ASSESSMENT OF THE BIOAVAILABILITY OF PAHS IN SOIL BY EARTHWORMS AND PASSIVE SAMPLING DEVICES

By

JASON BLAYNE WELLS

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Dean of the Graduate College

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NOMENCLATURE

CBR	critical body residue
FID	flame ionization detection
GC	gas chromatograph
HPLC	high performance liquid chromatograph
K_{OW}	octanol-water partition coefficient
LC_{50}	median lethal concentration
LD_{50}	medial lethal dose
MW	molecular weight
PAHs	polycyclic aromatic hydrocarbons
PHE	phenanthrene
PSD	passive sampling device
SPE	solid phase extraction
SPMD	semipermeable membrane device
SPME	solid phase micro-extraction fiber
UV/VIS	ultra-violet/visible light detection

CHAPTER 1

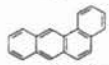
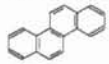
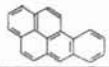
INTRODUCTION

Sources, Distribution, and Properties of PAHs

Increasing environmental pollution is an important issue especially with the growing industrialization of human society. Industries, such as petrochemical refineries and coal burning electric plants, may generate many types of wastes including a group of environmental contaminants known as polycyclic aromatic hydrocarbons (PAHs). These contaminants are ubiquitously distributed across the globe and are a part of our natural environment (Wagrowski and Hites 1997, Utvik and Johnsen 1999). PAHs are found in crude oil, used motor oil, soot, smoke, creosote, and even in charbroiled steaks (Agency for Toxic Substances and Disease Registry 1996; Connell et al. 1997; Van Brummelen 1995).

Environmental contamination by PAHs stems from the incomplete combustion (pyrogenic) of organic matter at high temperatures and from petrochemical (petrogenic) releases (Lane 1988). During combustion of organic matter (> 500° C), some of the C-C and C-H bonds are broken to form free radicals. In the absence of oxygen, these fragments will react with other fragments around them forming more complex fragments leading to PAHs. The structural makeup of PAHs typically consists of molecules containing two or more fused aromatic rings. Physical properties of these compounds exhibit low water solubility (non-polar) with an affinity for fatty tissues (Table 1). Aqueous solubility and low vapor pressures are important factors influencing environmental chemical activity (fugacity).

Table 1. Molecular structures and physical-chemical values for selected PAHs. K_{OW} is the octanol-water partition coefficient, which is calculated as the ratio of a compound dissolved in the organic phase to the aqueous phase at equilibrium. MW=Molecular weight.

Compound	Molecular Structure[1]	MW[2]	Log K_{OW}[2]	Water Solubility[2] [mg/L]	Vapor Pressure[2] [Pa]
Naphthalene		128.18	3.3	30	1.04×10^{1}
Phenanthrene		178.24	4.46	1.29	2.27×10^{-2}
Anthracene		178.24	4.45	7.5×10^{-2}	1.44×10^{-3}
Pyrene		202.26	5.18	1.35×10^{-1}	3.30×10^{-4}
Benz[a]anthracene		228.30	5.61	1.0×10^{-2}	1.47×10^{-5}
Chrysene		228.30	5.61	2.0×10^{-3}	6.00×10^{-7}
Benzo[a]pyrene		252.32	6.5	3.8×10^{-3}	6.67×10^{-7}

1 ISIS™ Draw 2.1.3d. 1997. MDL Information Systems, Inc.
2 Mode of values at 25 °C, Mackay et al. 1992.

The octanol/water partition coefficient (K_{ow}) mimics how nonpolar organic chemicals partition to animal tissues. It has been used to determine bioaccumulation potential in aquatic and terrestrial animals (Belfroid et al. 1995; Belfroid et al. 1996). K_{ow} values of PAHs (log K_{ow} 3 to 7.6) suggest tendencies to dissolve in nonpolar solvents more readily than into polar solvents. Lipophilicity of these compounds greatly increases with molecular weight, thus higher molecular weight PAHs would be expected to bioaccumulate in animal adipose tissues and bind to organic matter in soil and sediments, persisting in the environment for extended periods (Utvik and Johnsen 1999).

Bioavailability and Toxicity of PAHs to Soil-dwelling Organisms

In order for a toxicant to exert an effect on an organism, the toxicant must pass from the environment into the organism across cell membranes, regardless of route of uptake. Of the three principal mechanisms by which toxicants cross cell membranes (passive diffusion, facilitated diffusion and active diffusion), passive diffusion is the predominant mechanism for PAHs due to high K_{ow} values (Connell et al. 1997). Primary routes of uptake for soil-dwelling organisms are a) inhalation of soil air, b) absorption across the skin, and c) ingestion of contaminated soil or food. In order for a toxicant to enter an organism, the toxicant must be "bioavailable", or not bound to soil particles or be sterically hindered (too large) from entering cells. Many definitions of bioavailability exist. This concept is described as a measure by which a contaminant in a source has the potential for entry into or onto a human or ecological receptor and be able to react with its metabolic system. Bioavailability of a contaminant is specific to the receptor, route of entry, duration of exposure, and the matrix in which it is contained (Newman and Jagoe 1994).

Bioavailability Modifying Factors

Total chemical concentrations of PAHs in soil may be assessed by using vigorous non-polar solvent extraction techniques. These total chemical values have little relevance to biological observations in soil and sediment systems (Landrum et al. 1997, Conder and Lanno 2000). Constituents of soil and sediment (e.g., humic substances) coupled with soil pore-spaces of varying sizes and shapes compete for binding of lipophilic contaminants (Carmichael et al. 1997; Burgos et al. 1999). Soil matrices can be highly

variable in composition and can be referred to as a heterogeneous mixture of components. Unlike water, soil heterogeneity may not be predicted or modeled efficiently. This heterogeneous mixture of soil components coupled with environmental factors such as temperature, moisture content, and organic matter content makes toxicity prediction very difficult for risk assessments in soil systems (Lanno and McCarty 1997). These environmental factors are considered modifying factors of bioavailability. A modifying factor is any abiotic or biotic factor responsible for contributing to the altered effect of an ambient environmental contaminant concentration. Thus, total chemical concentrations are not appropriate estimates of exposure when accounting for the influence of so many modifying factors. Hypothetically, a sandy soil contaminated by PAHs containing little organic matter or humic substances may have a total chemical concentration of 850 mg/kg (dry soil wt.) and a bioavailable concentration (labile) of 600 mg/kg. A clay-based soil with copious amounts of organic matter and humic substances also having a total chemical concentration of 850 mg/kg may have a bioavailable portion of only 50 mg/kg.

A species of soil-dwelling organism may have a threshold exposure dose of only 200 mg/kg PAH bioavailable fraction (dry soil wt.) before a biological impact is observed. Organisms living in the sandy soil may be exposed to the toxicant in a bioavailable form at a concentration of 600 mg/kg. These organisms would exceed their threshold (via passive diffusion across the gradient) for these compounds and an effect would be observed. Organisms living in the clay soil (50 mg/kg bioavailable exposure) wouldn't exceed their threshold and no effect would be observed.

Toxicity tests adopted from traditional toxicity evaluations have been applied to environmental problems with the assumption that total chemical concentrations in the

environment (soil or water) is the dose and the observed effect is correlated to that measured dose (Spurgeon and Hopkin 1995). This information, combined with toxicological pathways for humans, has been used to develop clean-up guidelines for risk based corrective action (RBCA) following releases of contaminants to the environment (ASTM 1995; ASTM 1998). Ecologically relevant concentrations that include estimates of bioavailability have not been implicitly considered for risk assessments.

Some soil toxicity tests use a standardized artificial soil (Spurgeon and Hopkin 1995; ASTM 1997). This technique may be used to test responses of earthworms to various concentrations of single or multiple contaminants. Standardization of test protocols is done to make observations reproducible and data from multiple investigations comparable. By observing and sampling test organisms at prescribed intervals (e.g., hourly, daily, geometric series), important observations may be made regarding organism responses with contaminant kinetics to determine relative potency and efficacy of those contaminants. Commonly, exposure dose is equated with exposure concentration. By using whole body or specific tissue residues of soil-dwelling organisms, exposure dose can be more accurately assessed. Critical body residues (CBRs) are whole body or specific tissue residues of (a) contaminant(s) from an exposed organism correlated with a measurable ecologically relevant response from that organism (Fitzgerald et al. 1996). Commonly used organism responses may include the induction of a metabolic response, reduced growth, reduced fecundity, or mortality.

Surrogates for the Estimation of Bioavailability

Bioavailability of contaminants may be determined using CBR data obtained from organisms exposed in field soil as well as artificial soil. By definition, bioavailability can be assessed only in living organisms (Weston and Mayer 1998a; Weston and Mayer 1998b; Van Brummelen et al. 1998). Weak chemical extractions have been used with limited success as a surrogate for bioavailability of PAHs (Kelsey et al. 1997, Tang and Alexander 1999). Problems associated with weak chemical extractions are that the solvent extraction systems are either analyte or medium-specific and often not well correlated with biological responses or bioaccumulation. Passive sampling devices (PSDs) that are biomimetic may be used in place of living organisms. PSDs are specific to classes of contaminants (organic vs. inorganic, polar vs. non-polar) but not specific to individual contaminants within a class. The ability of PSDs to predict toxicity could be used as a screening tool to assess the efficacy of bioremediation, which could replace conventional chemical and biological testing methods.

Two new technologies being investigated for use as PSDs are semipermeable membrane devices (SPMDs) and solid phase microextraction (SPME) fibers (Figs 1 & 2). SPMDs are constructed of lay-flat low-density polyethylene (LPDE) dialysis tubing filled with a known volume or mass of material suitable for sequestration of lipophilic contaminants. The dialysis tubing has been shown to efficiently mimic the function of the bipolar lipid membrane in biological systems in the uptake of lipophilic environmental contaminants. Media used in SPMDs for storage of contaminants have been neutral lipid (triolein), hexane, and C18 sorbent. The triolein-filled SPMDs are most commonly used due to the similarities with the neutral lipids found in many

organisms. Residue analysis is followed by dialysis of the sampler in a nonpolar solvent (e.g. n-hexane).

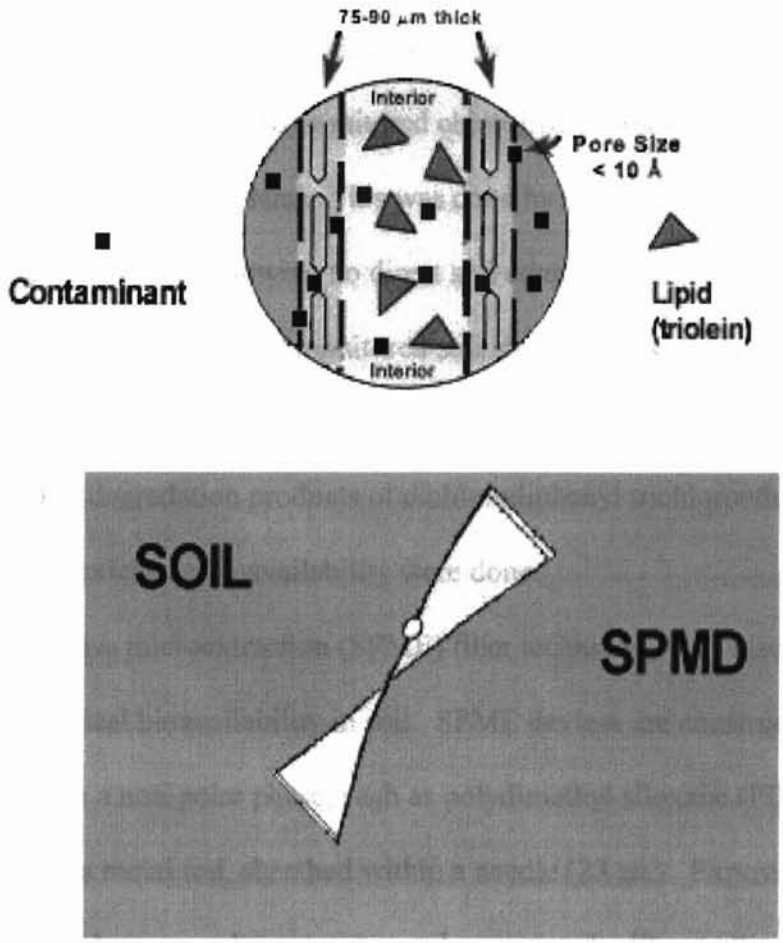


Fig 1 – Semipermeable membrane device (SPMD) with cut-away view of membrane (adapted from Huckins et al. 1999).

SPMDs have been used to detect and accumulate lipophilic contaminants from aquatic and atmospheric media (Petty et al. 1993, Huckins et al. 1996). The assessment of bioavailability of organic lipophilic contaminants has been very effective in aquatic and sediment systems, but the application to soil systems has been pursued with limited

success. At least two investigative applications of SPMDs to terrestrial monitoring have been conducted. Strandberg et al. (1997) exposed SPMDs directly in compost fortified with various congeners of PCBs. Uptake of PCBs by SPMDs was measured, but no relationships with toxicity or bioavailability were established.

Rantalainen et al. (1998) monitored chlorohydrocarbons (CHCs) in contaminated lake-shore soil in Central Finland. This was done by burying SPMDs enclosed in a stainless steel mesh tube (allowing no direct soil contact) along 350 m of contaminated lake shoreline. This experiment monitored soil air for semi-volatile organic contaminants such as hexachlorobenzene, various congeners of polychlorinated biphenyls (PCBs), and DDE and DDD (degradation products of dichlorodiphenyl trichloroethane - DDT). No correlations to toxicity or bioavailability were done.

Solid phase microextraction (SPME) fiber technology may also be useful in estimating chemical bioavailability in soil. SPME devices are constructed of an optical fiber coated with a non-polar phase, such as polydimethyl siloxane (PDMS). The coated fiber is fused to a metal rod, sheathed within a needle (23 ga.). Exposure is accomplished by depressing a plunger on the apparatus and exposing the fiber to the sample medium. The fiber adsorbs analytes by diffusion from the substrate by exploiting the fugacity, (tendency for a chemical to escape from one phase into another) of the compounds of interest. This sampling technology has the benefit of no liquid chemical extractions or concentration procedures with the ability to derivatize samples as collected. Analysis is most often done by conventional gas chromatography (GC) using a specialized narrow bore (0.75 mm ID) inlet liner (Lord and Pawliszyn 1998). The protective sheath needle is inserted into the heated injection port of the GC and the optical fiber deployed for

thermal desorption of analytes. Desorption times vary from seconds to minutes depending on the fiber coating and analyte of interest. The analytes condense on the cooler analytical column and separation is done by conventional thermal gradient programming.

Fig 2 - Solid phase micro-extraction fiber assembly with sample vial and magnetic stir bar.

Sampling has been effective in aquatic, atmospheric, tissue, and soil systems. Currently direct contact measurements using SPME fibers have only been made for aquatic and atmospheric media. Soil and tissues have been sampled by equilibrium headspace analysis only. The direct measurement of soil contaminants has been investigated with limited success (Parkerton and Stone 1998).

The idea behind using a passive sampler is that it would mimic the uptake by soil dwelling organisms. The PSD would take up PHE from the soil similarly to earthworms but there is one major difference in that PSDs do not metabolize contaminants. This aspect of using PSDs is attractive for monitoring acutely toxic levels of soil contaminants. A threshold value may be determined by correlating residues from the PSDs and those from earthworms at mortality. This could be done by assessing the mean fresh mass of a species of soil dwelling organism combined with its LD_{50} and confidence limits (or a fraction of the LD_{50}) for a chemical or class of chemicals. The result would be the number of molecules on average that would be responsible for a 50% probability (or a fraction of probability) of mortality for that species in that fresh mass range. A

number of molecules of chemical could be collected by a passive sampling device and at steady state be compared to the number of molecules required to kill a group of animals.

These proposed surrogates for soil-dwelling organisms could be exploited as inexpensive and rapid screening tools for the determination of toxicity soils contaminated by PAHs or other lipophilic toxicants. SPMDs and SPME fibers are attractive alternatives to using living organisms for many reasons (Table 2). The principles involved could be used to reduce cleanup costs and time spent remediating areas impacted by organic lipophilic contaminants.

Table 2. Comparison of using living organisms and passive sampling devices in laboratory and field conditions.

	Ease of Sampler Identification	Ease of Sampler Deployment	Ease of Sampler Retrieval	Integrative sampler	Variation among samplers
Organism					
Earthworm					
Lab	Easy	Easy	Easy	Yes	Moderate
Field	Difficult	Difficult	Difficult		
PSDs[1]					
SPMD[2]					
Lab	Easy	Easy	Easy	Yes	Low
Field	Easy	Moderate	Moderate		
SPME[3]					
Lab	Easy	Easy	Easy	No	Low
Field	Easy	Easy	Easy		

1 PSD = Passive Sampling Devices
2 SPMD = Semi-permeable Membrane Device
3 SPME = Solid-phase Microextraction Fiber

The experimental approach for this project was to conduct two exposures of earthworms and PSDs (i.e.., SPMDs and SPMEs) to phenanthrene-spiked artificial soil. Tests were conducted in a laboratory setting where modifying factors such as temperature

and moisture were controlled. The purpose for Experiment I was to derive a LC_{50} for phenanthrene (PHE) toxicity (mortality by non-polar narcosis) to *Eisenia fetida* and determine if SPMDs would take up PHE from soil and to what degree. The purpose for Experiment II was to determine how modifications to physical and chemical attributes affect PHE bioavailability (i.e., changing organic matter content) and to compare uptake by SPMDs and SPME fibers. Relationships between chemical residues in PSDs and earthworms were examined to determine if PSDs could be used as surrogate measures to estimate bioavailability and predict toxicity of nonpolar organic chemicals in soil. My hypotheses are:

1) Passive sampling devices will take up a portion of the total amount of nonpolar organic contaminant present in soil. The fraction of contaminant taken up by PSDs will better approximate that fraction available for biological uptake relative to the total chemical fraction. Correlations of PHE residues from PSDs and earthworms with nominal chemical concentrations in artificial soil were used to test this hypothesis.

2) Alteration of organic matter content level in soil will change bioavailability of organic contaminants in soil. This hypothesis was tested using PHE-spiked artificial soil with 1% and 10% peat as organic matter while maintaining a sand to clay ratio (3.45:1) and relative free water content (125 % of field moisture-holding capacity by dry weight) between soil types. PSDs and earthworms were exposed to various concentrations in both organic matter treatments to assess PHE uptake and biological responses.

CHAPTER 2

METHODS AND MATERIALS

Experiment I: *Eisenia fetida* Phenanthrene LC_{50} Determination and Phenanthrene Uptake by SPMDs

Earthworms were exposed simultaneously and in parallel with SPMDs in this experiment. Treatments for the earthworm assay included six soil concentrations (0.56, 1.01, 1.80, 3.14, 5.61, and 8.64 mmol PHE/kg dry wt soil) of phenanthrene (98 %, Aldrich) with an acetone (HPLC grade, EM Science) solvent control using three replicates per treatment. This range was established to encompass the predicted LC_{50} from a previous rangefinder assay (LeBlanc 1997, unpublished data). Treatments for the SPMD uptake assay included an acetone solvent control and 3.14 mmol PHE/kg soil with two replicates per treatment.

Experimental Organisms

Earthworms *Eisenia fetida* were originally obtained from Granny's Hillside Farms, Gore, OK, and cultured in antibiotic-free composted horse manure (24-27 °C) at Oklahoma State University-Ecotoxicology and Water Quality Research Laboratory (OSU-EWQRL). Bedding moisture was maintained by spraying weekly with reconstituted moderately hard water.

Passive Sampling Devices (SPMDs)

Semipermeable membrane devices (SPMDs, 2.54 cm x 15.24 cm, 0.167 g triolein, Environmental Sampling Technologies (EST), St. Joseph, MO) were received from the manufacturer in hexane-rinsed, sealed tin cans. One SPMD was deployed per 200 g (dry soil) replicate.

Artificial Soil Preparation

Artificial soil used in this experiment was composed (% dry weight) of 69% silica sand (60 mesh, Blasting Specialties, Tulsa, OK), 20% pulverized EPK Kaolin clay (Tomorrow's Treasures, OKC, OK), 10% *Sphagnum* peat (passed through a 2-mm sieve, Wal-Mart, Stillwater, OK), and 1% calcium carbonate ($CaCO_3$, Fisher Scientific). Each soil treatment was prepared in batch series.

Before the preparation of artificial soil, the water content of the sieved peat was determined by placing three peat samples (~10 g each) into a drying oven (overnight 105 °C, Topp 1993), with measurements of the weights before and after drying. The amount of fresh sieved peat used to prepare the artificial soil was adjusted for moisture content. Sand was weighed into shallow Pyrex® pans (33 cm x 27 cm x 5 cm) and spiked with the appropriate spiking solution of PHE dissolved in acetone. Following the spiking procedure, acetone was allowed to evaporate from the sand overnight in a dark fume hood until the sand was dry. All artificial soil components were combined (200 g total dry wt.) and placed into cleaned, acetone-rinsed 500-ml glass jars and fitted with screw top lids. Contents of the test containers were thoroughly mixed using a rotary mixer (25 rpm) for 1 hour. Ventilation holes (1-2 mm dia.) were made in the lids with an ice pick.

Hydration of the soil was achieved by the addition of reagent grade water (RGW, pH 7.0, resistivity-18 MΩ) to 35 % of the dry weight and mixing by hand using acetone-rinsed stainless steel utensils until the soil was uniform in color and texture. Temperature and moisture equilibration of the test containers was for 24 h in a Percival Scientific® environmental chamber (24 ± 1 °C in continuous light) before the addition of earthworms or PSDs.

Following the completion of the test, soil moisture (loss by evaporation overnight in a drying oven at 105 °C, Topp 1993), and pH (10 g dry wt soil/ 20 ml 0.01M $CaCl_2$, Hendershot et al. 1993) were evaluated.

Earthworm Toxicity Test

Toxicity tests were conducted according to the Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation Tests With Lumbricid Earthworm *Eisenia fetida* (ASTM 1997) with minor modifications. Two hundred and ten (210) clitellate adult earthworms were collected by hand-sorting from a mass culture and placed on moistened filter paper to allow depuration of gut contents for 24 h (24 ± 1 °C). Earthworms were randomly assigned in groups of 10 worms per replicate test container, weighed, and placed in test containers after test soil equilibration was complete. Observations for mortality were made following a geometric time scale (i.e., 2, 4, 8, 16 to 24 h) then in 24 h intervals to 336 h. Earthworms determined to be dead (no response from stimulation with blunt probe) were removed from the containers and rinsed with RGW, wrapped in n-hexane-rinsed aluminum foil, sealed in a ziplock bag and frozen

(-20 °C). Any earthworms remaining alive at end of the test were sacrificed and frozen as above.

Semipermeable Membrane Device (SPMD) Uptake Test

Deployment

Two modes of deployment were used in this portion of the assay. Static deployment involved burying the SPMD in the soil with no disturbances throughout the exposure period. A second deployment involved burying the SPMD in the soil for 48 h, then removing the SPMD from the container, mixing the soil to renew the contact gradient, and reburying the SPMD. This procedure was repeated in 48 h intervals.

Collection of SPMDs from spiked soil-containers was done after 8, 16, 32, and 64 days of exposure for static deployments (n=2 for each sampling period) and after 8, 16, and 32 days of exposure for turned deployments (n=2 for each sampling period). After removal from the soil, each SPMD was rinsed with RGW to remove any debris, sealed in its original can, and frozen (-20° C) until dialysis. Trip blank, manufacture blank, and spiked recovery SPMDs were used. Trip blanks were opened at various intervals during deployment to account for any volatile contaminants taken up by SPMDs. Manufacturer blanks were not opened until dialysis. Spiked recovery was done by injecting 100 µl of PHE certified standard (Chem Service) through the membrane and resealing the membrane. No references could by found regarding the direct application of SPMDs to artificial soil.

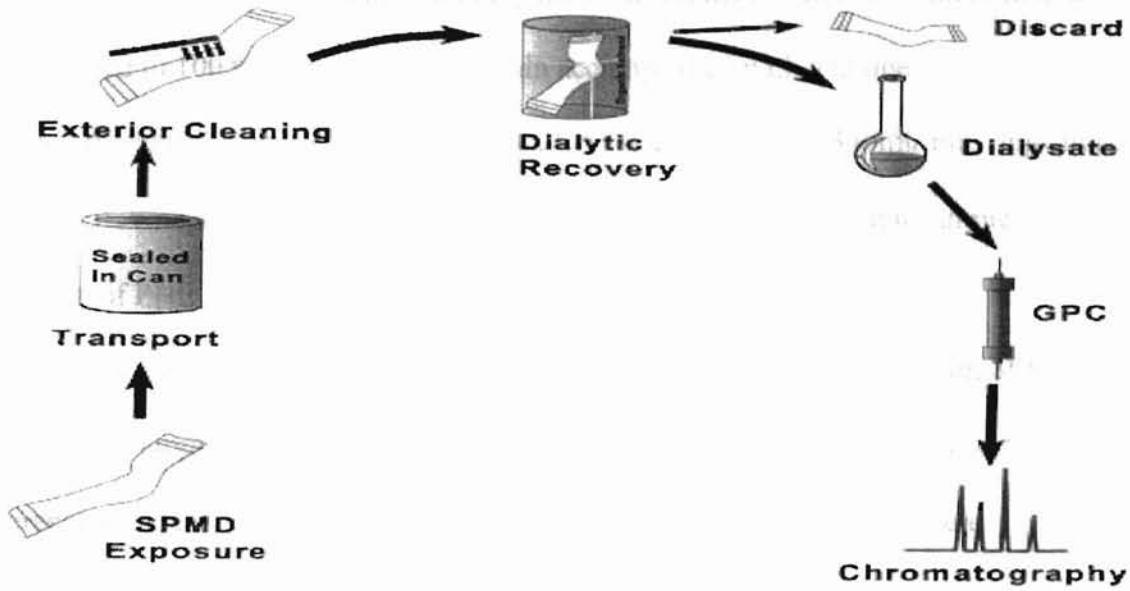


Fig 3. Dialysis procedure for semipermeable membrane devices (SPMDs) used by EST (from Huckins et al. 1999). SPMDs are cleaned by scrubbing with a nylon brush, rinsed with 2N HNO_3, and dialyzed with n-hexane for 48 h with solvent replacement after 24 h. Combined dialysates were condensed using Kaderna-Danish condenser and Snyder column assemblies. Automated gel-permeation cleanup was used to enrich the dialysates. Cleaned extracts were then sealed in 5-ml ampules for shipment.

Dialysis and Residue Analysis

SPMDs were sent by courier to Environmental Sampling Technologies (EST) for dialysis and gel permeation cleanup of extracts (Fig 3). SPMD extracts were received from EST in 5-ml ampules. The samples were quantitatively transferred to 15-ml graduated centrifuge tubes (Baxter, ± 0.05 ml) and adjusted to analytical volume. Dialysates were analyzed for phenanthrene residues using high performance liquid chromatography (HPLC). A Supelcosil LC-PAH (Supelco), 5 cm x 4.6 mm ID x 3 μm reversed-phase analytical column was used at ambient temperature for PHE quantification. Acetonitrile (Optima grade, Fisher Scientific) and RGW were used as the

mobile phase in a gradient mixture initially 60:40 acetonitrile:water, 0.3 min hold and then ramped to 100 % acetonitrile at 4.0 min accomplished with a Dionex GP-50 quaternary pump (Dionex Corp., Sunnyvale, CA) at a flow rate of 3.0 ml/min. Injections were 25 µL with a Dionex LC-10 autoinjector from a Dionex AS-40 autosampler and detection of PHE using a Waters™ 484 deuterium arc lamp UV/VIS detector. Integrations were carried out at 254 nm wavelength with an optical bandwidth of 8nm. Peaks from samples were identified by comparison of retention times with a certified reference standard (Chem Service, F81MS, 100 µg/mL). Concentrations were determined by peak area from standard curves. PHE calibration standards were made fresh for each analysis run from stock dissolved in acetonitrile. Calibration standards were checked using the certified reference standard. Results were expressed as mass PHE/ SPMD surface area. Residues were not corrected for 80% spike recovery. All data collection and chromatogram analysis was done using Dionex Peaknet® software ver. 4.2 and 5.1 (Dionex 1995-1999).

Experiment II:
Earthworm Toxicity and PSD Uptake Comparison
Artificial Soil Organic Matter (OM) Alteration Test
(1% Peat vs. 10% Peat)

Range-finder Toxicity Test

The range-finder test for the high organic matter (10% OM) soil was composed of three nominal concentrations of PHE (0.31, 3.1, and 6.3 mmol PHE/kg) and a negative control, while the low organic matter (1% OM) soil was composed of three nominal concentrations (0.031, 0.31, and 3.1 mmol PHE/kg) and acetone solvent control. Ten

earthworms per test container (in duplicate) for each exposure concentration were used. Observations for mortality were done at 1, 2, 4, 8, and 16 days. The range-finder toxicity test was done to improve planning for the definitive toxicity test.

Test Design

The design from Experiment I was followed with minor exceptions. Soil components and *E. fetida* were from the same sources and lots as in Experiment I. Earthworms were exposed simultaneously and in parallel to semipermeable membrane devices (SPMDs) during this experiment.

Exposures involved *E. fetida*, semipermeable membrane devices (SPMDs), and soil only (used for physical/chemical analyses and solid-phase microextraction (SPME) fiber determinations). Treatments involved high and low organic matter artificial soil with three nominal concentrations (0.31, 2.05, and 3.1 mmol/kg dry wt. soil) of PHE with an acetone solvent control (in triplicate) for each exposure regime. This range was established to further encompass the predicted LC_{50} of PHE for *E. fetida* from Experiment I and the range-finder test in Experiment II. Durations of exposure (for treatments other than control) were 0, 2, 4, 8, and 16 d for *E. fetida* and soil only exposures and 0, 4, 8, 16, and 32 d for the SPMD exposure. Exposure durations for the control soil containers were 0 and 16 d for the three exposure regimes.

Passive Sampling Devices

Semi-permeable Membrane Devices

A smaller version of the SPMD (2.54 cm x 5.08 cm, 0.0556 g triolein, Environmental Sampling Technologies (EST), St. Joseph, MO) was received from the manufacturer in hexane-rinsed, sealed tin cans. One SPMD was deployed per 200 g (dry soil) replicate.

Solid-phase Microextraction Fibers

Solid phase microextraction fibers (SPME, 7μm polydimethyl siloxane (PDMS) with manual holders, Supelco. Bellefonte, PA) were used to assess uptake of phenanthrene directly from the aqueous phase of soil (diluted pore water). Ten SPME fiber assemblies were used in this study. Each determination was made using 0.500 g freeze-dried artificial soil.

Artificial Soil Preparation

The composition of the standardized artificial soil was the same as for Experiment I. Composition of the low organic matter artificial soil was 76.67 % silica sand, 22.23 % Kaolin clay, 1 % *Sphagnum* peat (2-mm sieved), and 0.1 % $CaCO_3$.

Each test container was prepared individually, yielding 258 independent experimental units. The appropriate amount of sand was measured into test containers and spiked from the appropriate spiking solution of phenanthrene dissolved in acetone. Acetone was allowed to evaporate overnight from the sand in a dark fume hood until dry. Once dry, all artificial soil components were combined into test containers, fitted with

screw top lids, and mixed end-over-end in a rotary mixer (25 rpm) for one hour. Moisture content for both soil types was to adjusted to 125% field moisture capacity by adding RGW and mixing until uniform in color and texture (using acetone rinsed stainless steel impeller, 5-cm dia., attached to ¾-hp drill press at 190 RPM). This was done to ensure that both soil types shared the same proportion of free soil water. Field capacity of both soil types was assessed by using the Standard Test Method for Capillary-Moisture Relationships for Coarse- and Medium-Textured Soils by Porous-Plate Apparatus (0.3 atmosphere positive pressure, ASTM 1994). This concept can be described as the maximum amount of water a soil will retain at one-third atmosphere of pressure after excess gravitational water has drained away and after the rate of downward movement of water has materially decreased (Cassel and Nielson 1986; Smith 1990). Artificial soil (saturated with RGW for 48 h) was placed into support rings on a primed ceramic pore plate within a pressure plate apparatus (Soil Moisture, Inc.). Pressure was applied (0.3 atm) and expelled water was collected for 36 h or until equilibrium was reached. The soil was removed and moisture content determined (percent difference by dry mass, Topp 1993). Moisture content for both soil types was increased to 125% of percent moisture at field capacity, as *E. fetida* were desiccating in both soil types at field capacity (from range-finder assay in Experiment II). The equilibration procedure, and physical/chemical analysis for the test soils was the same as for Experiment I.

Definitive Earthworm Toxicity and Bioavailability Test

Earthworms were treated as in Experiment I, with the exception that 12 earthworms were used per replicate in this assay and observations were made daily. Selection of earthworms for this assay was based on an initial minimum fresh mass of 250 mg without regard to sexual maturity. Exposed earthworms from the standard ASTM artificial soil (0.31 mmol PHE/kg) were removed from test containers (in triplicate) following a geometric time scale (2, 4, 8, 16 d) to determine residues in living organisms.

Earthworm Body Burden Analysis

Tissue Extraction

Baseline residue analysis was done using 12 unexposed earthworms from the OSU-EWQRL culture population. Exposed test earthworms were randomly selected for body burden analysis at each mortality event from each exposure concentration. Earthworms were removed from storage (-20 °C), thawed to room temperature, placed into scintillation vials, fresh mass recorded (±0.00005 g, Mettler-Toledo Model AT261, Columbus, Ohio) and freeze-dried for 12 h (<100 µg Hg, Lyph-Lock 12, Labconco). Dry mass of earthworms was calculated by difference. Individual, freeze-dried earthworms were then transferred to 10-ml Teflon Oakridge centrifuge tubes with sealing cap assemblies (VWR Scientific) and 3 ml of RGW was added. Mechanical tissue homogenization of the whole earthworm was conducted for two minutes (OMNI International, Warrenton, VA). Three (3) ml n-hexane (95%, Fisher Scientific) was

added to the homogenate and ultrasonic extraction was done for two minutes at 40% duty cycle (Fisher M300). Following sonication, rotary extraction was conducted for one hour in total darkness (Rotamix, ATR Inc. Laurel, MD, 40 rpm). Extracts were then centrifuged to separate hexane from RGW (Sorvall RC2-B Superspeed, 5000 g for 5 min.). The hexane layer was removed using n-hexane-rinsed Pasteur pipettes, and concentrated to 2.0 ml using a gentle stream of N_2 for silica-gel cleanup.

Cleanup and Sample Preparation

Solid phase extraction cartridges (SPE, 500 mg silica gel, 3-ml volume, BakerBond J.T.Baker, Phillipsburg, NJ) topped with a 1-cm layer of sodium sulfate (Na_2SO_4, Fisher Scientific) were used for cleanup of earthworm extracts. SPE columns were conditioned before use by rinsing with three column volumes of n-hexane. Following column conditioning, the sample was quantitatively transferred to the SPE column. Three column volumes of n-hexane were used to elute the sample from the SPE cartridge. The elution rate from the SPE column was set to 5 mL/min by adjusting vacuum pressure. Eluate containing PHE was collected in 40-ml glass, graduated centrifuge tubes (± 0.05 ml, Kontes), and concentrated to analytical volume using a gentle stream of N_2. One cartridge was used per sample.

Analysis

Earthworm whole body extracts were analyzed for phenanthrene residues using high performance liquid chromatography as above for SPMDs in Experiment I. Results were expressed as mass PHE/ earthworm fresh mass or surface area. Spiked-recovery was done by adding 100 µl PHE certified standard (Chem Service F81MS, 100 µg/ml) to

reference worms and homogenized and treated as above. Earthworm PHE spike recovery was 86.5% (95% CL = 14.14%) and earthworm residues were not corrected for recovery (Appendix C). Blank analyses were done to correct for any impurities encountered.

Passive Sampling Device Uptake and Analysis

Semi-permeable Membrane Devices

SPMDs were statically deployed in triplicate, simultaneously and in parallel to the earthworm toxicity and bioavailability test. Manufacturer blanks and spiked recovery (100 µl injection o-terphenyl 2000 mg/L) SPMDs were also used. SPMDs were removed from respective containers after exposures of 4, 8, 16, and 32 d (n=3 at each time). Acetone control SPMDs were removed after 16 d (n=3). Residue analysis and SPMD treatment was as in Experiment I.

Solid-phase Microextraction Fiber

Freeze-dried, spiked artificial soil (0.500 g, collected at 2, 4, 8, 16 d post equilibration from the soil only exposure regime), 15 ml RGW vol., and a Teflon® coated magnetic stir bar (0.3 cm x 1.3 cm) were combined into screw top amber SPME vials (15 ml headspace with Teflon septum, Supelco). A ten-place magnetic stirrer (1200 RPM, IKA) was used with ten sample vials and ten SPME fiber assemblies to obtain steady-state data for PHE concentrations in soil suspensions. A support stand was constructed to hold ten SPME manual holders simultaneously during exposure. The needle of the SPME apparatus was inserted through the Teflon septum of the sample vial and the fiber was deployed. Each vial was aligned on the magnetic stirrer for optimum

stirring velocity (~1,000 rpm). Each SPME fiber was exposed until steady-state wasachieved (5 h). Steady state was determined by exposing SPME fibers to 1 mg PHE/L RGW over a geometric time interval (e.g., 0.5, 1, 2, 4, 8 and 16 h). No differences of PHE uptake existed between the 4, 8, and 16 h exposure ($p<0.05$).

Residue analysis was accomplished by conventional gas chromatography. A Tracor 565 gas chromatograph using flame ionization detection (GC-FID) with a megabore fused silica capillary column (DB-5, 30 m X 0.53 mm ID X 1.5 μm, J&W Scientific), 0.75-mm ID SPME-inlet liner (Supelco) and JADE septum-less injector with SPME adapter (0.56 mm ID, Alltec) was used to quantify extracts. Helium (High Purity, Sooner Airgas) was used as the carrier and makeup gas. The flow rate for the carrier gas was set to 35-cm/sec linear velocity make-up flow rate was set to 45 ml/min. Hydrogen (fuel for FID, High Purity, Sooner Airgas) flow rate was 35 ml/min and breathing air (oxidant, Grade D, Sooner Airgas) flow rate was 350 ml/min. The temperature program for direct injection GC analysis was: injection port temp-290 °C, detector temp-300 °C, initial oven temp-160 °C (5 minute hold) with 35 °C/min ramp to 210 °C (7 minute hold).

Each new fiber was conditioned (320 °C) for 4 h to remove any adhesive from the fiber as per instructions from the manufacturer. For each analysis, thermal desorption and conditioning of the SPME fiber was accomplished by exposing the fiber while inserted into the heated injection port (290 °C) of the GC for five minutes. This resulted in adequate desorption followed by blank analyses of each fiber to ensure no carry-over problems existed. SPME fiber performance was determined before and after each soil determination by measuring a reference standard solution (1 mg PHE /L RGW). Integration of peaks was done by external calibration using injections from phenanthrene

standards with certified PHE check standard (Chem Service, F81MS). Chromatogram data was collected and analyzed using PeakNet® chromatography software (version 5.1, Dionex 1999).

Statistical Analyses

Data interpretation was done using SAS® (Statistical Analysis System version 6.12, SAS Institute, Inc. 1989-1996), Origin® (version 6.0, Microcal™1991-1999), and Excel 2000 (version 9.0.2720, Microsoft® 1983-1999). Standard descriptive statistics, ANOVA, linear and non-linear regression techniques (SAS PROC REG) were used for SPME fibers and SPMDs. Earthworm body burden or dose for mortality was determined by SAS PROC PROBIT (Appendix B). Correlations were made between earthworm CBRs and SPME fiber determinations with nominal soil concentrations of phenanthrene using probit analysis (Origin®).

CHAPTER THREE

RESULTS

Experiment I

Earthworm Mortality Data

Occurrences of mortality events increased as nominal PHE soil concentrations and time increased (Fig 4). Time to 50% mortality decreased as exposure concentration increased. Physical-chemical data for the soil from Experiment I included pH and moisture content (Table 3).

Table 3. Selected physical-chemical data from Experiment I worm exposed soil at the termination of exposure. Values are expressed as mean ± sd.

Nominal Soil Concentration (mmol PHE/kg dwt)	pH[1]	% Moisture[2]
Control	6.28 (0.49)	15.37 (0.552)
0.56	7.37 (0.03)	17.43 (10.927)
3.1	6.54 (0.77)	16.84 (6.510)
8.6	6.94 (0.10)	27.52 (9.085)

1 Hendershot et al. 1993
2 Topp, GC 1993

Semipermeable Membrane Devices

Residues from static SPMDs ranged from 1.6×10^{-4} mmol PHE/cm^2 to 3.45×10^{-4} mmol PHE/cm^2. Residues increased over time with the exception of one data point influencing the quadratic curve fit demonstrated by $r^2 = 0.6471$ (Fig 5).

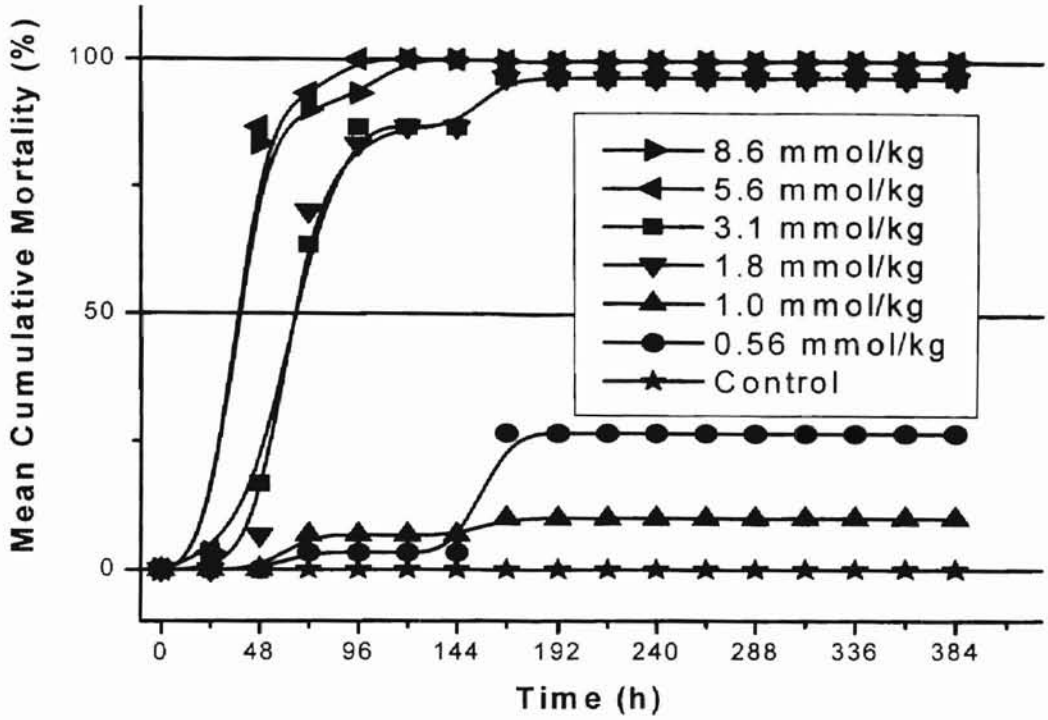


Fig 4. Cumulative mortality of earthworms from Experiment I. The predicted LC_{50} for PHE to *E. fetida* was between 1.0 and 3.1 mmol PHE/kg soil (dwt). The LC_{50} approximation was sufficient for the design of the toxicity test for Experiment II.

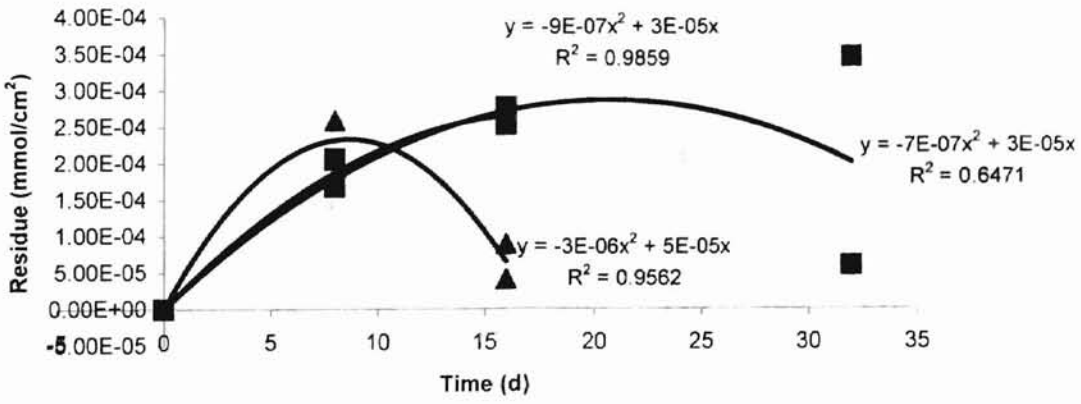


Fig 5. Non-linear fit plots of semipermeable membrane device uptake of PHE spiked artificial soil (3.1 mmol/kg dwt) over 32 d. Goodness and of fit and correlation for static deployment (■) SPMDs differed for 16 and 32 d. Uptake by turned SPMDs (▲) was similar to the static SPMDs at 8 d, then sharply declined by 16 d. No residues were detected for turned 32 d exposures.

Experiment II

Earthworm Mortality Data

The occurrences of mortality in the high (10%) SOM artificial soil (Fig 6) were comparable to those from Experiment I (Fig 4), but greatly different from the low (1%) organic matter soil toxicity test (Fig 7). Sigmoid curve fits were weighted to the standard error of the mean percent mortality at each observation period.

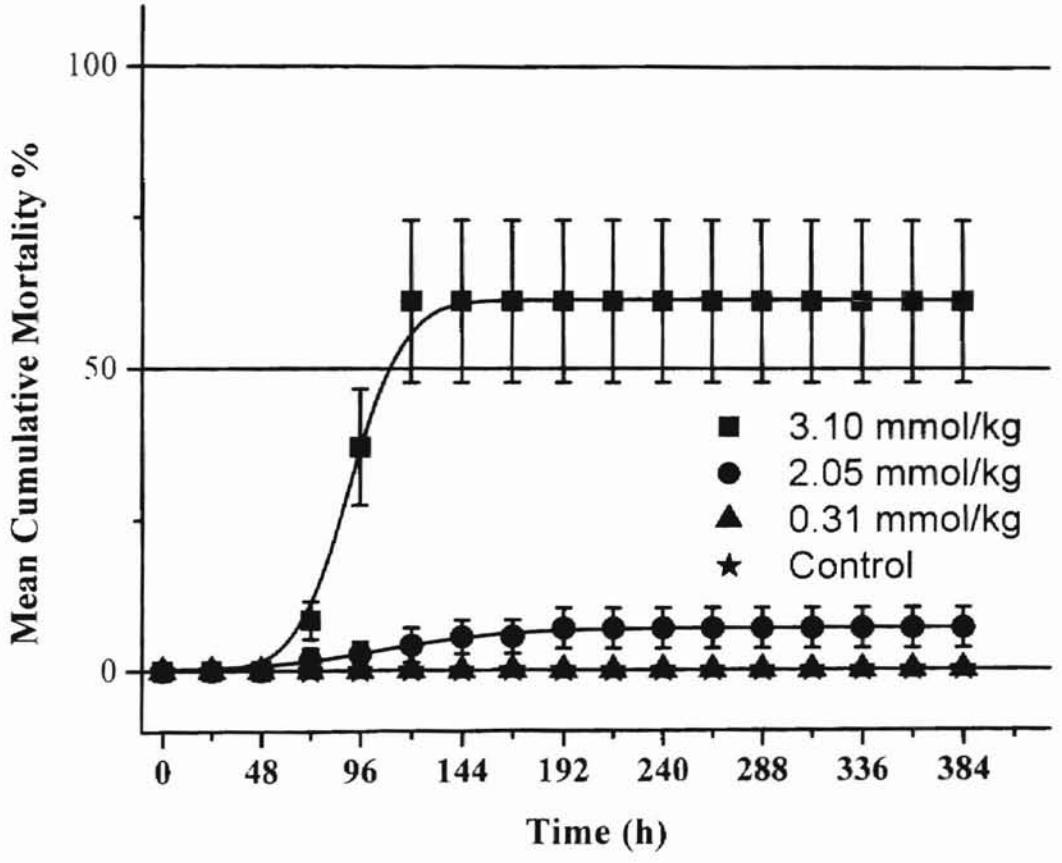


Fig 6. Effects of PHE on mortality of *E. fetida* exposed to several concentrations in an acute toxicity test in 10% OM artificial soil. Values are mean percent cumulative mortality ± SEM.

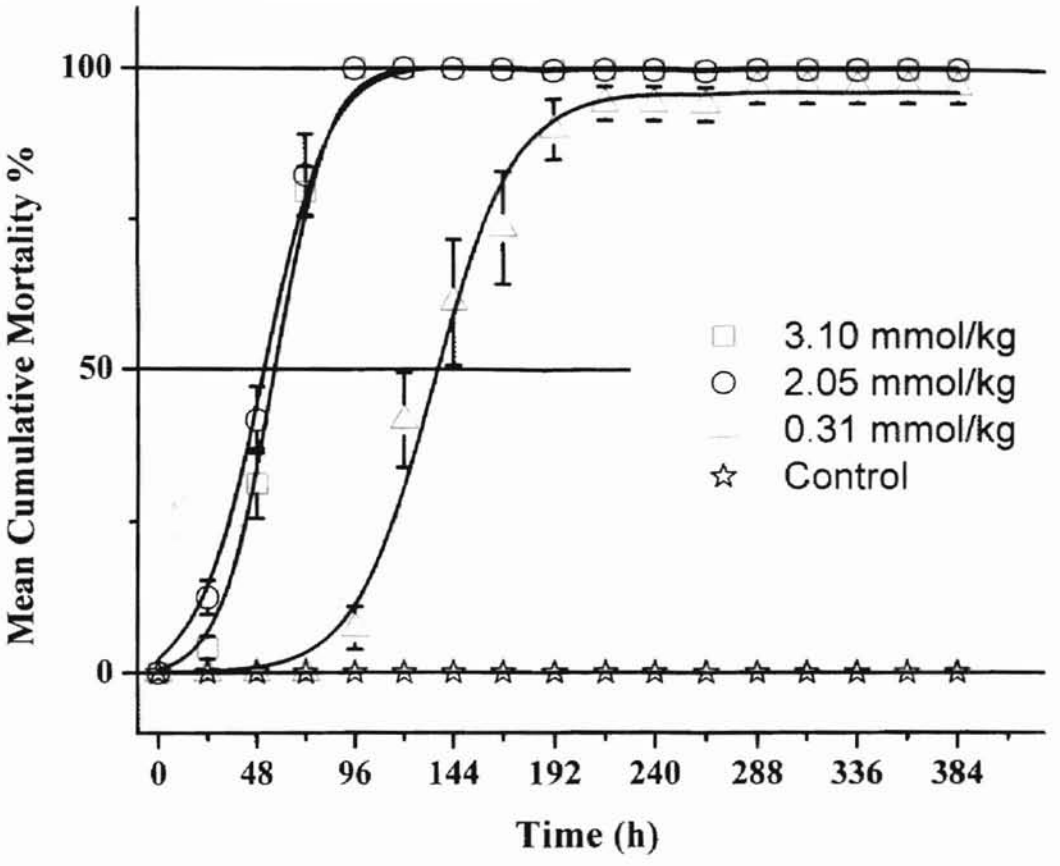


Fig 7. Effects of PHE on mortality of *E. fetida* exposed to several concentrations in an acute toxicity test in 1% OM artificial soil. Values are mean percent cumulative mortality ± SEM.

Critical Body Residue Analysis

Body burdens of PHE in background and control worms were below detection limits (nd < 7.01E-08 mmol PHE HPLC-UV/VIS). The body burden in earthworms increased with exposure concentration and time (Fig 8). CBR (mortality) for PHE was estimated to be > 0.24 mmol/kg wwt. Probit analysis (Fig 9) of residue data predicted the LD_{50} (PHE) = 0.114 mmol/kg wwt. (95% fiducial limits 0.072, 0.172). This was done using body burden data from alive and dead earthworms.

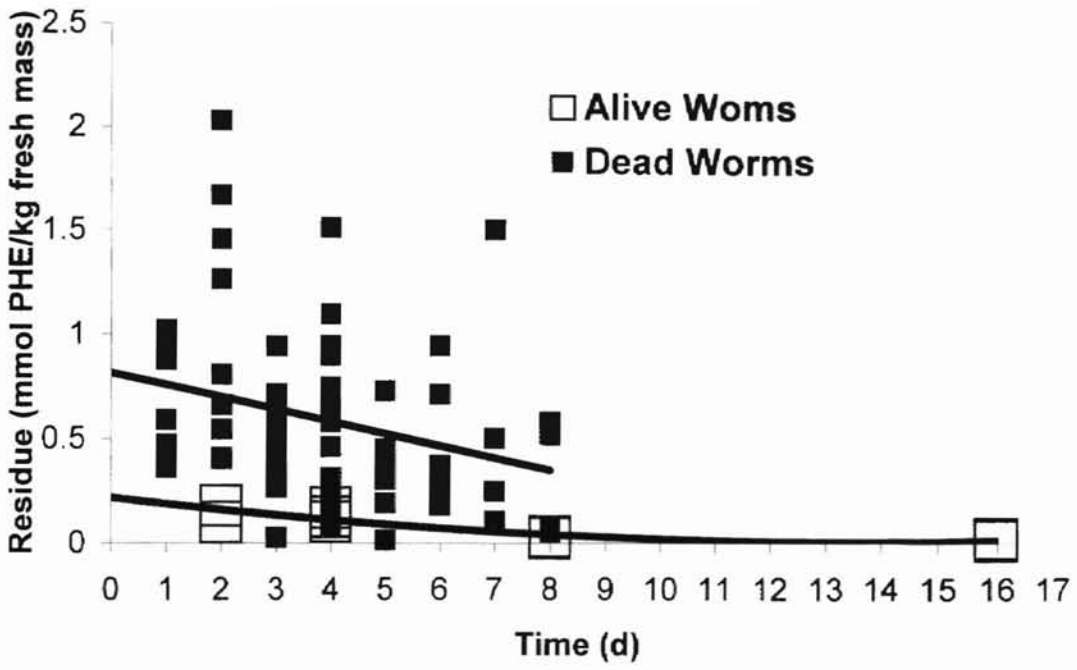


Fig 8. Body burden analysis of *E. fetida* exposed to PHE in artificial soil. Residues for alive worms were significantly different from residues found in dead worms ($p<0.05$).

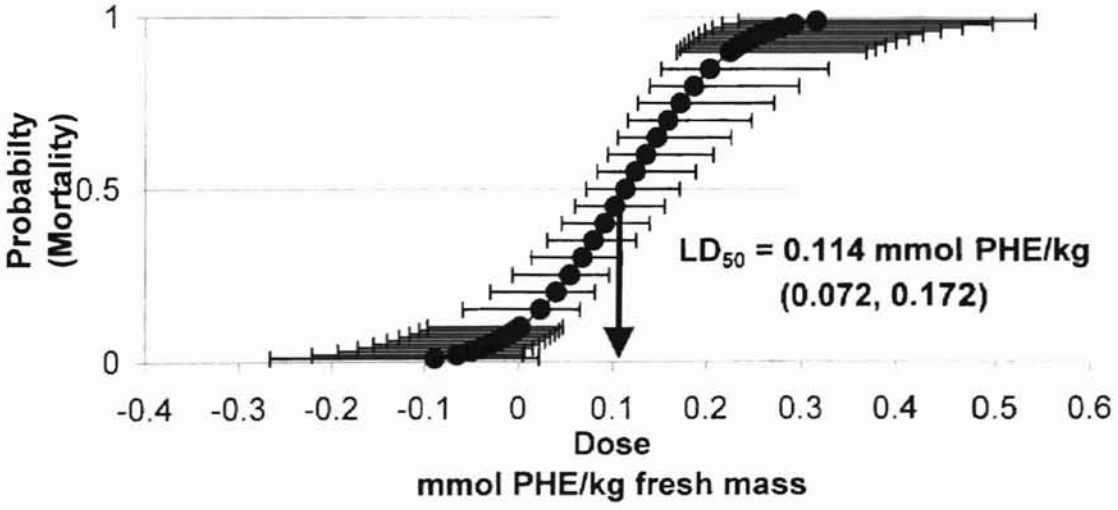


Fig 9. Probit analysis of PHE CBRs of E. fetida. LD_{50} = 0.114 mmol/kg fresh mass with 95% fiducial limits.

Variation of semipermeable membrane device uptake of PHE was acceptable for the 4, 8, and 16 d deployments. Variation among SPMDs for the 32 d exposure was not acceptable for exposure concentration 10% OM, 3.1 mmol/kg (Fig 10). SPMD variation appeared to increase as exposure duration increased. Steady state between SPMDs and soil was not confirmed due to high variation in the day thirty-two exposure period. This is important because the amount of PHE taken up by SPMDs cannot be directly compared if steady state does not exist.

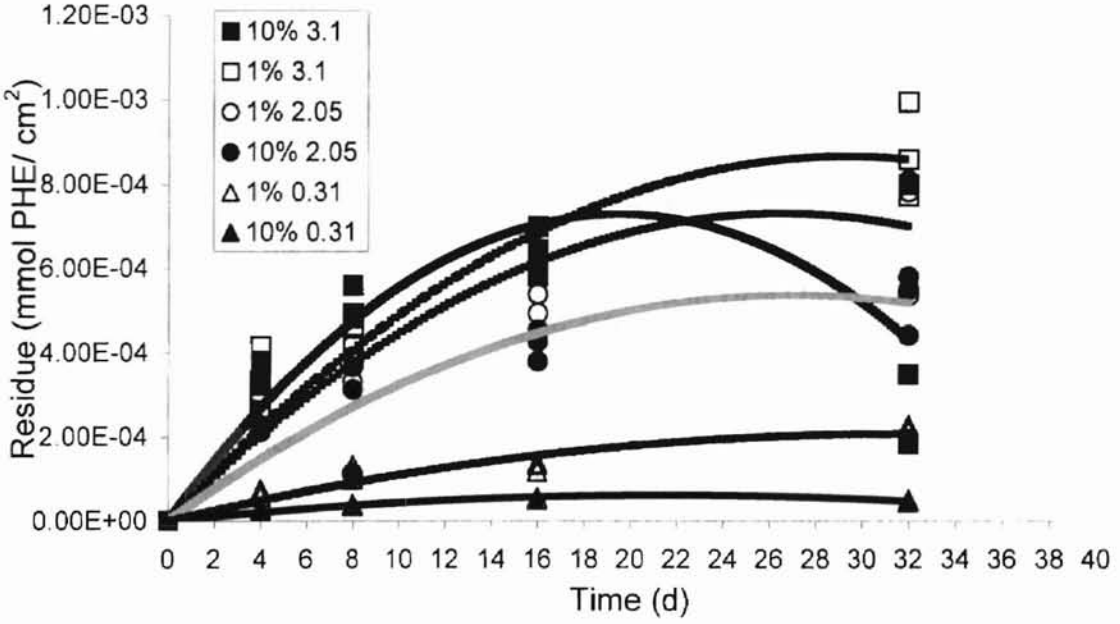


Fig 10. Semipermeable membrane device uptake of PHE over 32 d from Experiment II.

The rates of PHE uptake by SPMDs (slope) were investigated to determine differences between exposure soil type and concentration (Fig 11). The linear phase of uptake was determined by linear regression analysis (ANOVA) of data points through each cumulative time interval. The liner phase of PHE uptake was determined to be during the first eight days of exposure. No significant differences were detected between

slopes for common soil concentrations regardless of soil type. Slopes for SPMDs exposed to 1% and 10% OM, 0.31 mmol/kg were not significantly different from zero.

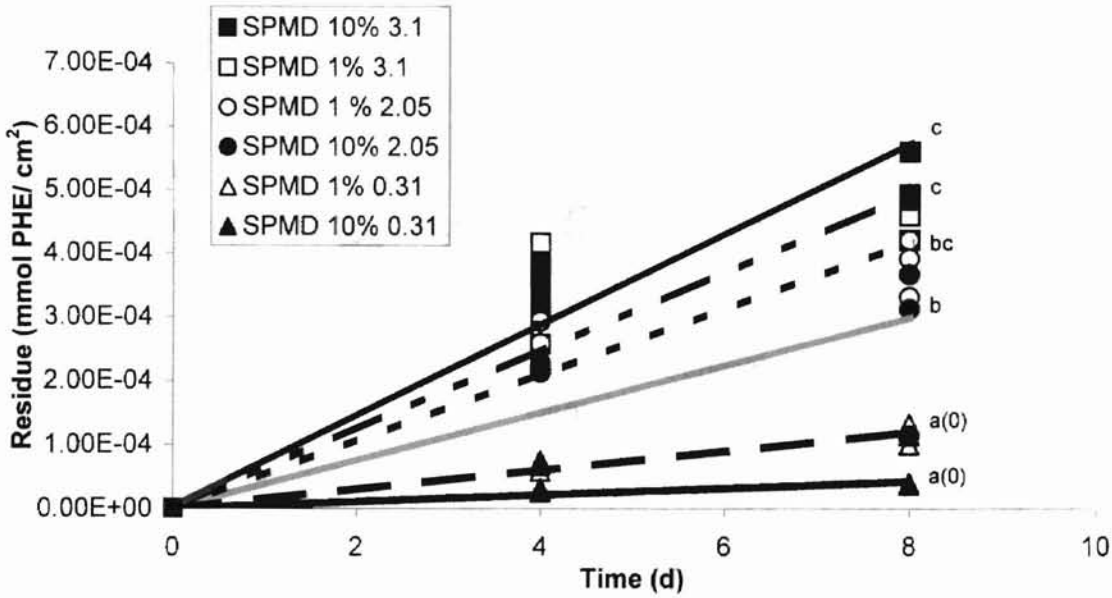


Fig 11. Linear phase uptake by SPMDs over 8d. Common letters indicate no significant differences ($p \geq 0.05$). Uptake rates of SPMD exposed in 10% OM 0.31 mmol/kg and 1% OM 0.31 mmol/kg soil were not significantly different from zero.

Solid phase microextraction fiber analysis (Fig 12) by ANOVA indicated significant differences among soil types and soil PHE concentrations ($p<0.05$). No significant differences were detected between day zero and day sixteen spiked soils. To increase sample size, day zero and day sixteen SPME soil determinations were pooled. An exception to this was in 1% OM 0.31 mmol/kg, no detection was observed at day sixteen and these data were not pooled with those from day zero. Being able to detect differences among residues from SPME fiber determinations, surrogate residues from the fibers could be used in place of earthworm residues to develop a dose-response curve (Fig 13).

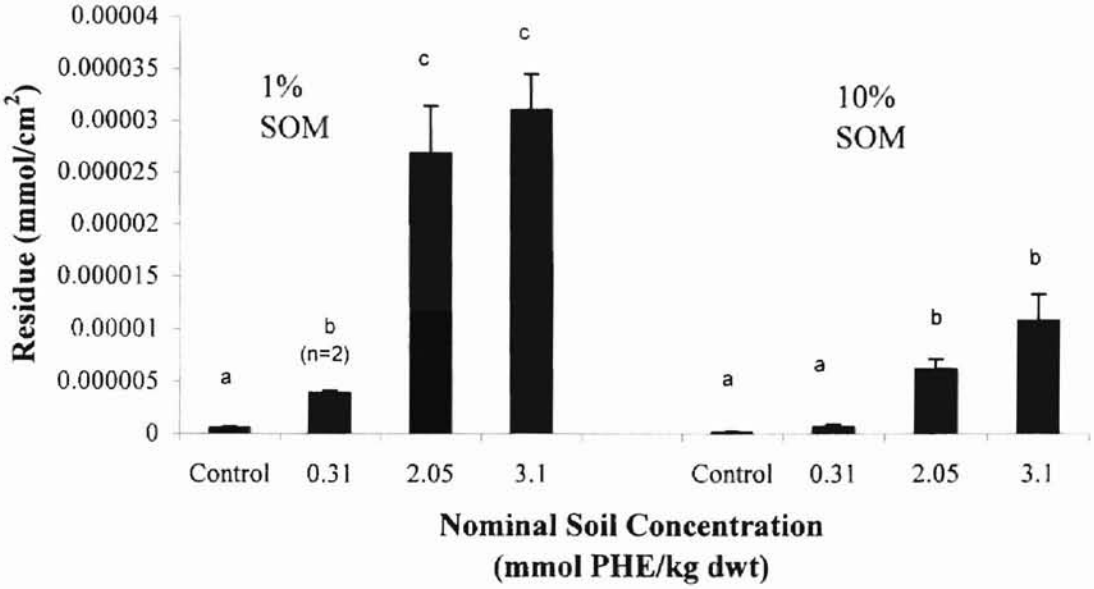


Fig 12. Solid-phase microextraction fiber analysis of 1% OM and 10% OM artificial soil spiked with PHE at various concentrations. Values are means ± SEM (n=4). Bars with common letters are not significantly different ($p \geq 0.05$).

The SPME residues (surrogate dose) from each soil type and concentration were correlated to earthworm mortality (response) from those soil types and concentrations. The data were plotted to generate the surrogate LD_{50} = 4.08E-7 mmol PHE (95% fiducial limits 2.68E-7 – 1.06E-6).

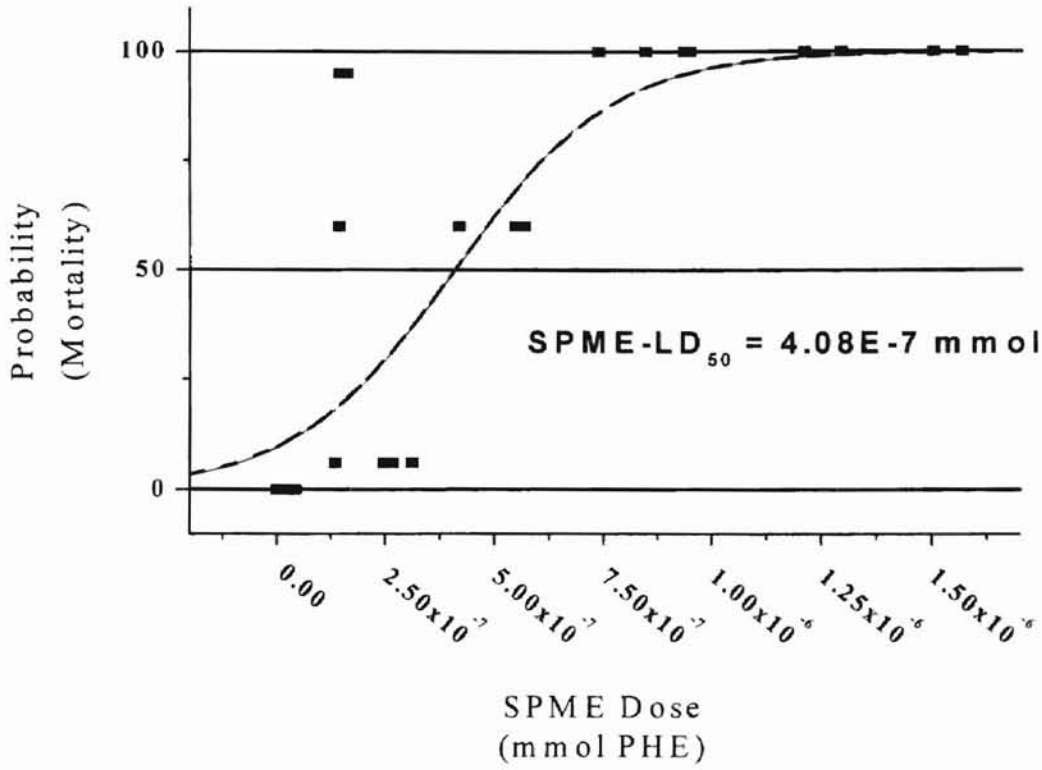


Fig 13. Solid-phase microextraction fiber analysis of SPME-Dose v. Probability of earthworm mortality.

CHAPTER FOUR

DISCUSSION

Earthworm Toxicity Tests

The final LC_{50} determined from the toxicity test in Experiment I and rangefinder toxicity test in Experiment II was adequate to design the toxicity and passive sampler uptake tests for Experiment II. A two-fold difference was observed in the results of the previous range-finder assays. This level of variation is not unusual and has been observed in many soil toxicity evaluations. However, the LC_{50}, as total PHE, is not the correct expression for exposure of toxicity for chemicals in soil systems as the LC_{50} changes with soil type, temperature, and moisture conditions. A better method for expressing the toxicity or potency of a chemical in soil is by determining the bioavailable fraction of chemical or that which is readily available to the animal (Experiment II).

Whole body residue analysis of the earthworms from Experiment II (Fig 8) indicated there were large differences in PHE residues between living and dead earthworms. Earthworms from the sub-lethal concentrations appeared to exhibit a metabolic response (Fig 8) as internal residues decreased by a factor of ten over a period of eight to twelve days.

Large differences were observed in the first two days of exposure for living and dead earthworms. This is when the more sensitive earthworms succumbed to the non-polar narcotic effects of PHE in the higher PHE exposures. Critical body residues for lethality were reached faster in earthworms exposed to high concentrations of PHE in the low organic matter soil than those exposed to high PHE concentrations in high organic

matter soil. Metabolism and excretion of PHE by the earthworms in the higher concentrations was not enough to compensate for the high internal body residues, resulting in mortality. Internal residues from more robust earthworms (worms that died after longer exposures at the same soil exposure concentrations) were slightly lower than the more sensitive earthworms indicating a stronger metabolic response. Great variability was observed in the residues from dead earthworms. This may be attributed to a number of reasons: 1) Location of the earthworm in the soil at the time of mortality-If the earthworm was buried (total surface contact) and had been dead for a long period (>12 h), the concentration gradient of PHE from the soil to the worm would allow PHE diffusion to continue after death and result in an artificially high PHE residue into the worm tissues. Earthworms lying on the surface of the soil would experience the same concentration gradient, but would not have the same surface contact as worms that were buried, also resulting in artificially high PHE residues. 2) Reduced metabolism-Earthworms that died could not continue metabolism to compensate for elevated residues of PHE, with the result of internal body residues continuing to increase to artificially elevated CBRs. 3) Natural variability in the sensitivity of earthworms to PHE-Theoretically, in a population of earthworms, sensitivities of earthworms would be distributed normally. That is, there would be a given number of organisms that were very sensitive to PHE and a given number of organisms that could resist the effects of PHE or compensate by metabolizing and excreting PHE.

This concept leads to the discussion of probit analysis of earthworm critical body residue analysis (Fig 9). From acute toxicity studies, the dose that may kill one animal may not kill another of the same species or strain. We may conceive of each animal

having an associated lethal dose (Salsburg 1986). By plotting the proportion of animals killed by a given dose, it is noted that few animals are killed from low doses and that there is a precipitous rise in the number of animals that would be killed as the dose increases until a dose is reached that will kill all animals of that species or strain. The dose resulting in a 50% probability of mortality is referred to as the LD_{50}. For this experiment the LD_{50} = 0.114 mmol/kg wwt with lower and upper 95% fiducial limits of 0.072 and 0.172 mmol/kg wwt. respectively.

The LD_{50} is chosen most often as the 'best reportable number' due to the lower errors associated with deriving that number. From this point the LD_{50} may be 'adjusted' to set regulatory levels. The median lethal dose (LD_{50}) is often associated and confused with the median lethal concentration (LC_{50}). All protective regulatory soil-screening levels are expressed using the LC_{50} values from soil toxicity tests. The soil screening levels do not implicitly consider environmental bioavailability of the exposure concentration, only total chemical levels. The results from Experiment II (Fig 6 &7) explain why total chemical exposure concentrations are inappropriate for regulatory soil screening levels. Mortality data from the two soils of differing composition spiked with the same nominal PHE concentrations unequivocally show drastic differences in mortality. If soil-screening levels are developed considering bioavailability, the total chemical estimates are usually site specific and are of no practical use for other sites. Conversely, CBRs in animals do not change appreciably from site to site (Spurgeon and Hopkin 1995).

The 'fast' or most readily available chemical fraction seems to be the most important factor in determining bioavailability and toxicity of organic lipophilic

contaminants in soil. The fast-fraction is most often associated with the amount of chemical dissolved in the pore water and interstitial space water in soil (Belfroid et al. 1996). This can be measured by using a surrogate sampler such as an SPMD or SPME fiber.

Semi-permeable Membrane Devices

The semipermeable membrane devices used in Experiment I were successful in taking up PHE from the soil (Fig 5). The static deployment appeared to have little variation between SPMDs through 16 d. Non-linear regression proved a good model for PHE uptake during static and turned deployments. Goodness of fit (R^2=0.9859) for static deployment through 16 d suggested the increased variation at 32 d (R^2=0.6471) may be due to extraneous factors. With only two data points at each time interval, interpretation was difficult. The data from turned deployment SPMDs were very consistent at each time interval, however the SPMDs appeared to lose PHE after eight days. This could be due to soil aeration after renewing the contact gradient at 48 h intervals as well as the degradation of PHE. Degradation of PHE may have been due to exposure to light or mineralization by bacteria in the soil. The turned deployment concept was abandoned because of many uncertainties involved and feasibility of turning the SPMDs in possible field deployments.

SPMDs from Experiment II showed that the samplers were capable of differentiating one soil concentration from another, but did not have the resolution needed to predict toxicity in earthworms exposed to the same soil concentrations (Fig 11). Mean rates of PHE uptake by SPMDs exposed in the high and low organic matter

soils at 0.31 mmol/kg did not significantly differ from each other or zero over the first eight days. This duration was chosen for uptake data because steady state was not achieved (Fig 10) in this experiment and this period was determined to be the linear portion of the exponential uptake curve. The reason for insignificant differences among the slopes could be due to the depletion of PHE in the immediate area of soil contact by the SPMDs. The rate of PHE replenishment to restore equilibrium in the soil in contact with the SPMD may have been the limiting factor in the rate of PHE uptake by SPMDs. This is plausible because the rates of uptake for each soil concentration were not statistically different. The steady-state residues of PHE by SPMDs would have been valuable in determining differences between soil type and concentration interactions. For an SPMD to come to steady state with its surroundings, sufficient time needs to pass for the uptake and back-diffusion to equalize. It would appear PHE degrades too rapidly in soil or PHE replenishment is too slow for SPMDs to come to steady state with soil concentrations. Since SPMDs will "lose" analyte (back-diffusion) to maintain steady state with surrounding conditions, modeling of soil concentrations over time would be necessary to determine exactly when SPMDs come to steady state. This was not feasible, nor practical in this experiment. The monitoring of steady-state conditions in soil with SPMDs would be too costly and time-consuming to be of much benefit in this case.

The application of SPMDs for qualitative measures in soil contaminated with lipophilic organic contaminants is without bound. For estimating relative bioavailability and qualitative analysis of soil contamination, steady state need not be achieved.

Solid-phase Microextraction Fibers

The SPME fibers were able to detect differences in soil concentrations between different soil types (Fig 12). This was due to the ability of SPME fibers to achieve steady state within 5 h. The resolution and sensitivity was such that the prediction of toxicity of PHE in this experiment was possible (Fig 13). Probit analysis of SPME residue as dose and probability of mortality from earthworms revealed the SPME-LD_{50} = 4.08E-7 mmol PHE. The reason the sigmoid curve fit of the probit model did not pass through the origin was that the presence influential data altering the fit of the model. With so few data points, influence by those data could be significant. Removal of data points did force the model through the origin, but did not affect the SPME-LD_{50}. Having no impact on the determination of SPME-LD_{50}, the data points were allowed to remain.

The concept of using this technology to evaluate soil contaminated by PAHs or other non-polar contaminants having similar modes of acute toxic action (acute non-polar narcosis) and routes of contaminant uptake could save many resources and much time. For instance, if a SPME soil determination revealed bioavailable residues above the SPME-LD_{50} the probability for mortality in earthworms would be greater than 50%. This could be an important tool for preliminary screening of contaminated soils suspected to be toxic. This tool could also be used to determine the efficacy of remediation for contaminated soil. The ability of SPME fibers to predict toxicity relies on the contaminant being at steady state with the soil suspension. The SPME fiber is measuring the fast fraction (water soluble) or environmentally bioavailable fraction of PHE in artificial soil.

The cost ($60 per fiber) and convenience of using SPME fibers for bioavailability

determination is very attractive. The durability of the fibers was such that at least eight bioavailability determinations could be made without need for correction of fiber degradation (Appendix G).

Conclusions

The "fast fraction" of PHE in artificial soil was taken up by earthworms, semi-permeable membrane devices, and solid-phase microextraction fibers. The earthworm toxicity test results indicated differences in bioavailability of PHE for the one percent and ten percent organic matter soils among the same nominal soil concentrations used in Experiment II. This was, as far as I know, the first study performed in soil using lipid-containing SPMDs as an organism surrogate where the SPMD sampled the soil directly and uptake was compared to toxicity and bioavailability derived from toxicity tests. The SPMD sampled the soil air and soil pore water in this experiment. Since the vapor pressure of PHE is very low (2.27E-2 Pa), the soil pore water was likely the predominant source of available PHE in the soil. Thus, PHE dissolved in soil pore water represents the "fast fraction" of the environmentally bioavailable PHE in soil. The semi-permeable membrane devices used in this experiment did not reach steady state and did not have the resolution needed to predict toxicity based on residues from the linear phase of uptake of PHE from soil. Therefore, SPMDs could not be considered for use as predictive tools for estimating toxicity of PHE to earthworms in artificial soil. Longer exposure periods for SPMDs should be implemented to achieve steady state for lipophilic contaminants with similar physical-chemical attributes as PHE. Since there were no statistically significant differences between slopes (rates of uptake) of SPMDs during the linear phase of uptake in both soil types, it can be reasoned that SPMDs removed the "fast fraction" of PHE

before any observations were made (before four days) regarding PHE uptake by SPMDs. During observations, SPMDs may have been removing PHE during the repletion of PHE to the soil area around the SPMD or monitoring the release of the "slow fraction" of PHE in the soil.

The design and intended use of solid-phase microextraction fibers was for the determination of total chemical measurements from various media. Modifying factors, such as dissolved organic matter and suspended particulate matter, negatively affect total chemical determinations by SPME. The SPME fibers were used in this experiment for the determination of PHE bioavailability in artificial soil. The detriment of modifying factors for total chemical determination was the benefit for SPME technology to detect bioavailable differences among soil suspensions at varying soil concentrations from different soil types.

The SPME fibers sampled the diluted soil pore water from spiked artificial soil in this experiment. PHE residues collected by SPME fibers (assessed at steady-state) were used for probability estimation of toxicity to earthworms in artificial soil. The use of SPME fibers in soil for earthworm toxicity prediction is more reliable than using total chemical levels assessed using vigorous solvent extractions coupled with bioavailability estimates determined by conducting soil toxicity evaluations. The critical body residues from earthworms were associated with high variability, while SPME estimates of toxicity were much more precise. SPME fibers also appeared to measure the "fast fraction" of PHE from spiked soil. Since steady state was achieved in less than five hours, it is intuitive that the SPME fiber was not able to measure the "slow fraction" or the repletion of PHE, which is bound tightly to organic matter in the soil.

For both passive samplers the question arises regarding the presence of an infinite source of PHE. In this study, it was concluded there are two sources of PHE in soil, the "fast fraction" being one source of PHE and the "slow fraction" being another source. The fast fraction may not be considered part of an infinite source since it is assimilated by the passive samplers and earthworms rapidly. The slow fraction may be considered a pseudo-infinite source since PHE was present in the soil at much higher proportions (>98% for SPMDs, >99.99% for worms, > 99.7% for SPME fibers – as nominal by mass balance for PHE) than was in SPMDs, earthworms, or SPME fibers. In this experiment, the removal of PHE from the soil was primarily the "fast fraction" however; a relatively small portion of the "slow fraction" was also removed. This small portion of the "slow fraction" may be considered negligible, as the fast fraction in this case at most comprises less than two percent of the spiked nominal soil level.

All things being equal, the costs involved in conducting bioassays using earthworms, chemical analysis of soil and tissues, inaccuracy involved determining bioavailability factors and time used are great for risk analysis compared to correlating toxicity observed using earthworms and SPME soil determinations of organic contaminants in soil. In this study, I have shown both the SPMD and SPME fiber methods to be applicable for screening organic lipophilic contaminant availability in soil.

BIBLIOGRAPHY

ATSDR. 1996. Polycyclic aromatic hydrocarbons (PAHs). Agency for Toxic Substances and Disease Registry. *ATSDR ToxFAQs* 4.

ASTM. 1994. Standard Test Method for Capillary-Moisture Relationships for Coarse- and Medium-Textured Soils by Porous-Plate Apparatus. D2325-68(1994) e1. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1995. Standard Guide for Risk-Based Corrective Action Applied at Petroleum Release Site. E 1739-95. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1997. Standard Guide for Conducting a Laboratory Soil Toxicity or Bioaccumulation Tests With the Lumbricid Earthworm Eisenia foetida. E 1676-97. American Society for Testing and Materials, Philadelphia, PA. Annual Book of ASTM Standards Vol. 11.04. pp. 1056-1074.

ASTM. 1998. Standard Provisional Guide for Risk-Based Corrective Action. PS104-98. American Society for Testing and Materials, Philadelphia, PA.

Belfroid AC, Seinen W, Van Gestel CAM, Hermens JLM, and Van Leeuwen KJ. 1995. Modelling the accumulation of hydrophobic organic chemicals in earthworms. *Environ. Sci. & Pollut. Res.* 2:5-15.

Belfroid AC, Sijm DTHM, and Van Gestel CAM 1996. Bioavailability and toxicokinetics of hydrophobic aromatic compounds in benthic and terrestrial invertebrates. *Environ. Rev.* 4:276-299.

Burgos WD, Munson CM, and Duffy CJ. 1999. Phenanthrene adsorption-desorption hysteresis in soil described using discrete-interval equilibrium models. *Water Resour. Res.* 35:2043-2051.

Carmichael LM, Christman RF, and Pfander FK. 1997. Desorption and mineralization kinetics of phenanthrene and chrysene in contaminated soils. *Environ. Sci. Technol.* 31:126-132.

Cassel DK and Nielson DR. 1986. Field capacity and available water capacity. In Klute A. ed. Methods of Soil Analysis. Part 1--Physical and Mineralogical Methods, Second Edition. Madison, WI, USA. p. 903.

Conder JM and Lanno RP. 2000. Evaluation of surrogate measures of cadmium, lead, and zinc bioavailability to *Eisenia fetida*. *Chemosphere* - in press.

Connell DW, Hawker DW, Warne M, and Vowles PP. 1997. *Basic Concepts of Environmental Chemistry.* CRC Press,Boca Raton, FL, USA.

Fitzgerald DG, Warner KA, Lanno RP, and Dixon DG. 1996. Assessing the effects of modifying factors on pentachlorophenol toxicity to earthworms: applications of body residues. *Environ. Toxicol. Chem.* 15:2299-2304.

Hendershot WH, Lalande H, and Duquette M. 1993. Soil reaction and exchangeable acidity. In Carter MR, ed. *Soil Sampling and Methods of Analysis*, Lewis Publishers, Boca Raton, FL, USA, pp. 141-145.

Huckins JN, Petty JD, Lebo JA, Orazio CE, Prest HF, Tillitt DE, Ellis GS, Johnson TB, and Manuweera GK. 1996. Semipermeable membrane devices (SPMDs) for the concentration and assessment of bioavailable organic contaminants in aquatic environments. In Ostrander GK, ed. *Techniques in Aquatic Toxicology*, CRC Press, Boca Raton, FL, USA, pp. 625-656.

Huckins JN, Petty JD, Lebo JA, Orazio CE, Clark RC, and Gibson VL. 1999. *SPMD Technology Tutorial, Second Edition.* http://wwwaux.cerc.cr.usgs.gov/spmd/SPMD-Tech_Tutorial.htm

Kelsey JW, Kottler BD, and Alexander M. 1997. Selective Chemical Extractants to predict bioavailability of soil-aged organic chemicals. *Environ. Sci. Technol.* 31: 214-217.

Landrum PF, Gossiaux DC, Kukkonen J. 1997. Sediment characteristics infuencing the bioavailability of nonpolar organic contamnants to *Diporei*a spp. *Chemical Speciation and Bioavailability* 9:43-55.

Lane DA. 1988. The fate of polycyclic aromatic hydrocarbons in the atmosphere and during sampling. In Vo-Dinh T, ed. *Chemical Analysis of Polycyclic Aromatic Compounds*, 101. Wiley & Sons, Inc., New York, NY, USA, pp. 31-58.

Lanno RP and McCarty LS. 1997. Earthworm bioassays: Adopting techniques from aquatic toxicity testing. *Soil Biol. Biochem.* 29:693-697.

Lord HL and Pawliszyn J. 1998. Recent advances in solid-phase microextraction. *LC-GC,* May Supplement: S41-S46.

Mackay D, Shiu WY, and Ma KC. 1992. *Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals.* Lewis Publishers, Chelsea, MI.

Newman MC and Jagoe CH. 1994. Ligands and the bioavailability of metals in aquatic environments. In: Hamelink JL, Landrum PF, Bergman HL, and Benson WH, eds. *Bioavailability: Physical, Chemical, and Biological Interactions.* Lewis Publishers, Boca Raton, FL, USA, p. 39.

Parkerton TF and Stone MA. 1998. Ecotoxicity on a stick: A novel new analytical method for assessing the toxicity of hydrocarbon-contaminated samples. Bioavailability Workshop Procedings, Pinawa, Manitoba - in press.

Petty JD, Huckins JN, and Zajicek JL. 1993. Application of semipermeable membrane devices (SPMDs) as passive air samplers. *Chemosphere* 27:1609-1624.

Rantalainen AL, Paasivirta J, Herve S. 1998. Uptake of chlorohydrocarbons from soil by lipid-containing semipermeable membrane devices (SPMDs). *Chemosphere* 36: 1415-1427.

Salsburg, DS. 1986. Statistics for Toxicologists. In: DiCarlo, FJ and Oehme, FW, eds. *Drug and Chemical Toxicology, vol 4.,* Marcel Dekker, Inc., New York, NY, USA, pp. 3-62.

Smith RL. 1990. *Ecology and Field Biology, Fourth Edition.* HarperCollins Publishers Inc. New York, NY, USA. p. 79.

Spurgeon DJ and Hopkin SP. 1995. Extrapolation of the laboratory-based OECD earthworm toxicity test to metal-contaminated field sites. *Ecotoxicology* 4:190-205.

Strandberg B, Wagman N, Bergqvist P-A, Haglund P, and Rappe C. 1997. Semipermeable membrane devices as passive samplers to determine organochlorine pollutants in compost. *Environ. Sci. Technol.* 31:2960-2965.

Tang J and Alexander M. 1999. Mild extractability and bioavailability of polycyclic aromatic hydrocarbons in soil. *Environ. Toxicol. Chem.* 18:2711-2714.

Topp GC. 1993. Soil water content. In: Carter MR, ed. *Soil sampling and methods of analysis*, Lewis Publishers, Boca Raton, FL, USA, pp. 541-557.

Utvik TIR and Johnsen S. 1999. Bioavailability of polycyclic aromatic hydrocarbons in the North Sea. *Environ. Sci. Technol.* 33:1963-1969.

Van Brummelen, TC. 1995. Distribution and ecotoxicity of PAHs in forest soil. Vrije Universiteit, Amsterdam, The Netherlands.

Van Brummelen TC, Van Hattum B, Crommentuijn T, and Kalf DF. 1998. Bioavailability and ecotoxicity of PAHs. In: Neilson AH, ed. *The Handbook of Environmental Chemistry*, Vol. 3 part J. Springer-Verlag, New York, NY, USA, pp. 205-245.

Wagrowski DM and Hites RA. 1997. Polycyclic aromatic hydrocarbon accumulation in urban, suburban, and rural vegetation. *Environ. Sci. Technol.* 31:279-282.

Weston DP and Mayer LM. 1998a. In vitro digestive fluid extraction as a measure of the bioavailability of sediment-associated polycyclic aromatic hydrocarbons: sources of variation and implications for partitioning models. *Environ. Toxicol. Chem.* 17: 820-829.

Weston DP and Mayer LM. 1998b. Comparison of in vitro digestive fluid extraction and traditional in vivo approaches as measures of polycyclic aromatic hydrocarbon availability from sediments. *Environ. Toxicol. Chem.* 17:830-840.

APPENDIX A – RAW DATA FROM EARTHWORM WHOLE BODY RESIDUE ANALYSIS

ID#	Status	SOM	Soil Conc. (mmol/kg) (dwt)	Exposure Period (d)	Body Residue (mmol/kg) (wwt)
1	Alive	1%	Control	16	nd
2	Alive	1%	Control	16	nd
3	Alive	1%	Control	16	nd
4	Alive	1%	Control	16	nd
5	Alive	1%	Control	16	nd
6	Alive	1%	Control	16	nd
7	Alive	10%	Control	16	nd
8	Alive	10%	Control	16	nd
9	Alive	10%	Control	16	nd
10	Alive	10%	Control	16	nd
11	Alive	10%	Control	16	nd
12	Alive	10%	Control	16	nd
13	Alive	10%	0.31	2	0.0982
14	Alive	10%	0.31	2	0.1777
15	Alive	10%	0.31	4	0.1685
16	Alive	10%	0.31	4	0.124
17	Alive	10%	0.31	4	0.1275
18	Alive	10%	0.31	4	0.1009
19	Alive	10%	0.31	8	0.0239
20	Alive	10%	0.31	8	0.0294
21	Alive	10%	0.31	8	0.0305
22	Alive	10%	0.31	16	0.0145
23	Alive	10%	0.31	16	0.0127
24	Dead	10%	0.31	16	0.0228
25	Dead	10%	0.31	16	0.0127
26	Dead	1%	0.31	4	0.2425
27	Dead	1%	0.31	4	0.2116
28	Dead	1%	0.31	4	0.271
29	Dead	1%	0.31	4	0.3134
30	Dead	1%	0.31	5	0.1922
31	Dead	1%	0.31	5	0.361
32	Dead	1%	0.31	5	0.0136
33	Dead	1%	0.31	6	0.2776
34	Dead	1%	0.31	6	0.7114
35	Dead	1%	0.31	6	0.1791
36	Dead	1%	0.31	6	0.9448
37	Dead	1%	0.31	6	0.3711
38	Dead	1%	0.31	7	1.5016
39	Dead	1%	0.31	7	0.2491

40	Dead	1%	0.31	7	0.1049
41	Dead	1%	0.31	7	0.5008
42	Dead	1%	0.31	8	0.5186
43	Dead	1%	0.31	8	0.5789
44	Dead	1%	0.31	8	0.0526
45	Dead	1%	0.31	8	0.5343
46	Dead	1%	2.05	1	0.4463
47	Dead	1%	2.05	1	0.5882
48	Dead	1%	2.05	1	0.9718
49	Dead	1%	2.05	1	0.3586
50	Dead	1%	2.05	1	0.4727
51	Dead	1%	2.05	2	1.4543
52	Dead	1%	2.05	2	0.4022
53	Dead	1%	2.05	2	0.5429
54	Dead	1%	2.05	2	0.4093
55	Dead	1%	2.05	2	0.8042
56	Dead	1%	2.05	3	0.561
57	Dead	1%	2.05	3	0.6059
58	Dead	1%	2.05	3	0.0252
59	Dead	1%	2.05	3	0.2668
60	Dead	1%	2.05	3	0.3596
61	Dead	1%	2.05	4	0.5782
62	Dead	1%	2.05	4	0.909
63	Dead	1%	2.05	4	0.6901
64	Dead	1%	2.05	4	0.8974
65	Dead	1%	3.1	1	0.8748
66	Dead	1%	3.1	1	1.0181
67	Dead	1%	3.1	1	0.9976
68	Dead	1%	3.1	1	0.3726
69	Dead	1%	3.1	2	1.2618
70	Dead	1%	3.1	2	0.6596
71	Dead	1%	3.1	2	2.0293
72	Dead	1%	3.1	2	1.6655
73	Dead	1%	3.1	3	0.573
74	Dead	1%	3.1	3	0.5279
75	Dead	1%	3.1	3	0.9413
76	Dead	1%	3.1	3	0.4168
77	Dead	1%	3.1	4	1.0958
78	Dead	1%	3.1	4	1.5113
79	Dead	1%	3.1	4	0.6279
80	Dead	1%	3.1	4	0.0812
81	Dead	10%	2.05	3	0.3143
82	Dead	10%	2.05	3	0.4341
83	Dead	10%	2.05	3	0.3292
84	Dead	10%	2.05	4	0.1173
85	Dead	10%	2.05	8	0.0799

86	Dead	10%	3.1	3	0.6043
87	Dead	10%	3.1	3	0.7103
88	Dead	10%	3.1	3	0.7125
89	Dead	10%	3.1	3	0.4332
90	Dead	10%	3.1	4	0.9435
91	Dead	10%	3.1	4	0.7463
92	Dead	10%	3.1	4	0.4612
93	Dead	10%	3.1	4	0.6958
94	Dead	10%	3.1	5	0.7278
95	Dead	10%	3.1	5	0.4499
96	Dead	10%	3.1	5	0.4397
97	Dead	10%	3.1	5	0.3047
98	Alive	10%	0.31	8	0.4776
99	Alive	10%	0.31	2	0.9463

APPENDIX B – SAS CODE FOR EARTHWORM WHOLE BODY RESIDUES

```
DATA ONE;
INFILE 'A:WORMSCSV.DAT';
INPUT ID Status$ SOM Conc Days Residue;

IF RESIDUE > .4 AND STATUS = 'Alive' THEN DELETE;

PROC PROBIT;
CLASS STATUS;
MODEL STATUS = RESIDUE/LACKFIT INVERSECL;

DATA TWO;
SET ONE;
IF STATUS = 'Alive';
DAYS2 = DAYS*DAYS;

PROC PLOT;
PLOT RESIDUE*DAYS;

PROC REG;
MODEL RESIDUE = DAYS;

PROC REG;
MODEL RESIDUE = DAYS DAYS2;

TITLE 'JASON WELLS';
TITLE2 'WORM LETHAL DOSE DATA';
TITLE3 'EXPERIMENT II';
RUN;
```

APPENDIX C - EARTHWORM PHE SPIKED RECOVERY ANALYSIS

Added earthworm and RGW to extraction tube
Added 100 µl of 100 µg/ml PHE std (Chem Service F81MS) to extraction tube
Homogenized and extracted as normal
Cleaned up as normal

PHE Spike Recovery Sample	Initial Reading (ng/µl)	Calibration Correction Factor	Corrected Reading (ng/µl)	Spike Amount (ng/µl)	Spike Recovery %
1 no spike	nd	0.1544	nd	0	nd
2	4.18	0.1544	4.83	5	96.51
3 no spike	nd	0.1544	nd	0	nd
4	2.96	0.1544	3.42	5	68.34
5	4.44	0.1544	5.13	5	102.51
6	4.17	0.1544	4.81	5	96.28
7	4.25	0.1544	4.91	5	98.12
8	2.4	0.1544	2.77	5	55.41
H_2O Spike A	3.46	0.1544	3.99	5	79.88
H_2O Spike B	4.12	0.1544	4.76	5	95.12
H_2O Blank A	nd	0.1544	nd	0	nd
H_2O Blank B	nd	0.1544	nd	0	nd

nd = No Detect limit <0.5 ng/µl

Spike Recovery %		
		L 95% CL
Mean	86.5223	72.39
Standard Error	5.97853	
Median	95.6998	**U 95 % CL**
Mode	#N/A	100.66
Standard Deviation	16.9098	
Sample Variance	285.943	
Kurtosis	-0.0526	
Skewness	-1.1112	
Range	47.0995	
Minimum	55.4112	
Maximum	102.511	
Sum	692.178	
Count	8	
Confidence Level(95.0%)	14.137	

APPENDIX D – SPMD RAW DATA

ID#	SOM	Soil Conc. (mmol/kg)	Exposure Period (d)	Residue (mmol/cm^2)
1	1%	Control	16	5.41E-07
2	1%	Control	16	6.24E-07
3	1%	Control	16	9.41E-07
4	1%	0.31	4	7.43E-05
5	1%	0.31	4	5.80E-05
6	1%	0.31	4	6.72E-05
7	1%	0.31	8	1.00E-04
8	1%	0.31	8	1.15E-04
9	1%	0.31	8	1.32E-04
10	1%	0.31	16	1.38E-04
11	1%	0.31	16	1.18E-04
12	1%	0.31	16	1.34E-04
13	1%	0.31	32	1.96E-04
14	1%	0.31	32	2.28E-04
15	1%	0.31	32	2.14E-04
16	1%	2.05	4	2.93E-04
17	1%	2.05	4	2.58E-04
18	1%	2.05	4	2.99E-04
19	1%	2.05	8	4.21E-04
20	1%	2.05	8	3.31E-04
21	1%	2.05	8	3.92E-04
22	1%	2.05	16	6.97E-04
23	1%	2.05	16	4.94E-04
24	1%	2.05	16	5.39E-04
25	1%	2.05	32	5.35E-04
26	1%	2.05	32	8.09E-04
27	1%	2.05	32	7.83E-04
28	1%	3.1	4	3.06E-04
29	1%	3.1	4	4.16E-04
30	1%	3.1	4	2.57E-04
31	1%	3.1	8	4.59E-04
32	1%	3.1	8	4.21E-04
33	1%	3.1	8	4.84E-04
34	1%	3.1	16	5.82E-04
35	1%	3.1	16	6.39E-04
36	1%	3.1	16	6.02E-04
37	1%	3.1	32	7.73E-04
38	1%	3.1	32	9.96E-04
39	1%	3.1	32	8.59E-04

40	10%	Control	16	4.29E-07
41	10%	Control	16	1.56E-07
42	10%	Control	16	1.89E-05
43	10%	0.31	4	2.57E-05
44	10%	0.31	4	2.78E-05
45	10%	0.31	4	3.10E-05
46	10%	0.31	8	3.68E-05
47	10%	0.31	8	4.67E-05
48	10%	0.31	8	9.93E-05
49	10%	0.31	16	5.12E-05
50	10%	0.31	16	5.16E-05
51	10%	0.31	16	5.55E-05
52	10%	0.31	32	4.60E-05
53	10%	0.31	32	5.01E-05
54	10%	0.31	32	4.77E-05
55	10%	2.05	4	2.19E-04
56	10%	2.05	4	2.28E-04
57	10%	2.05	4	2.13E-04
58	10%	2.05	8	3.13E-04
59	10%	2.05	8	3.67E-04
60	10%	2.05	8	1.14E-04
61	10%	2.05	16	4.54E-04
62	10%	2.05	16	4.28E-04
63	10%	2.05	16	3.80E-04
64	10%	2.05	32	5.50E-04
65	10%	2.05	32	4.42E-04
66	10%	2.05	32	5.81E-04
67	10%	3.1	4	3.81E-04
68	10%	3.1	4	3.54E-04
69	10%	3.1	4	3.23E-04
70	10%	3.1	8	5.60E-04
71	10%	3.1	8	4.93E-04
72	10%	3.1	16	7.00E-04
73	10%	3.1	16	6.45E-04
74	10%	3.1	16	5.87E-04
75	10%	3.1	32	1.84E-04
76	10%	3.1	32	8.00E-04
77	10%	3.1	32	3.28E-04

APPENDIX E - SAS CODE FOR ANALYZING SEMIPERMEABLE MEMBRANE DEVICES FOR EXPERIMENT II

```
LIBNAME SPMDS 'a:\';
DATA SPMDS.SPMD_ONE;
INFILE 'a:\SPMDCSV1.DAT';
INPUT ID$ SOM CONC DAYS RESIDUE;
LABEL        ID='SAMPLE ID';
LABEL              SOM='Soil Organic Matter';
LABEL     Conc='Nominal Soil Concentration (mmol/kg dwt)';
LABEL     Days='Length of Exposure (d)';
LABEL     Residue='PHE Residue (mmol/cm2)';

PROC SORT DATA=SPMDS.SPMD_ONE;
BY SOM CONC DAYS;

PROC MIXED;
CLASS SOM DAYS CONC;
MODEL RESIDUE = CONC|DAYS|SOM/DDFM=SATTERTH;
LSMEANS DAYS*CONC*SOM/SLICE=(CONC*DAYS CONC*SOM DAYS*SOM)
DIFF;

DATA TWO;
SET SPMDS.SPMD_ONE;

IF DAYS < 16;

PROC SORT REVERSE;
BY SOM DESCENDING CONC;

PROC MIXED ORDER=DATA;
CLASS SOM CONC;
MODEL RESIDUE = SOM*CONC DAYS SOM*CONC*DAYS/S;

PROC SORT;
BY SOM CONC;

PROC REG;
BY SOM CONC;
MODEL RESIDUE = DAYS;

RUN;
```

APPENDIX F – SOLID PHASE MICROEXTRACTION FIBER PHE DATA

ID#	SOM	Soil Conc.	Exposure Period (d)	PHE Residue (mmol)	PHE Residue ($mmol/cm^2$)
1	1%	Control	0	2.69E-08	6.91E-07
2	1%	Control	0	1.27E-08	3.27E-07
3	1%	Control	16	1.75E-08	4.49E-07
4	1%	Control	16	3.09E-08	7.93E-07
5	1%	0.31	0	1.61E-07	4.12E-06
6	1%	0.31	0	1.43E-07	3.66E-06
7	1%	0.31	16	nd	nd
8	1%	0.31	16	nd	nd
9	1%	2.05	0	9.46E-07	2.43E-05
10	1%	2.05	0	1.56E-06	4.01E-05
11	1%	2.05	16	9.35E-07	2.40E-05
12	1%	2.05	16	7.39E-07	1.89E-05
13	1%	3.1	0	8.47E-07	2.17E-05
14	1%	3.1	0	1.50E-06	3.84E-05
15	1%	3.1	16	1.29E-06	3.31E-05
16	1%	3.1	16	1.21E-06	3.10E-05
17	10%	Control	0	nd	nd
18	10%	Control	0	nd	nd
19	10%	Control	16	nd	nd
20	10%	Control	16	nd	nd
21	10%	0.31	0	3.30E-08	8.46E-07
22	10%	0.31	0	nd	nd
23	10%	0.31	16	4.29E-08	1.10E-06
24	10%	0.31	16	2.74E-08	7.02E-07
25	10%	2.05	0	2.47E-07	6.34E-06
26	10%	2.05	0	3.12E-07	8.00E-06
27	10%	2.05	16	1.36E-07	3.48E-06
28	10%	2.05	16	2.67E-07	6.84E-06
29	10%	3.1	0	5.47E-07	1.40E-05
30	10%	3.1	0	4.18E-07	1.07E-05
31	10%	3.1	16	5.67E-07	1.45E-05
32	10%	3.1	16	1.43E-07	3.68E-06

nd = 1.12E-08 mmol

APPENDIX G – SOLID PHASE MICROEXTRACTION FIBER DEGRADATION ANALYSIS

Run	SPME A	SPME B	SPME D	SPME E	SPME F	SPME G	SPME H	SPME I	SPME J
1	131.12	142.57	121.3	155.06	84.05	121.07	113.95	95.53	97.37
2	101.36	106.45	85.95	119.96	3.94	89.32	88.63	70.33	10.04
3	109.59	113.82	104.55	154.13	126.85	113.42	186.04	99.48	
4	103.25	97.62	79.76	135.17	87.16	100.57	91.18	80.62	

SPME A

Regression Statistics	
Multiple R	0.6120055
R Square	0.3745508
Adjusted R Square	0.0618262
Standard Error	13.225934
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	209.5083458	209.5	1.1977	0.387994467
Residual	2	349.8506542	174.9		
Total	3	559.359			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	138058.39	126048.6279	1.095	0.38769	-404285.4633	680402.238
Date	-3.7688136	3.443739872	-1.09	0.38799	-18.58604067	11.0484135

SPME B

Regression Statistics	
Multiple R	0.7725144
R Square	0.5967784
Adjusted R Square	0.3951677
Standard Error	15.137803
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	678.3051271	678.3	2.96005	0.227485632
Residual	2	458.3061729	229.2		
Total	3	1136.6113			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	248328	144269.5346	1.721	0.22734	-372414.1367	869070.141
Date	-6.781356	3.941548249	-1.72	0.22749	-23.74048113	10.1777692

SPME D

Regression Statistics	
Multiple R	0.6595359
R Square	0.4349876
Adjusted R Square	0.1524814
Standard Error	17.334535
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	462.6720068	462.7	1.53975	0.340464122
Residual	2	600.9721932	300.5		
Total	3	1063.6442			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	205095.31	165205.2963	1.241	0.34029	-505726.2073	915916.821
Date	-5.600678	4.513528433	-1.24	0.34046	-25.02083697	13.8194809

SPME E

Regression Statistics	
Multiple R	0.1030826
R Square	0.010626
Adjusted R Square	-0.484061
Standard Error	20.466168
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	8.997315254	8.997	0.02148	0.896917445
Residual	2	837.7280847	418.9		
Total	3	846.7254			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	28728.058	195051.0601	0.147	0.89641	-810509.5026	867965.618
Date	-0.781017	5.328936332	-0.15	0.89692	-23.70959537	22.1475615

SPME F

Regression Statistics	
Multiple R	0.4107109
R Square	0.1686834
Adjusted R Square	-0.2469749
Standard Error	57.545033
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1343.852481	1344	0.40582	0.589289146
Residual	2	6622.861719	3311		
Total	3	7966.7142			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-349296.08	548428.0018	-0.64	0.58936	-2708992.963	2010400.8
Date	9.5450848	14.9834505	0.637	0.58929	-54.92354425	74.0137139

SPME G

Regression Statistics	
Multiple R	0.2404208
R Square	0.0578022
Adjusted R Square	-0.4132967
Standard Error	16.669417
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	34.09360169	34.09	0.1227	0.75957917
Residual	2	555.7388983	277.9		
Total	3	589.8325			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	55753.923	158866.4443	0.351	0.75915	-627793.6929	739301.539
Date	-1.520339	4.3403464	-0.35	0.75958	-20.19535528	17.1546773

SPME H

Regression Statistics	
Multiple R	0.07631
R Square	0.0058232
Adjusted R Square	-0.4912652
Standard Error	55.571425
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	36.17694915	36.18	0.01171	0.923690035
Residual	2	6176.366451	3088		
Total	3	6212.5434			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-57202.896	529618.6928	-0.11	0.92385	-2335969.797	2221564
Date	1.5661017	14.46956654	0.108	0.92369	-60.69146162	63.823665

SPME I

Regression Statistics	
Multiple R	0.0728719
R Square	0.0053103
Adjusted R Square	-0.4920345
Standard Error	16.479752
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.899769492	2.9	0.01068	0.927128135
Residual	2	543.1644305	271.6		
Total	3	546.0642			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	16315.556	157058.8591	0.104	0.92674	-659454.6439	692085.755
Date	-0.4433898	4.290961865	-0.1	0.92713	-18.90592147	18.0191418

VITA

Jason Blayne Wells

Candidate for the Degree of

Master of Science

Thesis: ASSESSMENT OF THE BIOAVAILABILITY OF PAHS IN SOIL BY EARTHWORMS AND PASSIVE SAMPLING DEVICES

Major Field: Zoology

Biographical:

Education: Graduated from Altus High School, Altus, Oklahoma in May, 1992; received Associate of Science degree in Biological Sciences from Western Oklahoma State College, Altus, Oklahoma in May 1994; received Bachelor of Science degree in Biological Sciences from Oklahoma State University, Stillwater, Oklahoma in May, 1997. Completed the requirements for the Master of Science degree in Zoology at Oklahoma State University in May, 2000.

Experience: Employed by Oklahoma State University, Department of Zoology as a graduate research assistant, January, 1998 to present to evaluate using passive sampling devices in soil for organic contaminants.

Employed by Oklahoma State University, Ecotoxicology and Water Quality Research Laboratory as a technician, January, 1996 to January, 1998 to conduct bioassays on industrial effluents.

Professional Memberships:

Society of Environmental Toxicology and Chemistry
National Association of Graduate and Professional Students