/31/09	BDL	1.20	BDL	1.37	BDL	BDL	BDL	20.15
4.2 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	10.19
4.3 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	27.13
4.5 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	23.62
5.1 7/31/09	BDL	1.08	BDL	1.29	BDL	BDL	BDL	34.24
5.2 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	31.16
5.3 7/31/09	BDL	0.87	BDL	1.05	BDL	BDL	BDL	36.53
6.1 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	11.55
6.2 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	15.33
6.3 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	13.85
6.4 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	15.17
6.5 7/31/09	BDL	0.70	BDL	0.86	BDL	BDL	BDL	27.24
7.2 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	11.80
7.3 7/31/09	BDL	BDL	BDL	0.68	BDL	BDL	BDL	18.72

7.4 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	15.98
7.5 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	12.17
8.1 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	13.43
8.2 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	15.62
8.3 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	18.34
8.4 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	15.78
8.5 7/31/09	BDL	BDL	BDL	BDL	BDL	BDL	BDL	14.02

Appendix J – Congener contribution to the total concentration to each large-scale field sampled in 2009.

% Cong	**BDE 28**	**BDE 47**	**BDE 100**	**BDE 99**	**BDE 154**	**BDE 153**	**BDE 183**	**BDE 209**
ZC8	5.89	5.76	3.92	5.16	6.12	6.33	5.10	61.72
ZH12	1.44	5.12	0.96	6.09	1.50	1.55	1.25	82.08
ZK3	0.52	3.24	1.20	6.69	0.71	0.76	0.45	86.42
ZK5	1.24	4.09	1.34	7.37	1.29	1.33	1.07	82.26
SI2	0.99	3.74	0.66	5.60	1.03	1.07	0.86	86.05
SI4	1.06	3.83	1.04	5.85	1.11	1.14	0.92	85.04
SK2	0.26	4.15	1.23	5.67	0.27	0.28	0.23	87.90
SK1	0.36	5.22	1.51	7.07	0.55	0.72	0.31	84.27
SC11	2.86	2.80	1.90	2.50	2.97	3.07	2.48	81.42
SH10	2.45	3.37	1.63	3.84	2.55	2.63	2.12	81.41
SH15	1.00	3.48	0.90	4.55	1.03	1.07	0.86	87.10
SJ4	0.22	1.47	0.38	2.29	0.23	0.23	0.19	94.99
ZB5	2.17	3.87	1.44	5.14	2.26	2.33	1.88	80.91
ZI6	5.52	5.40	3.68	11.07	5.74	5.93	4.79	57.87
MA2	0.39	2.61	0.99	4.90	0.41	0.42	0.34	89.94
MA3	0.10	6.02	1.92	9.05	0.95	1.06	0.08	80.81
MA10	0.33	3.17	1.32	4.67	0.34	0.35	0.29	89.53
MD1	0.10	3.21	1.10	4.91	0.61	0.61	0.09	89.39

MD2	0.09	1.21	0.46	1.81	0.13	0.09	0.07	96.14
MG2	0.24	4.64	1.44	6.34	0.38	0.38	0.21	86.38
MG1E	0.34	4.02	1.13	5.17	0.77	0.76	0.29	87.53
MG1B	0.15	5.99	1.68	8.18	0.62	0.81	0.13	82.44
MH6C	0.08	1.96	0.65	3.11	0.26	0.28	0.07	93.60
MH5C	0.11	2.66	0.87	4.16	0.45	0.50	0.10	91.14
MI5	2.07	4.13	1.38	4.42	2.15	2.22	1.79	81.85
MI11	3.34	3.27	2.22	2.93	3.47	3.59	2.89	78.29
MI12	1.62	4.24	1.08	6.76	1.68	1.74	1.40	81.47

Appendix K – Congener concentration of soils collected in large-scale fields that never received biosolids application.

Concentration (µg/kg)	**BDE 28**	**BDE 47**	**BDE 100**	**BDE 99**	**BDE 154**	**BDE 153**	**BDE 183**	**BDE 209**
ZC8-1	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
ZC8-2	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
ZC8-3	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
ZC8-4	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
ZC8-5	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
ZC8-7	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
ZC8-8	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
ZC8-9	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
ZH12-1	BDL	1.15	BDL	1.51	BDL	BDL	BDL	24.03
ZH12-2	BDL	3.11	BQL	3.79	BDL	BDL	BDL	45.51
ZH12-3	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
ZH12-4	BDL	BQL	BDL	BQL	BDL	BDL	BDL	12.14
ZH12-5	BDL	BDL	BDL	BQL	BDL	BDL	BDL	BQL
ZK3-1	BDL	BDL	BDL	1.35	BDL	BDL	BDL	BQL
ZK3-2	BDL	BQL	BQL	2.47	BDL	BDL	BDL	22.32
ZK3-3	BDL	BQL	BQL	2.22	BDL	BDL	BDL	54.44
ZK3-4	BDL	6.44	2.17	11.42	1.22	1.42	BDL	125.83
ZK3-5	BDL	5.32	1.69	8.36	BQL	BQL	BDL	97.57
ZK3-6	BDL	1.52	0.79	3.09	BDL	BDL	BDL	75.09
ZK3-7	BDL	BDL	BDL	BQL	BDL	BDL	BDL	10.66

ZK3-8	BDL	BQL	BDL	1.08	BDL	BDL	BDL	BQL
ZK3-9	BDL	1.52	BQL	3.09	BDL	BDL	BDL	42.86
ZK3-10	BDL	1.59	0.82	3.41	BQL	BQL	BDL	40.12
ZK5-1	BDL	1.21	0.91	2.61	BDL	BDL	BDL	36.95
ZK5-2	BDL	3.36	BDL	6.63	BQL	BDL	BDL	65.39
ZK5-3	BDL	BDL	BDL	BQL	BDL	BDL	BDL	BQL
ZK5-4	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
ZK5-5	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
ZK5-6	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Appendix L – Congener concentration of soils collected in large-scale fields that received a single biosolids application.

Concentration (µg/kg)	**BDE 28**	**BDE 47**	**BDE 100**	**BDE 99**	**BDE 154**	**BDE 153**	**BDE 183**	**BDE 209**
SI2-1	BDL	2.38	BQL	3.22	BDL	BDL	BDL	44.43
SI2-2	BDL	1.38	BQL	1.93	BDL	BDL	BDL	20.97
SI2-3	BDL	BQL	BDL	1.08	BDL	BDL	BDL	20.36
SI2-4	BDL	BQL	BDL	BQL	BDL	BDL	BDL	13.86
SI4-1	BDL	BQL	BDL	BQL	BDL	BDL	BDL	BQL
SI4-2	BDL	1.95	BQL	2.57	BDL	BDL	BDL	26.73
SI4-3	BDL	3.55	0.91	4.45	BDL	BDL	BDL	55.45
SI4-4	BDL	BQL	BDL	1.27	BDL	BDL	BDL	21.72
SI4-5	BDL	BQL	BDL	1.35	BDL	BDL	BDL	22.68
SI4-6	BDL	BQL	BDL	BQL	BDL	BDL	BDL	14.66
SI4-7	BDL	BQL	BDL	BQL	BDL	BDL	BDL	14.00
SI4-8	BDL	1.38	BQL	2.26	BDL	BDL	BDL	25.66
SK2-1	BDL	2.21	0.93	3.23	BQL	BDL	BDL	73.40
SK2-2	BDL	8.49	2.15	11.36	BQL	BQL	BDL	99.44
SK2-3	BDL	5.86	1.58	7.82	BQL	BQL	BDL	184.34
SK2-4	BDL	3.40	1.05	4.01	BQL	BDL	BDL	75.78
SK2-5	BDL	2.70	1.02	4.57	BQL	BQL	BDL	47.05
SK1-1	BDL	1.23	BQL	1.94	BDL	BDL	BDL	24.47
SK1-2	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
SK1-3	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

SK1-4	BDL	9.59	2.38	12.41	BQL	1.23	BDL	138.24
SK1-5	BDL	3.56	1.70	6.22	BQL	BQL	BDL	71.91
SK1-6	BDL	9.12	2.40	11.50	1.27	1.25	BDL	159.57
SK1-7	BDL	5.03	1.34	6.84	BQL	BQL	BDL	69.66
SC11-1	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
SC11-2	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
SC11-3	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
SC11-4	BDL	BDL	BDL	BQL	BDL	BDL	BDL	12.69
SC11-5	BDL	BDL	BDL	BQL	BDL	BDL	BDL	12.23
SC11-6	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BQL
SC11-7	BDL	BQL	BDL	BDL	BDL	BDL	BDL	18.68
SC11-8	BDL	BDL	BDL	BQL	BDL	BDL	BDL	BDL
SC11-9	BDL	BDL	BDL	BQL	BDL	BDL	BDL	15.00
SH10-1	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BQL
SH10-2	BDL	BQL	BDL	BQL	BDL	BDL	BDL	BQL
SH10-3	BDL	1.20	BQL	1.84	BDL	BDL	BDL	31.16
SH10-4	BDL	BDL	BDL	BQL	BDL	BDL	BDL	BQL
SH10-5	BDL	BDL	BDL	BQL	BDL	BDL	BDL	BQL
SH10-6	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BQL
SH10-7	BDL	BDL	BDL	BQL	BDL	BDL	BDL	15.32
SH10-8	BDL	BQL	BDL	BQL	BDL	BDL	BDL	14.86
SH15-1	BDL	1.57	BQL	2.04	BDL	BDL	BDL	22.40
SH15-2	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BQL
SH15-3	BDL	BQL	BDL	BQL	BDL	BDL	BDL	18.76
SH15-4	BDL	BQL	BDL	BQL	BDL	BDL	BDL	16.67
SH15-5	BDL	1.19	BDL	1.49	BDL	BDL	BDL	29.39
SH15-6	BDL	3.40	0.81	4.18	BDL	BDL	BDL	65.35
SH15-7	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BQL
SH15-8	BDL	BQL	BDL	1.23	BDL	BDL	BDL	33.30
SH15-9	BDL	1.48	BQL	1.88	BDL	BDL	BDL	34.43
SJ4-1	BDL	4.88	1.52	7.74	BQL	BQL	BDL	103.83
SJ4-2	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
SJ4-3	BDL	1.35	BQL	2.19	BDL	BDL	BDL	325.08
SJ4-4	BDL	2.06	BQL	3.33	BDL	BDL	BDL	487.67
SJ4-5	BDL	2.38	0.84	3.63	BDL	BDL	BDL	53.67
SJ4-6	BDL	3.45	1.04	4.69	BDL	BDL	BDL	39.10
SJ4-7	BDL	1.28	BQL	1.91	BDL	BDL	BDL	62.73
SJ4-8	BDL	BQL	BDL	1.04	BDL	BDL	BDL	12.33

SJ4-9	BDL	1.38	BQL	2.30	BDL	BDL	BDL	39.20
SB5-1	BDL	BQL	BDL	1.11	BDL	BDL	BDL	29.10
SB5-2	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
SB5-3	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
SB5-4	BDL	BQL	BDL	BQL	BDL	BDL	BDL	BQL
SB5-5	BDL	1.67	BQL	1.97	BDL	BDL	BDL	23.15
SB5-6	BDL	BDL	BDL	BQL	BDL	BDL	BDL	BQL
SI6-3	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BQL
SI6-4	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
SI6-5	BDL	BQL	BDL	1.22	BDL	BDL	BDL	BQL

Appendix M – Congener concentration of soils collected in large-scale fields that received multiple biosolids applications.

Concentration (µg/kg)	**BDE 28**	**BDE 47**	**BDE 100**	**BDE 99**	**BDE 154**	**BDE 153**	**BDE 183**	**BDE 209**
MA2-1	BDL	2.23	1.01	3.91	BDL	BDL	BDL	72.73
MA2-2	BDL	1.32	BQL	1.99	BDL	BDL	BDL	32.71
MA2-3	BDL	1.04	BQL	1.90	BDL	BDL	BDL	44.94
MA2-4	BDL	3.04	1.17	5.20	BQL	BQL	BDL	87.85
MA2-5	BDL	1.90	0.86	3.27	BDL	BDL	BDL	42.52
MA2-6	BDL	4.10	1.44	7.17	BQL	BQL	BDL	95.90
MA2-7	BDL	BQL	BQL	1.98	BDL	BDL	BDL	87.22
MA2-8	BDL	BQL	BQL	1.35	BDL	BDL	BDL	33.42
MA2-9	BDL	3.07	1.33	5.69	BQL	BQL	BDL	98.54
MA3-1	BDL	26.58	7.23	35.48	3.41	3.74	BQL	287.71
MA3-2	BDL	7.72	3.90	16.62	2.04	2.21	BDL	166.24
MA3-3	BDL	28.64	8.10	39.05	3.91	4.44	BQL	378.10
MA3-4	BDL	9.07	3.47	15.76	1.83	2.06	BDL	139.61
MA3-5	BDL	17.73	5.98	27.99	2.97	3.32	BDL	232.31
MA10-1	BDL	BQL	BQL	1.25	BDL	BDL	BDL	40.19
MA10-2	BDL	2.99	1.29	4.17	BQL	BQL	BDL	63.57
MA10-3	BDL	2.80	1.16	3.75	BQL	BQL	BDL	76.97
MA10-4	BDL	2.32	1.04	3.07	BDL	BDL	BDL	67.31
MA10-5	BDL	3.40	1.46	5.22	BQL	BQL	BDL	83.38

MA10-6	BDL	3.79	1.54	5.89	BQL	BQL	BDL	108.84
MA10-7	BDL	3.67	1.32	5.05	BQL	BQL	BDL	58.63
MA10-8	BDL	2.10	0.88	2.86	BDL	BDL	BDL	40.26
MA10-9	BDL	3.88	1.51	5.76	BQL	BQL	BDL	186.67
MA10-10	BDL	2.35	1.13	3.65	BQL	BQL	BDL	54.39
MD1-2	BDL	11.73	3.54	16.64	1.90	1.90	BDL	306.21
MD1-3	BDL	9.35	3.07	14.43	1.72	1.75	BDL	213.66
MD1-5	BDL	6.45	2.80	11.04	1.58	1.54	BDL	247.35
MD2-1	BDL	5.27	1.95	7.99	BQL	BQL	BDL	385.53
MD2-3	BDL	3.10	1.25	4.57	BQL	BQL	BDL	146.93
MD2-4	BDL	2.60	1.09	3.99	BQL	BQL	BDL	146.06
MD2-5	BDL	2.97	1.31	4.84	BQL	BQL	BDL	161.86
MD2-6	BDL	1.68	0.89	2.89	BDL	BDL	BDL	29.68
MG2-1	BDL	2.33	1.20	4.12	BQL	BQL	BDL	39.01
MG2-2	BDL	3.82	1.20	5.53	BQL	BQL	BDL	60.21
MG2-3	BDL	4.14	1.38	5.65	BQL	BQL	BDL	90.07
MG2-4	BDL	6.86	2.16	9.05	BQL	BQL	BDL	118.72
MG2-5	BDL	5.67	1.76	8.17	BQL	BQL	BDL	123.93
MG2-6	BDL	7.17	1.96	9.36	BQL	BQL	BDL	137.76
MG2-7	BDL	8.87	2.43	11.26	1.35	1.31	BDL	154.44
MG1E-2	BDL	BQL	BDL	BQL	BDL	BDL	BDL	25.74
MG1E-3	BDL	1.46	BQL	1.88	BDL	BDL	BDL	45.31
MG1E-4	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BQL
MG1E-5	BDL	8.56	2.51	11.14	1.37	1.35	BDL	153.64
MG1B-2	BDL	6.44	2.09	10.04	BQL	1.23	BDL	104.12
MG1B-3	BDL	23.74	5.86	31.83	2.64	3.14	BQL	242.19
MG1B-4	BDL	6.24	2.08	8.26	BQL	BQL	BDL	114.25
MG1B-5	BDL	8.43	2.50	11.08	1.38	1.36	BDL	156.40
MH6C-1	BDL	3.57	1.21	5.61	BQL	BQL	BDL	314.85
MH6C-2	BDL	3.63	1.32	5.75	BQL	BQL	BDL	354.05
MH6C-3	BDL	4.92	1.90	9.01	BQL	BQL	BDL	276.08
MH6C-4	BDL	4.83	1.63	7.99	BQL	BQL	BDL	192.60
MH6C-5	BDL	10.12	3.25	15.55	1.62	1.78	BDL	322.00
MH6C-6	BDL	7.92	2.79	12.30	1.53	1.54	BDL	335.48
MH6C-7	BDL	11.91	3.49	18.26	1.81	2.09	BQL	449.88
MH5C-1	BDL	9.30	2.90	15.02	1.56	1.78	BDL	444.54
MH5C-2	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BQL
MH5C-3	BDL	1.47	0.82	2.48	BDL	BDL	BDL	51.25

MH5C-4	BDL	9.16	2.63	13.96	1.36	1.57	BDL	176.69
MH5C-5	BDL	7.76	2.70	11.85	1.50	1.59	BDL	275.11
MI5-1	BDL	BDL	BDL	BQL	BDL	BDL	BDL	BQL
MI5-2	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
MI5-3	BDL	BDL	BDL	BQL	BDL	BDL	BDL	BQL
MI5-4	BDL	BDL	BDL	BDL	BDL	BDL	BDL	27.87
MI5-5	BDL	1.75	BQL	2.06	BDL	BDL	BDL	19.98
MI11-1	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BQL
MI11-2	BDL	BQL	BDL	BQL	BDL	BDL	BDL	14.19
MI11-3	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BQL
MI12-1	BDL	BQL	BDL	1.38	BDL	BDL	BDL	21.22
MI12-2	BDL	1.12	BQL	1.62	BDL	BDL	BDL	18.70
MI12-3	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BQL
MI12-4	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BQL
MI12-5	BDL	1.79	BQL	2.49	BDL	BDL	BDL	26.27

Appendix N – Picture for the project of bioavailabity of DDT, DDE, DDD, and dieldrin.

Figure N.1 – Bulk soil collection at Henry A. Wallace Beltsville Agricultural Research Center, Maryland, USA.

Figure N.2 – Soil sieving and native earthworm collection.

Figure N.3 – Earthworms in soil from pots.

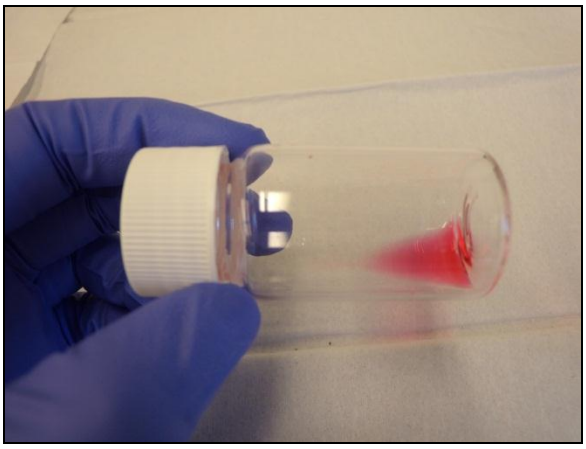


Figure N.4 – Scintillation vial with EVA solution.

Appendix O – Polymer coated vial preparation and extraction schedule.

Orchard Soil Experiment

Week day	Prepare on	Week day	Extract on	Incubation time	# incubation
Tuesday	08/02/11	Tuesday	08/02/11	30 minutes	1
Tuesday	08/02/11	Tuesday	08/02/11	1 hour	2
Tuesday	08/02/11	Tuesday	08/02/11	2 hours	3
Tuesday	08/02/11	Tuesday	08/02/11	4 hours	4
Tuesday	08/02/11	Tuesday	08/02/11	8 hours	5
Monday	08/01/11	Tuesday	08/02/11	1 day	6
Monday	08/01/11	Wednesday	08/03/11	2 days	7
Monday	08/01/11	Friday	08/05/11	4 days	8
Monday	08/01/11	Tuesday	08/09/11	8 days + blanks	9
Monday	08/01/11	Tuesday	08/16/11	15 days	10
Monday	08/15/11	Friday	09/09/11	25 days	11
Wednesday	08/03/11	Wednesday	09/07/11	35 days	12
Monday	08/01/11	Thursday	09/15/11	45 days + blanks	13

4-mo. Compost Experiment

Week day	Prepare on	Week day	Extract on	Incubation time	# incubation
Tuesday	08/02/11	Tuesday	08/02/11	30 minutes	1
Tuesday	08/02/11	Tuesday	08/02/11	1 hour	2
Tuesday	08/02/11	Tuesday	08/02/11	2 hours	3
Tuesday	08/02/11	Tuesday	08/02/11	4 hours	4
Tuesday	08/02/11	Tuesday	08/02/11	8 hours	5
Monday	08/01/11	Tuesday	08/02/11	1 day	6
Monday	08/01/11	Wednesday	08/03/11	2 days	7
Monday	08/01/11	Friday	08/05/11	4 days	8
Monday	08/01/11	Tuesday	08/09/11	8 days + blanks	9
Monday	08/01/11	Tuesday	08/16/11	15 days	10
Monday	08/15/11	Friday	09/09/11	25 days	11
Wednesday	08/03/11	Wednesday	09/07/11	35 days	12
Monday	08/01/11	Thursday	09/15/11	45 days + blanks	13

2-yr. Compost Experiment

Week day	Prepare on	Week day	Extract on	Incubation time	# incubation
Wednesday	08/03/11	Wednesday	08/03/11	30 minutes	1
Wednesday	08/03/11	Wednesday	08/03/11	1 hour	2
Wednesday	08/03/11	Wednesday	08/03/11	2 hours	3
Wednesday	08/03/11	Wednesday	08/03/11	4 hours	4
Wednesday	08/03/11	Wednesday	08/03/11	8 hours	5
Monday	08/01/11	Tuesday	08/02/11	1 day	6
Monday	08/01/11	Wednesday	08/03/11	2 days	7
Monday	08/01/11	Friday	08/05/11	4 days	8
Monday	08/01/11	Tuesday	08/09/11	8 days + blanks	9
Monday	08/01/11	Tuesday	08/16/11	15 days	10
Monday	08/15/11	Friday	09/09/11	25 days	11
Wednesday	08/03/11	Wednesday	09/07/11	35 days	12
Monday	08/01/11	Thursday	09/15/11	45 days + blanks	13

Biosolids Compost Experiment

Week day	Prepare on	Week day	Extract on	Incubation time	# incubation
Thursday	08/04/11	Thursday	08/04/11	30 minutes	1
Thursday	08/04/11	Thursday	08/04/11	1 hour	2
Thursday	08/04/11	Thursday	08/04/11	2 hours	3
Thursday	08/04/11	Thursday	08/04/11	4 hours	4
Thursday	08/04/11	Thursday	08/04/11	8 hours	5
Monday	08/01/11	Tuesday	08/02/11	1 day	6
Monday	08/01/11	Wednesday	08/03/11	2 days	7
Monday	08/01/11	Friday	08/05/11	4 days	8
Monday	08/01/11	Tuesday	08/09/11	8 days + blanks	9
Monday	08/01/11	Tuesday	08/16/11	15 days	10
Monday	08/15/11	Friday	09/09/11	25 days	11
Wednesday	08/03/11	Wednesday	09/07/11	35 days	12
Monday	08/01/11	Thursday	09/15/11	45 days + blanks	13

CHAR Experiment

Week day	Prepare on	Week day	Extract on	Incubation time	# incubation
Thursday	08/04/11	Thursday	08/04/11	30 minutes	1
Thursday	08/04/11	Thursday	08/04/11	1 hour	2
Thursday	08/04/11	Thursday	08/04/11	2 hours	3
Thursday	08/04/11	Thursday	08/04/11	4 hours	4
Thursday	08/04/11	Thursday	08/04/11	8 hours	5
Monday	08/01/11	Tuesday	08/02/11	1 day	6
Monday	08/01/11	Wednesday	08/03/11	2 days	7
Monday	08/01/11	Friday	08/05/11	4 days	8
Monday	08/01/11	Tuesday	08/09/11	8 days + blanks	9
Monday	08/01/11	Tuesday	08/16/11	15 days	10
Monday	08/15/11	Friday	09/09/11	25 days	11
Wednesday	08/03/11	Wednesday	09/07/11	35 days	12
Monday	08/01/11	Thursday	09/15/11	45 days + blanks	13

Appendix P – Ions monitored for chromatographic analysis.

Compound	Ions
Dieldrin	79, 263, 277
4,4’-DDT	235, 237, 165, 199
4,4’-DDE	318, 246, 248, 176
4,4’-DDD	235, 237, 165, 199
$^{13}C_{12}$4,4’-DDT	258, 330, 260, 188

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