### APPROVAL SHEET

Title of Dissertation: Measuring and Reducing Bioavailability of PAHs in Soils

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### ABSTRACT

Title of Document:

Measuring and Reducing Bioavailability of PAHs in Soils

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Polycyclic aromatic hydrocarbons (PAHs) are a group of organic contaminants that are widely distributed in soils. Some PAHs are potent human carcinogens. When released into soils, PAHs are often emitted within a certain source matrix (e.g. soot, coal tar or oils). In addition to the diversity of the PAH source matrix, soils are also very heterogeneous mixtures containing many different components, such as sand, clay, organic matter and black carbon which can have varying capacity and affinity for sorbing hydrophobic organic contaminants. Together, these different matrices in soil control the PAH bioavailability to receptors such as soil invertebrates and human beings via various exposure pathways. In terms of soil remediation, numerous recent studies have demonstrated the prominent effectiveness of condensed, black carbon particles, such as biochar or activated carbon (AC), in reducing the bioavailability of hydrophobic contaminants in ecological receptors.

The primary objective of this research was to investigate how different PAH source materials and geochemical soil components interact with each other and affect the

overall sorption capacity for PAHs and the freely dissolved concentrations measured by passive samplers. Subsequently how these interactions can further affect the uptake of PAHs by both ecological receptor, such as soil invertebrates, as well as human beings through dermal contact and incidental ingestion of contaminated soils are investigated. For each of these exposure pathways, equilibrium and kinetic models have been developed to explain and predict PAH bioavailability to different receptors. The performance of the models has been evaluated under different source materials. In general, these models can give adequate predictions of PAH bioavailability with deviation falling within one log unit when evaluated across a wide range of soil concentrations. In terms of soil remediation, biochar amendment has exhibited promising and varying effectiveness in reducing PAH bioavailability from soils to different receptors. The effectiveness also varies with different source materials.

### MEASURING AND REDUCING BIOAVAILABILITY OF PAHS IN SOILS

By

Huan Xia

### Dissertation submitted to the Faculty of the Graduate School of the University of Maryland, Baltimore County, in partial fulfillment of the requirements for the degree of

### **Doctor of Philosophy**

2017

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# Dedication

To my beloved parents who have supported and allowed me to become who I am.

### Acknowledgements

This has been a truly wonderful journey. There are so many amazing people I would like to thank for enriching my life here in Baltimore. First of all, I would like to express my sincere gratitude to my advisor, Dr. Upal Ghosh for his guidance when I was lost, his encouragement when I'm in doubt, and his patience when I was stumbling along my way in research. Thank you again for all your support during the past six years. Second, I would like to thank Dr. Jose Gomez-Eyles for being such a great mentor and friend to me, for encouraging me, teaching me, taking care of me in and out of the lab through the tough times. Third, I would also like to thank my committee members: Dr. Chris Hennigan, Dr. Govind Rao, Dr. Judy Lakind and Dr. Lee Blaney for all your invaluable help and advices on my Ph.D. work. Also, I would like to thank my best friend Hilda Fadaei for being there for me through all the ups and downs. Thank you and all the many other awesome people for helping make this journey a memorable one. In the end, special thanks to Chesley Patterson, for all your love, support, and always being prouder of me than I am.

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## List of Abbreviations

- AC Activated carbon
- BAF Bioaccumulation factor
- BC Black carbon
- BCF Bioconcentration factor
- DOC Dissolved organic carbon
- DOM Dissolved organic matter
- GAC Granular activated carbon
- HOC Hydrophobic organic contaminant
- Kow Octanol-water partitioning coefficient
- MGP Manufactured gas plant
- NAPL Non-aqueous phase liquid
- NOM Natural organic matter
- OC Organic carbon
- PAC Powdered activated carbon
- PAH Polycyclic aromatic hydrocarbon
- PE Polyethylene
- PCB Polychlorinated biphenyl
- PDMS Polydimethylsiloxane
- POM Polyoxymethylene
- TOC Total organic carbon

### Chapter 1: Introduction

#### **1.1 PAH Sources to Soils**

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic contaminants which are ubiquitous in the environment. They are of particular concern due to their toxic, mutagenic and/or carcinogenic properties to both human beings and ecosystem receptors. PAHs are released into the environment either as by-products of incomplete combustion of carbon fuels (such as diesel) and pyrolysis processes (pyrogenic PAHs), or as spills of petroleum products (petrogenic PAHs). Table 1 summarizes the PAH sources of natural, industrial, and non-industrial origins, and the primary PAH-bearing materials produced by these sources (Ruby et al., 2016). Pyrogenic PAHs are often emitted within a matrix of tar, pitch, or black carbon (BC)-like products such as coke, char, or soot, while petrogenic PAHs are released within a non-aqueous phase liquid (NAPL) matrix (e.g. crude oil, fuel oil or other petroleum distillates). These matrices, especially BCs, have been shown to provide strong sorption domains for PAHs (Accardi-Dey and Gschwend, 2001; Cornelissen et al., 2006; Cornelissen et al., 2005a; Jonker and Koelmans, 2002; Jonker et al., 2003a; Khalil et al., 2006). The partitioning in BCs is found to be up to two orders of magnitude stronger than in natural organic matter (Cornelissen et al., 2005a; Hong et al., 2003).

### **1.2 PAH Sorption to Black Carbon and Organic Matter**

Black carbons are mainly comprised of chars and soot that may have PAHs occluded within their structure. The residual solid carbon mass that remains after partial

combustion or pyrolysis of biomass is termed char (e.g., charcoal from wood). Coke is a form of char that is produced from the pyrolysis of coals and may be found in significant quantities in soils and sediments near industrial sites that historically used coal for energy or raw material (Khalil et al., 2006). Chars can have a wide range of particle sizes, depending on the size of the starting material and the nature of the physical breakdown process. Soot is produced through a condensation mechanism from vapor phase organics during a combustion process under an insufficient oxygen environment. It can be produced in oil flames, diesel engines, forest fires, and also through industrial manufacturing processes. Because of the widespread production, use, and release of BCs in the environment, significant amounts of BCs are found in soils. Cornelissen et al. found BC typically constitutes 1-6% of total organic carbon (TOC) in a range of agricultural and urban soils (Cornelissen et al., 2005a), although values of BC of up to 45% of the TOC in a soil have been reported in soils with ancient fire management (Skjemstad et al., 2002). BC products reduce the availability of the PAHs occluded within them, but also have the ability to sorb other PAHs and organic compounds they contact, rendering them unavailable. This strong sorption of PAHs and other hydrophobic organics has led to remediation of contaminated soil and sediment through the addition of BCs such as activated carbon or bio-chars to reduce contaminant bioavailability (Zimmerman et al., 2004, Beesley et al., 2011b; Ghosh et al., 2011).

Aside from BC-like materials, PAHs also bind to other types of organic matter usually present in soils, like natural organic matter or coal (Cornelissen et al., 2005a). The dual-mode sorption concept (Xing and Pignatello, 1997) provides a simplified way of looking at the complex mixture of carbons in soils:

$$K_D = f_{OC} K_{OC} + f_{BC} K_{BC} C_W^{n-1}$$
 equation (1)

where  $f_{OC}$  and  $f_{BC}$  are the mass fractions of OC and BC in the soil;  $K_{OC}$  and  $K_{BC}$ are the sorption coefficients for OC and BC, respectively; C<sub>w</sub> is the HOC concentration in aqueous phase and n is the nonlinear coefficient. In this model, the organic carbon in the soil is assumed to be composed of two domains, one displaying linear and noncompetitive absorption or partitioning (amorphous organic matter) (Schwarzenbach et al., 2003), and the other showing nonlinear, extensive, and competitive surface adsorption (e.g. BC), which is mainly composed of elemental carbon and some inorganic compounds) (Cornelissen et al., 2005a). Most types of organic carbon in soil will have a mixture of both absorption and adsorption sites, ranging from absorption dominated sorption in natural organic matter to more competitive adsorption dominated sorption in more carbonized BC-like products (Chen et al., 2008). The PAH fraction weakly absorbed within the amorphous organic matter or adsorbed on mineral surfaces can be defined as the rapidly desorbing fraction (Cornelissen et al., 1998), and is widely regarded as the PAH fraction potentially available for uptake or biodegradation by soil organisms. The PAHs strongly adsorbed to the surface, or residing within narrow nanopores of more carbonized materials will have strong sorption to the carbon phase that will diminish their tendency to partition out of the sorbed phase into the aqueous phase. These PAHs are considered to be part of the slowly desorbing fraction. This distinction is depicted in Figure 1 in which certain types of organic carbon, like natural organic matter (NOM) or NAPL contain only rapidly-desorbing PAHs, BCs contain primarily slowly desorbing PAHs, and some types of organic carbon, such as pitch, contain a mixture of slowly and rapidly desorbing PAHs.

However, this dual-mode sorption concept is a simplification of the actual sorption processes occurring in soils. Contaminated soils usually contain a range of types of organic matter and PAH source materials of varying sorption capacities within them. In fact, particle scale partitioning studies of soil from former manufactured gas plant (MGP) sites have demonstrated that coal tar pitch particles dominate PAH sorption, not absorption into natural organic matter particles or adsorption onto BC-like materials (Ghosh and Hawthorne, 2010; Khalil et al., 2006). Coal tars are formed when coal is carbonized to make coke or gasified to make coal gas, and have high concentrations of PAHs within them. For this reason, MGP soils and sediments are amongst the contaminated sites most heavily impacted by PAHs. Coal tar pitch is a semi-solid resinous material left behind after distilling off the lighter fractions of coal tar. This material also accumulates fine coke and soot particles (collectively termed as quinolineinsolubles) within its structure, which could contribute to the mixture of slowly and rapidly desorbing PAHs in pitch (Figure 1). It is therefore important to realize that PAH sorption is controlled, in many cases by the original source materials of the PAHs in the soil.

#### **1.3 Competition and Saturation Effects**

Another issue that is important when considering PAH sorption in soils is that adsorption to BC-like material is competitive and non-linear, so the lower the concentration of organic compounds the more likely BCs are to dominate sorption (Cornelissen and Gustafsson, 2004a; Cornelissen et al., 2005a). However, at higher concentrations, which will include not only PAHs but also other organic contaminants

and native organic compounds found in soils (e.g. natural aromatic acids), competition effects can saturate the surface adsorption sites available (Cornelissen et al., 2005a). Absorption into amorphous organic matter will gain increasing importance in these conditions. Hong et al. found that for lampblack soot-impacted soils, at low PAH concentrations sorption behavior was dominated by binding to soot, while at high PAH concentrations, the soot phase was overwhelmed and the PAH binding was dominated by organic matter (Hong et al., 2003). In these tests, the governing phase for PAH partitioning resulted in as much as 2-3 orders of magnitude difference in aqueous equilibrium concentrations of PAHs. These differences indicate that there could be large differences in PAH-soil binding behavior (including those that affect bioavailability to humans) depending on the concentration of the PAHs in the soil and the saturation of sorption sites. The concentration of PAHs at which the saturation of sorption sites occurs will largely depend on the characteristics of the BC-like materials present in the soil. BC surface area and porosity varies between different type of BCs and these parameters have been shown to correlate strongly with the capacity of BCs to sorb organic compounds (Bucheli and Gustafsson, 2000; Kleineidam et al., 2002a).

### **1.4 Aging Effects**

It is also important to consider the processes that occur during the weathering (or aging) of PAHs in soils. In this context, aging is associated with the diffusion to and retention of organic compounds into remote and inaccessible regions within the soil matrix, thus occluding the compounds from abiotic and biotic processes (Northcott and Jones, 2001). Aging mechanisms were the focus of much of the early research on organic

compound availability in soils (Alexander, 2000; Northcott and Jones, 2001; White et al., 1997). Our understanding of PAH interactions in soils has improved since then, and it is now understood that the strong sorption of PAHs to the previously discussed sorption domains is the main factor controlling the availability of PAHs in soils. The diffusion of PAHs into less accessible regions, from less strongly sorbing NAPL or tar phases into more strongly sorbing BC phases, or into even more occluded nanopores within the BC particles is enhanced with time, and has been demonstrated to reduce the subsequent removal of PAHs from the soil. With time the rapidly desorbing PAHs in soils will volatilize or be degraded by soil organisms, so as soils age they will have an increasingly recalcitrant PAH fraction left within them.

#### **1.5 Human Exposure to PAHs in Different Environmental Matrix**

Human exposure to PAHs in the environment is much more complicated due to more complex human activities. Environmental sources of PAHs include automobile exhaust, urban air pollution, drinking water, and foods. The major routes for human exposure to PAHs for the general population are breathing polluted air, consuming PAH contaminated foods, smoking cigarettes. The inhalatory exposure to PAHs for smokers or occupationally exposed workers is often considered the prominent route (Menzie et al., 1992). And these occupational exposure to PAHs may occur from breathing exhaust fumes (such as mechanics, street vendors, or motor vehicle drivers) and those involved in mining, metal working, or oil refining (Müller et al., 2004). For non-smokers, the main route of exposure is through food, especially under the process through smoking or cooking at high temperatures (grilling, roasting, and frying) are major sources of

generating PAHs (Greenberg, 1993). Meanwhile, some crops (such as wheat, rye) may also absorb PAHs or from contaminated air, water, and soil (Greenberg, 1993). Other routes include dermal contact in both occupational and non-occupational settings, such as contacting PAH contaminated soils, PAH rich chemical products such as coal tar, petroleum product (Kim et al., 2013; Menzie et al., 1992). And often times, these different exposure routes may occur simultaneously. Studies in the past have compared the magnitudes of different exposure routes for human exposure to B[a]P in the environment: for example, studies by Menzie et al. estimated a mean total PAH intake of 3.12 µg/day for non-smokers with dietary intake contributing 96.2 %, air 1.6 %, water 0.2 %, and soil 1.9 %; while for smokers, a mean total PAH intake of 5 to 8  $\mu$ g/day was estimated (Menzie et al., 1992). Although less significant compared to dietary exposure pathway, depending on the exposure duration, individual's age, and soil ingestion rate, the exposure to contaminated soil matrix could still result in cancer risks comparable to those from food ingestion (Greenberg, 1993). In this context, it is noteworthy that soil ingestion can still be a major route of exposure to PAHs, especially for children (Wilson et al., 1999, 2000, 2001), and at hazardous waste sites (Greenberg, 1993).

### **1.6 PAH Toxicity Endpoints via Different Exposure Pathways**

With benzo(a)pyrene being the first chemical carcinogen discovered in the 19th century, the different endpoints of PAH toxicities have been extensively studied ever since (Phillips, 1983). The most significant toxicity endpoints associated with human exposure to PAHs include PAH induced carcinogenesis and mutagenesis (Greenberg, 1993). It is generally recognized that PAH carcinogenic mechanisms (e.g. for benzo(a)pyrene) include metabolic transformation which creates benzo(a)pyrene diol

epoxide that can form a covalently bonded adduct with guanine in DNA, making it appear as thymine during DNA replication or repair process (Phillips, 1983). And other mechanism such as the radical cation mechanism involves generation of unstable adducts and mutations in transforming enzymes (e.g. HRAS) was also proposed (Cavalieri and Rogan, 1997). Such carcinogenic activities occurs at the site of application and the respiratory tract are often considered as the most vulnerable and prominent sites of benzo(a)pyrene induced cancer (Godschalk et al., 2000a; Schneider et al., 2002). Studies in the past have discovered that various exposure routes have different effects on the formation of DNA adduct at different target organs (Godschalk et al., 2000b). As for exposure through inhalation of PAH laden fumes, laboratory studies have shown that animals exposed to benzo(a)pyrene and other carcinogenic PAHs suffered from lung cancer, which was caused through the diolepoxide mechanism (Hecht, 1999). Some other studies have suspected inhalation of PAH-laden fumes released from combustion of soft coal to be a probable cause of high rates of oesophageal cancer in China (Roth et al., 1998). As for oral ingestion routes, extensive research has demonstrated the role of carcinogenic PAHs such as benzo(a)pyrene in the causation of upper digestive tract cancers: stomach cancer was found to be induced from ingesting PAH contaminated foods (Latif et al., 2010); tumors of the epithelium of esophagus (e.g. esophageal and colorectal cancers) might also arise from chronic exposure to dietary-borne PAHs, and especially the tongue and esophagus were found to be the major targets of tumor formation in mice (Roth et al., 1998). Aside from upper digestive tract cancers, increase in the incidence and multiplicity of lung tumors was also observed in mouse on a diet containing coal tar (Weyand et al., 1995); and Dybdahl et al. also observed a significant

increase in the level of DNA strand breaks in the rat liver and colon following oral administration of diesel exhaust particles (Dybdahl et al., 2003). It has been established that PAHs, after metabolic activation following ingestion, are capable of inducing mutations in oncogenes. These carcinogenic PAHs can also cause changes in cellular gap-junction communication, thus acting through a promotional mechanism in addition to serving as tumor initiators (Baird et al., 2005). As for dermal exposure, while some mixtures of PAHs are known to cause skin irritation and inflammation (Unwin et al., 2006), other carcinogenic PAHs are mostly reported to cause skin cancers (Godschalk et al., 2000b; Kensler et al., 1987; Lynch and Rebbeck, 2013): e.g. increased incidence of skin cancer was found in chimney sweeps and workers dermally exposed to tar containing substantial amounts of PAHs (Boffetta et al., 1997); also in the study by Godschalk et al., significant increase of aromatic-DNA adduct level was observed in rat skin following application of Benzo(a)pyrene mixture. And the skin tumors were considered to be caused through both the diolepoxide and radical-cation mechanisms.

#### 1.7 Soil Geochemistry and Bioavailability of PAHs to Humans

Research over the last two decades has produced a defensible model for how PAHs are adsorbed and absorbed to different forms of organic carbon in soils and how this process controls the availability to ecological receptors (plants, microbes, and soil invertebrates). However, there have been few studies to date addressing the effect of different organic carbon forms on the oral bioavailability or dermal absorption of PAHs from soil in humans (Ruby et al. 2016). Whether the mechanisms that control PAH uptake to ecological receptors will also serve to explain the dermal and/or oral bioavailability of PAHs from soil to humans is an outstanding question. For example, the digestive process in the gastrointestinal tract is an aggressive extraction that contains components that will enhance PAH solubility (e.g., surfactants, lipids, and proteins), suggesting that reductions in oral bioavailability due to the presence of BC in soils could be less than the reductions in aqueous concentration, although this has yet to be demonstrated. Due to the likely differences in PAH binding associated with the different sorption sites in the complex matrix of soil, this work attempts to elucidate how PAHs are distributed within soil as a function of the different carbonaceous sorption materials present. Of particular use is assessing whether there is a significant mass transfer of PAHs within the matrix they have been emitted in, or migration to other more strongly sorbing matrices already present in the soil. Greater knowledge of these mechanisms can improve our understanding of how the bioavailability of PAHs from soils to both humans and ecological receptors is likely to vary with time, and can also help evaluate the potential remediation effectiveness of *in situ* carbon amendment to contaminated soils.

#### **1.8 Remediation of PAH Contaminated Soils**

A variety of remediation approaches have been developed in the last two decades with the goal of reducing total and bioavailable PAH concentrations in soils. Traditional remediation treatments like soil excavation and chemical oxidation can significantly disturb soil properties and cause great harm to the surrounding ecosystem (Gomes et al. 2013). Phytoremediation, as well as bioremediation approaches such as the introduction of PAH degrading bacteria, fungal inocula, and endogenic earthworms have all been investigated (Hickman and Reid 2008, Hickman and Reid 2008, Gan et al. 2009, Meynet et al. 2012, Winquist et al. 2014). While these methods are environmental friendly and sustainable, they usually require very long treatment durations as a self-adaptation process is needed for extrinsic flora and fauna to function, and their remediation efficiencies tend to be quite low. For example, one major mechanism for phytoremediation is diffusion and uptake through the plant rhizosphere, the effectiveness of which is highly dependent on the available PAH concentration in the rhizosphere and its degree of hydrophobicity, which does not favor the removal of high-molecular weight PAHs (Marchal et al. 2014). The PAH bacterial degradation process is a function of PAH porewater concentration, which is typically extremely low for the five- and six-ring PAHs that drive the risk to human health (Cébron et al. 2013). Another novel alternative treatment method is to introduce carbonaceous sorptive materials such as activated carbon and biochar into contaminated soils to reduce the bioavailable PAH concentration. This approach is receiving increasing attention in recent years as it is less expensive, ecologically sustainable and has immediate effectiveness as well as outstanding efficiency in reducing bioavailability of organic contaminants. For example, 84% reduction in PAH aqueous concentrations was first observed in marine sediment that was treated with 3.4 % activated carbon (Zimmerman et al., 2004). Similar results were also reported by Brändli et al., where 84% (median) reduction of PAH porewater concentration in urban soils was achieved after addition of only 2% of powered activated carbon (PAC) while 64% reduction was observed in soils applied with granular activated carbon (GAC) (Brändli et al. 2008). In addition, carbon application was also found to be effective in reducing the bioaccumulation in soil organisms; a study by Gomez-Eyles indicated a 45 % reduction in earthworm accumulation with soil amended with biochar

(Gomez-Eyles et al. 2011). Similarly, 69 to 99% reduction of PCB bioaccumulation in oligochaete worms was found in activated carbon amended sediment with reduced PCB aqueous concentration of over 93% (Beckingham and Ghosh 2011); and 48% to 99% dibenzo-p-dioxin and dibenzofuran bioavailability reduction was observed in soils treated with PAC (Fagervold et al. 2010). While the carbonaceous material amendments represent promising site remediation strategies with proven effectiveness in reducing bioavailability to ecological receptors, it is still unclear if these treatments would be effective in reducing the risk to humans through dermal and oral exposure pathways. The impact of PAH sources (e.g. fuel oil, soot, coal tar, creosote) and soil composition on bioavailability reduction potential also needs to be investigated in order to provide more realistic remediation designs and anticipation of performance.

#### **1.9 Research Objectives**

The purpose of this research was to address the effects of soil geochemistry and PAH source materials on bioavailability and to explore the effectiveness of novel remediation treatments on reducing PAH exposure from soils to both ecological receptors and humans. Several key research questions that motivate this research and form the basis of the thesis chapters are:

- 1. How does PAH partitioning behavior change with different source materials and depletion from the source material (Chapters 2&3)?
- 2. How do source materials affect dermal uptake of PAHs, and can this process be explained by a partitioning and diffusion model (Chapters 2&4)?

- 3. How do source materials affect PAH desorption in the gastrointestinal environment, and can this process be explained by a modified partitioning model (Chapter 5)?
- 4. How do PAH source materials and soil compositions affect PAH uptake in earthworms, and can this uptake process be predicted using a bio-uptake model (Chapter 6)?
- 5. How does PAH bioavailability to different receptors change in response to application of biochar amendment, and does the remediation efficiency vary with different sources materials (Chapters 4,5 &6)?

### **1.10 Outline of the Dissertation**

This dissertation is presented as a collection of six chapters. The first chapter provides an introduction to the topic areas of the dissertation, including PAH fate and transport in the environment, and various exposure pathways to humans and ecological receptors. Chapters 2 and Chapter 5 are written as stand-alone papers that are either published, or submitted to peer-reviewed journals. Chapters 3, 4, and 6 are being worked towards submission for publication.

In **Chapter 2**, the effects of PAH sources, soil properties, PAH concentration, and soil aging on PAH bioavailability have been investigated, as well as the relative importance of each of these factors and how they interact with each other. To address this knowledge gap, a library of 30 weathered artificial soils were constructed to have a better control of the different factors likely to affect PAH bioavailability. These factors include the effects of typical PAH source materials (fuel oil, soot and coal tar based skeet

particles), different forms of organic matter in soil (peat and humus), different PAH concentrations across four orders of magnitude, and other soil components such as clay, sand, and native black carbon. PAH sorption to the different PAH sources and soil components have been measured in isotherm studies and different modeling approaches have been tested to explain the PAH partitioning behavior. An in-vitro dermal uptake study has been performed to illustrate the effect of PAH partitioning on a key exposure pathway for humans using several selected soils. The soil construction and partitioning characterization part of this study was supported by SERDP Project ER-1743. I was primarily responsible for conducting laboratory experiments, data analysis and writing the manuscript, which was published in the journal of *Environmental Science and Technology*, 2016, 50 (7), pp 3444–3452.

**Chapter 3** was developed to address one interesting observation from Chapter 2, that the depletion of PAHs from weathered skeet particles led to an increased sorption coefficient. This observation is of particular importance to an improved understanding of the PAH partitioning in source materials and soil/sediment environment, and the effect of PAH depletion on typical source material partitioning has never been studied. It is therefore reasonable to hypothesize that this partitioning phenomenon observed with skeet may also be applied to other typical PAH sources like fuel oil and soot. The aim of this Chapter was to investigate of the effect of PAH depletion on partitioning behavior in other typical source materials including fuel oil No. 6 and soot.

The objective of **Chapter 4** was to extend the dermal uptake study initiated in Chapter 2 to explore the effect of PAH concentrations and biochar amendment on PAH dermal uptake. Modeling approaches including both steady state and non-steady state diffusion scenarios have been explored in order to explain and predict PAH dermal uptake by humans from soils. Eventually, a mechanistic model was developed, which was able to adequately predict the dermal uptake (estimated using pig skin as a surrogate for human skin) within a factor of 10 across a large range of soil concentrations. The model performance and the effectiveness of biochar amendment on dermal bioavailability has been evaluated under different source materials.

In Chapter 5, the other important human exposure pathway to PAHs in soils, oral ingestion of PAH impacted soils, has been discussed. The work in this Chapter improves methods for measuring *in vitro* PAH oral bioavailability from soils using physiologically based extraction tests and evaluates PAH bioaccessibility from a library of soils previously constructed with typical PAH source materials, different forms of soil organic matter and components and different PAH concentrations. PAH bioaccessibility was measured and compared under different PAH sources, concentrations and soil components (including biochar amendment), and modeling approaches using partitioning theory (e.g. freely dissolved PAH concentration and partition constants) have been tested to explain PAH oral bioaccessibility. This was a collaborative project with other researchers (J. Gomez-Eyles) and consultants (M. Ruby and Y. Lowney) under the SERDP Project ER-1743. My contributions to this work included improving the method for physiologically based extraction test (PBET), conducting all the PBET experiments with constructed soils, interpreting results, developing partitioning model to predict oral bioaccessibility and writing the manuscript, which has been submitted to the journal Environmental Pollution.

The objective of **Chapter 6** was to investigate the effects of PAH source materials and biochar amendment on PAH biouptake into soil invertebrates (*Eisenia fetida*) and to use kinetic uptake models to explain PAH bioaccumulation in ecological receptors from soils. In this Chapter, a bioaccumulation study was carried out by exposing adult earthworm *E. fetida* in both biochar-treated and untreated soils with a variety of PAH sources and PAH concentrations for 30 days. The uptake of PAHs in *E. fetida* was measured and assimilation efficiencies of PAHs via soil ingestion were measured for individual soils. With experimentally determined parameters (e.g. PAH soil and porewater concentrations) and those from literature (e.g. dermal uptake and elimination rates), a first-order kinetic model inclusive of both dermal and dietary ingestion and elimination pathways has been developed to explain the uptake of PAHs into *E. fetida* from soils. The performance of the kinetic model has been assessed by comparing with observed results.

**Chapter 7** summarizes the key findings and conclusions with regard to each of the research questions from previous chapters. It also points out the strength and limitations of the current work, and provides recommendations for future research.

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**Figure 1.** PAH availability for oral or dermal absorption as a function of PAH source materials and soil chemistry. (Ruby et al., 2016)

Type of		
source	PAH source	Primary PAH-bearing materials
Natural	Forest fires	Soot, char
	Grass fires	Soot, char
	Volcanic eruptions	Soot, char
	Oil seeps	weathered crude petroleum
Industrial	Manufactured gas plants	Coal tar, pitch, chars, soot
	Coking operations	Coal tar, coke, soot
	Aluminum production	Coal tar pitch (making and disposing of anodes)
	Foundries	Coal tar pitch, soot, creosote, fuel oil (used in making
		sand casts at some facilities)
	wood treating	Creosote
	Carbon black	Soot, various NAPLS (crude oil, fuel oil, diesel, etc.)
	manufacture	Soot oil tar
	manufacture	Various NAPLs (crude oil fuel oil waste oil diesel iet
	Fuel spills and/or disposal	fuel)
Non-industrial	Olivert	Or al tan (use of an his day in tanyata)
sources	Skeet	Coal tar (used as binder in targets)
	Asphalt sealants	Coal tar
	Landillis	Creosole (treated wood), soot, char
	hospital industrial)	Sect
	Open burning	Soot char
	Fire training	Soot char
	i ne training	(covered above)
	Auto/truck emissions	Soot

**Table 1.** Sources of PAHs to soils<sup>a</sup> (from (Ruby et al., 2016)).

<sup>a</sup> Includes both current and historical sources of PAHs to soils

## Chapter 2: Effect of PAH Source Materials and Soil Components on Partitioning and Dermal Uptake

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#### Abstract

The bioavailability of Polycyclic Aromatic Hydrocarbons (PAHs) in soils can be influenced by the source material they are emitted within, the properties of the receiving soil, weathering processes, and the concentration of PAHs. In this study 30 contaminated soils were constructed with common PAH sources (fuel oil, soot, coal tar based skeet particles) and direct spike with a solvent added to different types and contents of soil organic matter and minerals to achieve PAH concentrations spanning four orders of magnitude. Source material had the greatest impact on PAH partitioning. Soils containing skeet generally exhibited the highest K<sub>D</sub> values, followed by soot, fuel oil and solvent spiked soils. Among all soil compositions, the presence of 2% charcoal had the largest enhancement of  $K_{D}$ , with the biggest increase observed for soils spiked with solvent (one to two log unit increase). Partitioning behavior could not be predicted by an organic carbon and black carbon partitioning model. Including independently measured partitioning behavior of the soil components and PAH sources allowed better prediction but still suffered from issues of interaction (oil sorption in peat) and highly nonlinear partitioning with depletion (for skeet). Dermal absorption of PAHs measured using pig skin was directly related to the freely dissolved aqueous concentration in soil and not the total concentration in the soil. Overall, we show that PAH source materials have a

dominating influence on partitioning and need to be part of the matrix in soil bioavailability and risk assessments.

## **2.1 Introduction**

Polycyclic Aromatic Hydrocarbons (PAHs) are often released into soils either as by-products of combustion and pyrolysis processes such as coal tar, pitch, char, and soot, or as spills of petroleum products such as crude oil, fuel oil, or other petroleum distillates. These matrices, especially the black carbons (BC) such as soot or char have been shown to provide strong sorption domains for hydrophobic organic compounds (HOCs) like PAHs, (Accardi-Dey and Gschwend, 2001; Cornelissen et al., 2006; Cornelissen et al., 2005a; Jonker and Koelmans, 2002; Khalil et al., 2006) resulting in partition coefficients that are several orders of magnitude higher than sorption to natural organic matter (NOM) (Cornelissen et al., 2005a; Hong et al., 2003). The HOC fraction reversibly absorbed to natural organic matter, or weakly adsorbed onto mineral surfaces can be released rapidly and is regarded as potentially available for biodegradation or uptake by organisms. The HOCs strongly adsorbed to the porous surfaces or occluded within BC exhibit slow desorption behavior and low bioavailability to organisms (Cornelissen et al., 2005a). However, PAH source materials described above such as pitch have sorption characteristics that may be difficult to describe as either NOM or BC, and are difficult to model based on a simple two domain carbon model (Accardi-Dey and Gschwend, 2001). For example, particle scale partitioning studies of soil from former manufactured gas plant (MGP) sites have demonstrated that source coal tar pitch particles dominate PAH sorption, not absorption into NOM or adsorption onto BC (Ghosh and Hawthorne, 2010; Khalil et al., 2006). PAH sorption, and hence bioavailability, therefore depends not only on soil geochemical properties (e.g. NOM and BC content), but also on the source of PAH contamination which challenges the development of uniform cleanup criteria based on total concentration of PAHs in soils. For example, study from Stroo et al. demonstrated for lampblack impacted soils, that the estimated cancer risk was reduced by 97% when accounting for measured dermal absorption of native PAHs (Stroo et al., 2005).

Since PAH adsorption to BC such as soot and char is non-linear, in soils with lower PAH concentrations, sorption is more likely to be dominated by BC; while higher PAH concentrations can result in adsorption site saturation on carbonaceous materials, thereby increasing absorption into NOM phases (Cornelissen and Gustafsson, 2004a; Cornelissen et al., 2005a). Study from Hong et al. found that for oil and lampblack sootimpacted soils; at low PAH concentrations sorption behavior was dominated by binding to soot, while at high PAH concentrations, the soot phase was overwhelmed and the PAH binding was dominated by the residual oil phase (Hong et al., 2003).

While there is general understanding that PAH bioavailability in soil can be influenced by many factors including PAH sources, soil properties, PAH concentration, and soil aging, the relative importance of each of these factors and how they interact with each other is not well understood. To address this knowledge gap, we constructed a library of 30 weathered artificial soils to have a better control of the different factors likely to affect PAH bioavailability. These factors include the effects of typical PAH source materials (fuel oil, soot and coal tar based skeet particles), different forms of organic matter in soil (peat and humus), different PAH concentrations across four orders of magnitude, and other soil components such as clay, sand, and black carbon. PAH sorption to the different PAH sources and soil components were measured in isotherm studies and different modeling approaches were tested to explain the PAH partitioning behavior. Finally, an in-vitro dermal uptake study was performed to illustrate the effect of PAH partitioning on a key exposure pathway for humans.

#### 2.2 Materials and Methods

#### 2.2.1 Construction of PAH Contaminated Soils

A baseline synthetic soil (BSS) was used as the basis for all the soils constructed. The composition of this soil was adapted from an artificial ASTM soil used for toxicity testing (2004) and consisted of 10 percent peat moss (Miracle-Gro, Enriched Sphagnum Peat Moss), 20 percent kaolin clay (ACROS Organics, CAS:1332-58-7) and 70 percent silica sand (quality ground silica, SIL-CO-SIL). Calcium carbonate (0.4% by mass) was added to the soil mixture to adjust the pH to around 7. Since human exposure was part of the focus of this study, all individual soil components were ground and sieved down to < 150um, which simulated the size fraction that easily adhere to human skin (Ruby and Lowney, 2012).

Thirty 1 kg batches of BSS were prepared with different formulations as described in Table 1. These different formulations encompassed different amounts of PAH source materials including field weathered coal tar based skeet (shooting target) particles obtained from a Navy skeet range, Jacksonville, FL (provided by John Schoolfield, Naval Facilities Engineering Command), lampblack soot particles (from Fisher Scientific [Catalog No. 1333-86-4]) and fuel oil No.6 (from Chevron) to obtain a wide range of PAH concentrations. A series of soils were also spiked directly with a stock solution of PAHs in solvent (dichloromethane). PAH concentrations in this solution are given in Table S1. Due to the relatively low PAH levels in the fuel oil and soot, these source materials were also spiked with this stock solution to a target concentration of 200 mg/kg benzo(a)pyrene (B(a)P). Soot was spiked by adsorption from water using the method described in (Rust et al., 2004).

Changes were also made to the composition of the BSS in select soils as shown in Table 1. These included reduced peat content to 1%, reduced clay to 2%, substituting humus for peat or increased black carbon content by the addition of 2% charcoal (S79959, Fisher Scientific). Changes in the mass fraction of each soil ingredient were compensated by replacing it with silica sand. PAH source materials (solvent spike, fuel oil, soot and skeet particles) were then introduced and different PAH concentrations (as 0.1, 1, 10 and 100 mg/kg B(a)P) were targeted by spiking 0.1, 1, 10 and 100 ml of solvent stock solution; 0.5, 5, 50, 500 g of spiked soot particles; 0.022, 0.22, 2.22, 22.2 g of skeet particles (unspiked), and 0.5, 5, 50 g of spiked fuel oil, respectively, into the amended soils. Solvent spiked soils were left under the fume hood overnight for solvent dissipation after spiking. Deionized water was then added into the constructed soils at 4:1 mass ratio of water to soil in glass jars to create slurries, and were then placed on a roller for three weeks to ensure a homogenous distribution of PAH within the soils and weathered<sup>13</sup> for 8 weeks as described in the supplemental information.

#### 2.2.2 PAH Concentration in Soils and Source materials

Approximately 2 g of each weathered soil/source material samples were extracted in triplicate following EPA method 3550B (Test Methods for Evaluating Solid Waste, Physical/Chemical Methods) with three volumes of 40 mL each of acetone-hexane mixture (50:50) and sonicating the slurry for 6 minutes (pulsing for 30 seconds on and 30 seconds off). Silica gel cleanup was performed on the soil extracts following EPA Method 3630C, and PAHs were analyzed using an Agilent GC (Model 6890) with a mass spectrometer detector following EPA method 8270. Surrogate recovery was measured using deuterated [D-10] phenanthrene and was generally acceptable within the range of 85% to 110 %.

## 2.2.3 TOC and BC content in soils

Total organic carbon (TOC) was measured using a Shimadzu TOC analyzer with a solids sample module (TOC-5000A and SSM-5000A) by combustion at 900 °C after removal of inorganic carbon with hydrochloric acid. BC content in each soil was measured using a chemo-thermal oxidation method (CTO-375) (Grossman and Ghosh, 2009).

#### 2.2.4 Soil/Source Material Aqueous Equilibrium Experiment

The freely dissolved PAH concentrations in each test soil and source material were determined by equilibrating the soils/sources in a sterile aqueous solution (containing 100 mg/L sodium azide) with 76- $\mu$ m-thick polyoxymethylene (POM) strips (CS Hyde Company, IL, USA). The mass of POM used was adapted to each soil to ensure negligible depletion of the matrix or porewater concentration when equilibrium is reached (Table S6). The mixtures were placed on a shaker at a speed of 150 rpm and agitated for a month. POM strips were then removed, rinsed with DI water, cleaned with kimwipes and extracted in an acetone: hexane (50:50) mixture (3 x 24h, with sequential extracts pooled). The POM extracts were then cleaned up and analyzed in the same way as the soil extracts. Freely dissolved PAH concentrations (C<sub>w</sub>) were calculated using

experimentally determined PAH partitioning coefficients for POM ( $K_{POM}$ ):  $C_W = C_{POM}/K_{POM}$ ; (Hawthorne et al., 2011) and equilibrium partitioning coefficients ( $K_D$ ) for each soil was calculated using  $K_D = C_{SOIL}/C_W$ . The partition coefficients of the source materials ( $K_{skeet}$ ,  $K_{soot}$ , and  $K_{fuel-oil}$ ) were calculated similarly.

#### 2.2.5 Sorption Isotherms for Soil Components

Partitioning of four representative PAHs with different numbers of aromatic rings (phenanthrene (PHE), pyrene (PYR), B(a)P and benzo(g,h,i)perylene (B(ghi)P)) was investigated in four soil components including kaolin clay, sand, peat moss, and charcoal. A slurry was made by mixing soil components with sterile water (containing 100 mg/L sodium azide) at a mass ratio of 1:4 for sand, clay, and peat and a mass ratio of 1:1000 for charcoal. POM strips were added into the slurries to measure the aqueous PAH concentrations. PAHs were spiked into the slurry in an acetone solution (the volume of the acetone spike was always < 1% to avoid co-solvent effects). The mixture was then sealed, and placed on an orbital shaker for a month to allow for equilibrium. For each soil component, PAH equilibrium concentrations across four orders of magnitude were created by varying the amount of PAH stock spiked and the mass of POM used (Table S7). The concentration range selected was based on aqueous PAH concentrations for the solvent spiked soils in the previous aqueous equilibrium experiment. PAH concentrations in the soil components were calculated by mass balance assuming no PAH losses. Equilibrium partition coefficients (K<sub>i</sub>) for each soil component 'i' (sand, clay, peat) were calculated as the ratio of PAH concentration in soil component divided by concentration in water. Adsorption data for charcoal were fitted using a Freundlich equation, to describe the adsorption of PAHs on the charcoal material:  $C_{charcoal} = K_{F-charcoal} C_{W}^{n}$ ; where

 $C_{charcoal}$  is the PAH concentration in charcoal. Freundlich sorption coefficient  $K_{F-charcoal}$  and *n* were estimated by fitting the model to the measured adsorption isotherm.

#### 2.2.6 Dermal uptake of PAHs from soil.

PAH dermal uptake was measured in-vitro using pig skin as described in detail in the supplemental information.

#### **2.3 Results and Discussion**

#### 2.3.1 PAH Levels in Source Materials and Weathered Soils

The sum of the 16 EPA priority pollutant PAHs in skeet was 54,900 mg/kg, which was the highest level of native PAHs among the source materials (Figure S1). Soot and fuel oil were spiked with additional PAHs to reach target B(a)P concentration of 200 mg/kg and the achieved B(a)P concentrations were  $273\pm134$  mg/kg and  $197\pm3$  mg/kg in soot and fuel oil, respectively (Figure S1). After introducing the source materials and weathering for 8 weeks, each soil was analyzed for PAHs. As shown in Figure 1, the B(a)P concentrations in weathered soils were lower than the target concentration of 1 mg/kg in all soils, and this trend was also observed for other PAH compounds and in soils with different target concentrations (0.1, 10 and 100 mg/kg; Supplemental Table S2). The soils spiked with PAHs in solvent had the largest PAH losses (e.g. over 70% loss of B(a)P, as these PAHs were introduced freely into the soil and were therefore more prone to abiotic losses. There was also considerable PAH losses from the fuel oil soils, indicating that PAHs are available for losses in the degradable fuel oil matrix. For the skeet and soot spiked soils, the final concentration of B(a)P was close to 0.8 mg/kg compared to the target of 1 mg/kg for these soils. The smallest losses of PAHs were

observed in skeet soils, which was likely because PAHs are known to be strongly bound in the pitch matrix contained in skeet, especially after long-term field weathering. PAH concentrations in the soot soils showed higher variability compared to other source materials (Figure 1) despite multiple attempts to further homogenize these soils. It is likely that the soot particles were heterogeneous in PAH content and potential agglomeration of the hydrophobic soot particles during the wetting and drying cycles produced soot aggregates that were difficult to re-homogenize fully at the scale of samples taken for PAH analysis.

## 2.3.2 PAH Partition Coefficients for Soil Components and Source Material.

Measured partition coefficients for soil components and PAH source materials are reported for four representative PAHs (Figure 2 and Table S3). As expected, soil mineral components (sand and clay) showed the weakest sorption of PAHs compared to the organic components. Compared to sand and clay, PAH sorption to peat was nearly 3 orders of magnitude stronger and sorption to charcoal was nearly 5 orders of magnitude stronger. Despite some reports in the literature on the relevance of PAH sorption to clays (Yang et al., 2013), it is abundantly clear that in the presence of typical organic matter content of a few percent by weight, the influence of the mineral components is going to be negligible. The reduction of clay content has no influence on overall soil  $K_D$  (Figure S2). The K<sub>peat</sub> values measured in this study were in line with those reported by Gidley et al.(e.g. log K<sub>peat</sub> of 3.85 and 4.82 for phenanthrene and pyrene from (Gidley et al., 2012)). All soil components exhibited linear sorption except charcoal.

Sorption nonlinearity was observed with stronger sorption at lower PAH concentration in charcoal as shown in Figure 2. The observed sorption coefficients for

charcoal in this study (e.g. log K<sub>charcoal</sub> =  $5.40 \sim 7.10$  for phenanthrene) were in line with the findings on coal carbon (e.g. log K<sub>coal</sub> =  $6.3 \sim 6.8$ ), fusinite charcoal (e.g. log K<sub>charcoal</sub> = 5.57), and biochar (e.g. log K<sub>biochar</sub> =  $5.38 \sim 6.60$ ) in other literature (Cornelissen and Gustafsson, 2004b; Kleineidam et al., 2002b), but lower than those for activated biochar (e.g. log K<sub>biochar</sub> = 7.52) and activated carbon (e.g. log K<sub>AC</sub> = 8.71) reported by Gomez-Eyles et al., 2013.

While the sorption experiments with soil components were performed as isotherm studies with new PAHs being added, the studies with the source materials were performed based on desorption equilibrium of native PAHs. The PAH partition coefficients for the source materials were all high and comparable to charcoal (Figure 2). The log  $K_{SOOT}$  values ranged from 5.4 to 9.3 for different PAH compounds, which is within the range of those reported in other studies (Jonker and Koelmans, 2001, 2002). Coal tar pitch based skeet particles also exhibited high sorption for PAHs with log  $K_{SKEET}$ ranging from 5.1 to 8.6 for phenanthrene (with minimal depletion at lowest POM/skeet mass ratio of 0.125), which are much higher than those reported for coal tar pitch in the literature (e.g. log value of 4.55 to 5.08 for phenanthrene) (Ghosh and Hawthorne, 2010; Khalil et al., 2006) (Figure 2). These elevated partition coefficients are most likely the result of specific processing and extensive weathering in the field (Khalil et al., 2006). In addition, exceptionally high partition coefficients (from log value of 5.4 to 9.3) were also observed for fuel oil which was a dense, viscous phase consisting of petroleum hydrocarbons. Previous studies also found high sorption capacity for PCBs and PAHs in light gasoil (Distillate Marine grade A) and light crude oils (Arabian Crude Light), which were even superior to soot particles (e.g. log K<sub>OIL</sub> values close to 7.0 for PHE in both oils)

(Jonker and Barendregt, 2006; Jonker et al., 2003b). The  $K_{OIL}$  measured in this study was in the range of what would be estimated using Raoult's law for most PAH compounds (Table S3).

#### 2.3.3 TOC and BC Contents in Weathered Soils

The measured TOC content in BSS (about 2.6%) is consistent with the mass fraction of peat (10%) and its approximate 30% carbon content (Supplemental Table S4) (Gidley et al., 2012). Some peat in the prepared soils may have been degraded or lost during the weathering process. The lowest TOC and BC were observed in the solvent spiked soil of 1 mg/kg target B(a)P concentration with reduced peat while the highest TOC and BC were observed in soot spiked soil of 100 mg/kg target B(a)P concentration (mostly coming from the added soot).

## 2.3.4 Effect of Source Materials on Soil K<sub>D</sub>

The PAH source materials had a dominating influence on the overall  $K_D$  of the soils. As shown in Figure 3 for the spike level of 1 mg/kg B(a)P, solvent spiked soils had the lowest measured  $K_D$  and the skeet soils had the highest measured  $K_D$  (nearly 2 orders of magnitude higher). The fuel oil and soot soils showed intermediate  $K_D$  values. This trend was also observed among soils of other PAH concentrations (0.1, 10 and 100 mg/kg as target B(a)P concentration) (Figures S3-S5). The only exception to this general trend was the soils at the highest PAH level that showed a higher  $K_D$  for soot-spike than the soils spiked with skeet (Figure S5). The high  $K_D$  observed in soot spiked soils is consistent with the reported high sorption coefficients for black carbons in the literature (Brändli et al., 2008; Cornelissen and Gustafsson, 2004a). Compared to soot spiked soils, the skeet spiked soils surprisingly exhibited even higher  $K_D$  in the concentration range of

0.1 to 10 mg/kg of target B(a)P, even at the extremely low amount of skeet spiked into the BSS (up to 0.2 % by mass). The fact that the soot was freshly spiked before weathering, and the skeet particles were not spiked and had weathered for a much longer period in the field likely contributed to this phenomenon. The weathered coal-tar component of pitch is known to contain black carbon in the form of soot, coke, and cenospheres that get included in the tar during the production process. As the tar is further processed and weathers in the field and degradable components are lost, the residual matrix becomes enriched in the black carbon residue and takes the appearance of a hard coke-like substance. Similar enhanced sorption capacity for weathered coal tar pitch in soil/sediment has been documented in other studies (Khalil et al., 2006; Smith et al., 2011). Thus, PAH bioavailability assessments need to use field soils with natively weathered source materials to adequately characterize exposure and risk, as also suggested by (Arp et al., 2014). As illustrated in the Supplemental Figure S6, further weathering in the laboratory of soils prepared with source materials did not greatly impact partitioning, especially for the high molecular weight PAHs.

### 2.3.5 Effect of Soil Composition on PAH Partitioning in Weathered Soils

As expected based on the relative sorption capacity for PAHs, the mineral components had a small impact on overall partitioning and the biggest impact was from the presence of charcoal (Figure S2). The replacement of kaolinite with montmorillonite in solvent-spiked soils increased  $K_D$  by a factor of 2 to 24, with a more pronounced effect observed for lighter (3-ring) PAHs, but still noteworthy for the larger (5 and 6-ring) PAHs. This is in line with a study by Chai et al., where montmorillonite addition was found to reduce the desorption of hexachlorobenzene from soils by 17% (Chai et al.,

2007). The higher adsorption capacity for HOCs on montmorillonite relative to kaolinite may be attributed to the higher surface area and expandable interlayer structure associated with montmorillonite. Conversely, replacing peat with humus caused a decrease in overall soil  $K_D$ , which was likely due to the lower organic carbon content found in humus (26.3% in peat and 4.2% in humus).

To evaluate the effect of elevated native black carbon content, soils with all four PAH source materials were altered with 2% charcoal. The charcoal effect was larger for lighter PAH compounds; e.g. 151-fold increase in  $K_D$  was observed for PHE and 54-fold increase for B(a)P in the solvent spiked soil. The effect of charcoal was the greatest in the solvent spiked soils where the native soil  $K_D$  was the lowest, and the effect was lowest for the fuel oil and soot-spiked soils (Supplemental Figure S7). In the fuel oil spiked soils, we hypothesize that the charcoal surface was fouled due to the excess oil hydrocarbons rendering it less effective at increasing the  $K_D$  as greater fouling effects of oils on black carbons have been reported previously (Choi et al., 2012; Hong and Luthy, 2007). In the soot spiked soils, the sorption capacity of the source material was already high and the presence of charcoal had a smaller impact ( $K_D$  decrease by a factor of 3 to 18). For skeet spiked soils, an overall  $K_D$  increase of more than a factor of 10 was observed for most PAHs.

The extensive sorption of HOCs in black carbons such as charcoal has been widely documented in other studies (Accardi-Dey and Gschwend, 2001; Cornelissen and Gustafsson, 2004a; Ghosh and Hawthorne, 2010), and black carbon amendment has been shown to reduce pollutant bioavailability in soils (Beesley et al., 2011a; Denyes et al.,

2013; Gomez-Eyles et al., 2011b). We show here for the first time that PAH source material sorption capacity influences the observed effectiveness of soil black carbon in increasing partitioning.

## 2.3.6 Modeling Partitioning Based on TOC and BC

As shown in Figure 3, the model predictions based merely on natural organic carbon (OC) partitioning ( $K_D = f_{OC}*K_{OC}$ ) s(Xing and Pignatello, 1997) under-predicts sorption in the soils. The BC-inclusive dual domain model ( $K_D = f_{OC}*K_{OC} + f_{BC}*K_{BC}*C_w$ <sup>n-1</sup>) (Koelmans et al., 2006) appears to predict reasonably for some cases, especially phenanthrene and pyrene for solvent/soot/oil spiked soils, but greatly under-predicts partitioning for all compounds for skeet-spiked soils. Thus, the traditional approach for modeling HOC partitioning in soils and sediments using a OC + BC sorption model is not able to describe the observed behavior, especially in the presence of weathered source materials. When assuming all the carbon (OC+BC) in skeet and soot spiked soils sorb similarly to coal tar as done by Arp et al. 2014, the predicted  $K_D$  values are improved for soot and skeet spiked soils and fall within one order of magnitude difference from measured  $K_D$  (see supplemental information for modeling details). However, the coal-tar model is not able to predict the observed partitioning for B(a)P for solvent or fuel-oil spiked soils within an order of magnitude.

#### 2.3.7 Soil Components and Source Material Inclusive Sorption Model

An alternative model was constructed using the measured partition coefficients of the soil components and source materials and assuming all components come to a thermodynamic equilibrium:

$$C_{s} = f_{clay}K_{clay}C_{W} + f_{sand}K_{sand}C_{W} + f_{peat}K_{peat}C_{W} + f_{charcoal}K_{charcoal}C_{W}^{n} + f_{s}K_{s}C_{W} \quad (Eq \ 1)$$

where  $C_s$  is the PAH concentration in soil;  $f_{clay}$ ,  $f_{sand}$ ,  $f_{peat}$ ,  $f_{charcoal}$  and  $f_s$  are the mass fractions of clay, sand, peat, charcoal and source material in soil, respectively;  $K_{clay}$ ,  $K_{sand}$ ,  $K_{peat}$ ,  $K_{charcoal}$  and  $K_s$  are the partition coefficients for clay, sand, peat, charcoal and source material, respectively. PAH aqueous equilibrium concentration  $C_w$  was calculated using equation 1 and the overall soil partitioning coefficient  $K_D$  was predicted for each case and compared with measured values (Figure 4).

For the solvent spiked soil the overall soil sorption capacity for PAHs was estimated within an order of magnitude by the addition of each individual contributing component. The observed high  $K_{charcoal}$  explained the elevation of overall  $K_D$  in solvent spiked soils containing charcoal. The model generally underestimated soil  $K_D$  (by little over one log unit) for the case where peat content was reduced to 1%.

For the fuel oil spiked soils, the model generally overestimated soil  $K_D$  except for the lightest PAHs (PHE). Model overestimation of  $K_D$  increased with increasing fuel oil concentrations (Supplemental Figure S9). Based on the measured partition coefficients, fuel oil would dominate PAH sorption relative to sorption to peat especially for the high molecular weight compounds. However, previous studies have shown that at low oil fraction in soil, a distinct oil phase is absent as the individual hydrocarbons in oil are absorbed into sediment organic matter, leaving the organic matter as the dominant sorption domain in the system. (Jonker et al., 2003b) found that a separate oil phase was not present when oil accounted for less than 15% of the organic carbon in soils. This is confirmed by the similarity in  $K_D$  between the fuel oil and solvent spiked soils in this study except at the highest oil dose (10 mg/kg target BaP), where fuel oil accounted for 50% of the TOC (Supplemental Figure S10). For the soils spiked with PAH-laden soot, the model predictions of  $K_D$  are within an order of magnitude of the measured values (generally on the higher side). Based on Figure 4, no general trend was observed for the model performance with increasing  $K_{OW}$ . Based on the high  $K_{SOOT}$  values (Table S3), soot would be the dominating sorption phase over peat, sand, and clay for soils with soot mass fraction above 0.5%.

Using  $K_{SKEET}$  presented in Table S3 (with minimal depletion), the modeled soil  $K_D$  was often more than one log unit lower for most skeet spiked soils (Figure 4). For PHE and PYR, the only observed soil  $K_D$  that fell within an order of magnitude of our prediction were from the soil with charcoal addition at 1 mg/kg target B(a)P. In this case, our model predicted charcoal to dominate the sorption for PHE and PYR when the mass of skeet was low (as 0.02%). When charcoal was not present, peat was predicted to dominate the PAH sorption in all skeet soils for all PAHs investigated until the PAH concentration increased up to 100 mg/kg (as target B(a)P). At the high PAH concentration, the domination shifted from peat to skeet due to its increased mass fraction of up to 2.2% skeet and a reasonable prediction was observed for B(a)P and B(ghi)P.

### 2.3.8 PAH Distribution among Source and Soil Components

To better understand some of the observed deviations in the model predictions for soot and skeet, the PAH mass distribution between source material and soil components at thermodynamic equilibrium was calculated based on Equation 1 (Table S5). Using the  $K_D$  values measured at minimal depletion, at equilibrium up to 97% of the PAHs are predicted to redistribute from the skeet into the peat. However, previous studies with weathered pitch suggest such a large redistribution of PAHs is very unlikely. Ghosh et al., found that under strong extraction conditions (using Tenax beads as an infinite hydrophobic sink), the PAH mass fraction released from coal tar pitch materials in sediment did not exceed 40% to 70%. Therefore, it is unlikely that PAH redistribution of 97% from weathered skeet into peat would take place in our study, and the skeet impacted soils are either far from equilibrium or the K<sub>SKEET</sub> values are increasing drastically as PAHs are being desorbed into the soil matrix.

To test the latter hypothesis, a modified equilibrium study was performed using a range of skeet to passive sampler mass ratios to create different levels of PAH depletion from skeet. As shown in Supplemental Figure S11, with only 0.3% of B(a)P mass extracted from skeet (at POM:skeet mass ratio of 500), the K<sub>SKEET</sub> value increased by three orders of magnitude. The model predictions for skeet soils are generally within an order of magnitude of the observed values when  $K_{SKEET}$  is assumed to increase by 2-3 orders of magnitude as a result of depletion (Figure S12). Previous studies have shown that PAHs in geosorbents may reside in different fractions that have different affinities and undergo different rates of desorption (Barnier et al., 2014; Cornelissen et al., 1998). What is remarkable, and demonstrated for the first time for weathered skeet, is the drastic 2-3 orders of magnitude increase in partitioning with only a small fraction loss of PAHs. The residual PAHs after a small depletion appear to be very strongly bound resulting in increasing K<sub>SKEET</sub> values with depletion. The PAH redistribution from skeet to soil is much less after considering the highly nonlinear behavior of K<sub>SKEET</sub> with PAH depletion. This highly nonlinear partitioning behavior of native PAHs in skeet (and likely other weathered coal-tar pitch materials) needs to be taken into account in predictive models where they serve as the source of PAHs.

### 2.3.9 Implications for Potential Human Exposure

We show that PAH sources to soil play a dominating role in PAH partitioning, followed by soil composition, especially the presence of native black carbon. Previous studies have demonstrated that freely dissolved concentrations of PAHs in soils are good indicators of bioavailability to soil organisms (Marchal et al., 2014; Ruus et al., 2010). Dermal uptake of PAHs in animals and humans should also be driven by the freely dissolved concentration exposed to the external skin surface. A dermal exposure study was performed using pig skin to evaluate how absorption rate is influenced by PAH sources in soil (see supplemental information). As shown in Figure 5a, the dermal uptake flux for soils with PAH introduced with solvent, fuel oil, soot, and skeet ranged over an order of magnitude which could not be explained based on PAH concentration differences in soil. When the dermal flux is plotted against equilibrium aqueous concentration for the PAHs, a strong correlation is evident (Figure 5b). Thus, we demonstrate for the first time that dermal uptake is directly related to freely dissolved aqueous concentration in soil and not the total concentration in the soil. While, this would make theoretical sense, dermal uptake studies in the past often relate uptake to concentration in soil and do not measure or correlate to aqueous concentration or partitioning (Spalt et al., 2009).

Based on the results from the present study, the bioavailability of B(a)P in soils with skeet or soot as the PAH source material may be nearly two orders of magnitude lower than soils freshly spiked with PAHs in a solvent (even after 8 weeks of artificial weathering). Many studies on soil PAH bioavailability in the toxicology literature have used radiolabeled B(a)P spikes in solvent to assess PAH exposure from oral ingestion and dermal uptake (Cavret et al., 2003; Lapole et al., 2007). Even in the presence of original source material, spiking with a fresh radiolabeled compound results in a 5-10 fold increase in observed absorption in skin<sup>8</sup>. The present results highlight the importance of using weathered field soils with native PAH source materials for bioavailability assessment of soil. This challenges traditional approaches for PAH toxicity and bioavailability studies with animals and human cadaver skin that have relied largely on solvent spiked soils, not accounting for PAH source material effects.

#### 2.4 Acknowledgements

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**Figure 1.** PAH concentrations after weathering in baseline synthetic soil prepared with different PAH source materials targeted to achieve 1 mg/kg B(a)P (n=3, error bars represent  $\pm 1$  standard deviation).



**Figure 2.** Comparison of measured partitioning coefficients (log  $K_D$  [L/kg]) for soil components and source materials for PHE, PYR, B(a)P and B(ghi)P). The dark red square represents  $K_D$  for skeet measured with minimal depletion (at POM/skeet mass ratio of 0.125) while, the orange squares represent  $K_D$  measured with increasing depletion of PAHs from skeet (by increasing POM/skeet mass ratio). For soot and fuel oil,  $K_D$  was measured at only one POM/source mass ratio. Standard error within 0.1 log unit, n=3.



**Figure 3.** Comparison of measured and predicted overall soil  $K_D$  using a single domain model ( $K_D = f_{OC}*K_{OC}$ ; using generic  $K_{OC}$  or coal tar  $K_{OC}$ ) and dual domain model ( $K_D = f_{OC}*K_{OC} + f_{BC}*K_{BC}*C_w^{n-1}$ ; using generic  $K_{BC}$ ). Soils presented in the figure are BSS soils spiked with solvent (blue), fuel oil (black), soot (green) and skeet (orange) at target B(a)P concentration of 1mg/kg. Standard error within 0.2 log unit, n=3.



**Figure 4.** Comparison between predicted  $K_D$  from multi-domain sorption model and observed  $K_D$  for soils with different source materials shown for PHE, PYR, B(a)P, and B(ghi)P). Solid fills for each shape represent soils with addition of 2% charcoal.



**Figure 5.** PHE and B(a)P flux through pig skin as a function of concentration in soil (A) and equilibrium aqueous concentration (B).

PAH Sources	Baseline Synthetic Soil (BSS)	BSS-2 % charcoal	BSS-peat content reduced to 1 %	BSS – kaolin content reduced to 2 %	BSS - kaolin replaced with montmorillonite	BSS-peat replaced with humus		
Solvent	0.1,1,10,100 mg/kg BaP	0.1,1,10 mg/kg BaP	1 mg/kg BaP	1 mg/kg BaP	1 mg/kg BaP	1 mg/kg BaP		
Soot	0.1,1,10,100 mg/kg BaP	1 mg/kg BaP	-	-	-	-		
Skeet Particles	0.1,1,10,100 mg/kg BaP	1 mg/kg BaP	-	-	-	-		
Fuel Oil	0.1,1,10 mg/kg BaP	0.1, 1,10 mg/kg BaP	1 mg/kg BaP	1 mg/kg BaP	-	-		
Concentrations shown above are target values before weathering								

**Table 1.** Composition of experimental soils and their respective PAH concentrations (as target BaP levels).

# Chapter 3: PAH Depletion and Its Effect on Source Material Partitioning

## Abstract

Based on the observation from Chapter 2 that the PAH partitioning behavior in skeet material changes with depletion, the hypothesis has been raised that such phenomena may occur in other source materials. This chapter extended the investigation of the effect of PAH depletion in partitioning from skeet onto other sources including fuel oil No. 6 and lampblack soot. PE samplers were used as a passive extraction device to facilitate PAH depletion from source material, and different PE to source material mass ratios were employed to allow varying degrees of PAH depletion. The results showed that different source materials responded differently to PAH depletion. As for fuel oil and soot, increased PAH depletion from source materials did not significantly affect equilibrium partitioning coefficient. However, for skeet, with only 7% PAH depleted, the equilibrium partitioning coefficient increased by up to two orders of magnitude. The nonlinear partitioning behavior for skeet could be described by a Freundlich equation. While nonlinear partitioning based on adsorption isotherms is well documents for black carbon materials, the issue of nonlinear desorption kinetics from source materials has not been previously studied. The proposed model can be used to predict the skeet partitioning coefficients under a depletion environment. This model may benefit risk assessment by improving the accuracy of prediction of coal-tar pitch contaminated soil/sediment partitioning coefficient.
## **3.1 Introduction**

As discussed in Chapter 1, soil K<sub>D</sub> is usually described as a function of organic/black carbon content, the properties of the PAH compound and the soil aging profile, which control the bioavailability of PAHs in soils (Xing and Pignatello, 1997; Northcott and Jones, 2001). Nevertheless, in Chapter 2, it has been demonstrated that PAH bioavailability can also be affected, prominently by the type of source matrix within which PAHs are being introduced into the soil, and that the inclusion of source material partitioning in a multi-sorption model can improve the prediction of overall soil K<sub>D</sub> than traditional OC and/or BC models (Xia et al., 2016). More interestingly, in Chapter 2, we have also discovered that the depletion of PAHs from aged source material such as skeet, can dramatically change the source material partitioning behavior: e.g. the partitioning coefficient of benzo(a)pyrene increased by over one order of magnitude with only 0.5% of PAHs being depleted from skeet particles (Chapter 2, Figure S11). This observation is of particular importance to an improved understanding of PAH partitioning in source materials, as in most cases source material partitioning is not considered a function of depletion. And the effect of PAH depletion on partitioning has never been investigated before.

Based on the observation in Chapter 2, it was suspected that PAHs in skeet consist of different fractions which had varying affinities to different sorption domains in skeet particle. These different domains are featured with different PAH desorption patterns: one is fast, occupied by readily released PAHs, and the other one is extremely slow, occupied by strongly bound PAHs. It is possible that the observed variation in K<sub>SKEET</sub> is the result of different fractions of PAH being depleted from skeet at various equilibrium conditions. This chapter extended the investigation of the effect of PAH depletion on partitioning onto other sources including fuel oil No. 6 and lampblack soot. To do this, a slurry system containing passive samplers, water and source materials was created, where passive samplers served as the PAH extraction device. A series of  $C_{source}$  and  $C_w$  values, along with equilibrium partitioning values (source material partitioning coefficients, calculated as  $K_S = C_{source}/C_w$ ) was measured at different levels of depletion achieved by varying the mass ratios of source materials to extraction devices (passive samplers). The effect of PAH depletion on partitioning behavior was discussed under each source material. In addition, a dual sorption domain model was used to describe different PAH desorption characteristics from source materials.

## **3.2 Materials and Methods**

#### 3.2.1 PAH Source Material

The source materials described in Chapter 2 were also used in this study, which included field weathered coal tar based skeet (shooting target) particles obtained from a Navy skeet range, Jacksonville, FL (provided by John Schoolfield, Naval Facilities Engineering Command), lampblack soot particles (from Fisher Scientific [Catalog No. 1333-86-4]) and fuel oil No.6 (from Chevron).

# 3.2.2 Passive Extraction Device (Passive Samplers)

In this experiment, Low-density PE samplers (25 µm thickness), manufactured by Poly-America (Grand Prairie, TX) were used as a passive extraction device to facilitate PAH depletion from source material, and different PE to source material mass ratios (0.1, 1, 10, 100) were used to allow varying degrees of PAH desorption. The use of passive sampler also enabled the calculation of PAH aqueous concentration in the system, using published K<sub>PE</sub> values (equation 3.2.2) by (Choi et al., 2013):

$$C_{w} = \frac{C_{PE}}{K_{PE}}$$
 (equation 3.2.2)

Based on the mass balance of PAHs in the system, the following equation applies:

$$C_{pE}M_{pE} + C_{source}M_{source} = C_{source}^{0}M_{source}$$
 (equation 3.2.3)

Based on the above two equations:

$$\mathbf{C}'_{\text{source}} = \frac{C_{\text{source}}^0 M_{\text{source}} - C_{\text{PE}} M_{\text{PE}}}{M_{\text{source}}}$$
 (equation 3.2.4)

where  $C_{PE}$  is the PAH concentration in PE at equilibrium;  $M_{PE}$  is mass of PE in the system; C'<sub>source</sub> is the PAH concentration in the source material at equilibrium (after PAH depletion) and  $C^{0}_{source}$  is the initial PAH concentration in the source material, which was measured prior to the experiment, following EPA Method 3550B and 3630C. For each source material, four sets of mass ratios were carried out in triplicate. The masses of source materials and PE samplers used for each depletion level are presented in Table 1.

For each replicate, source materials were mixed with 50 mL of deionized, sterile water (with 100 mg/L NaN<sub>3</sub>) in a 60 ml amber glass vial, followed by addition of precleaned (with acetone rinse) dry PE strips into each solution, respectively. Vials were capped and placed on an end-over-end shaker for 30 days to allow partitioning to reach equilibrium. For fuel oil, one potential issue with this experiment set up was the fouling of oil on the passive samplers during equilibration. In order to prevent fouling, the fuel oil was first spiked into a 1 ml glass vial, which was then placed into the 60 ml glass vial, to prevent the direct contact between oil and sampler. After 30 days, PE strips were removed from the slurry and cleaned with deionized water and wiped with dry soft tissue paper. The PE strips were extracted with three rounds of hexane: acetone 1:1 solution overnight (Booij et al., 2002). The three solvent extracts were combined and analyzed for PAHs by GC-MS. The mass of PAHs in PE strips represents the fraction that has been desorbed from source material. With  $C'_{source}$  and  $C_w$  calculated from equation 3.2.2 and equation 3.2.4, the equilibrium source material partitioning constants were calculated as:  $K_S = C'_{source}/C_w$ , at each different depletion level for each source materials.

#### 3.2.3 Dual Sorption Domain Model

A dual sorption domain model was used to describe the partitioning behavior in different source materials due to depletion (Weber et al., 1992). Two sorption domains were included in the model: one with a low sorption coefficient representing those sites with fast and linear desorption pattern and the other with a high coefficient representing those following Freundlich nonlinear sorption behavior:

$$C_{\text{source}} = f_l K_l C_w + f_n K_n C_w^n$$
 (equation 3.1.1)

where  $C_{source}$  and  $C_w$  are the measured PAH concentrations in source material and aqueous phase, respectively;  $f_l$  and  $f_n$  are the mass fractions of the linear and non-linear sorption domains; and  $K_l$  and  $K_n$  are the linear and nonlinear sorption coefficients for the two domains. Source materials like skeet, soot and fuel oil are visually homogeneous; it is impossible to physically separate different domains and measure the fractions and sorption coefficient individually. Therefore, the parameters K and f were mathematically calculated using the Polymath software program.

## **3.3 Results and Discussion**

#### 3.3.1 Level of Depletion from Source Material

The percentage of PAHs extracted by passive samplers from the source materials after 30 days' equilibration was calculated as mass of PAHs in PE divided by initial mass of PAHs in source materials. The PAH extracted (expressed as %) varied greatly with different source materials. As shown in Table 2, the highest PAH extractability was observed in fuel oil, followed by soot and skeet particles. This trend was consistent across all four depletion levels (represented as mass ratios of PE/source materials, from 0.1 to 100). This trend was expected as skeet particles have the strongest sorption coefficient for PAHs, followed by soot and fuel oil based on the K<sub>s</sub> results measured in Chapter 2. As the mass ratios of PE to source increased from 0.1 up to 100, the percentage of total PAHs desorbed from source materials generally increased, although the degrees of increase differ dramatically with different source. For skeet particles, the mass fraction of PAHs desorbed increased from 0.4% to only 7.2% as the extraction capacity by PE increased by 3 orders of magnitude (as mass ratio of PE/source materials goes from 0.1 to 100). Unlike skeet, for soot, the mass fraction desorbed increased by over 200 fold (from 0.25% to 64.3%). However, for fuel oil, nearly 80% of all PAHs are depleted at a PE/source mass ratio of only 10. The percentage of mass depleted from fuel oil at the ratio of 100 was due to analytical error encountered: nearly 100% or more depletion was calculated for some PAHs.

#### 3.3.2 Effect of Depletion on Source Material Partitioning $(K_S)$

The K<sub>S</sub> values at each depletion level were calculated and presented in Figure 2. The effect of depletion on K<sub>S</sub> varied greatly with the source material. In Figure 2, K<sub>S</sub>

values of four representative PAHs (each with a different number of aromatic rings) were presented for all three source materials: fuel oil, soot and skeet. As for fuel oil, no significant difference in equilibrium  $K_S$  was observed at different mass ratios for all four PAHs investigated (Anova test, p = 0.9). For soot, no significant trend on Ks was observed either, with PAH depletion by PE for all four PAHs. However, for skeet particles, as mass ratio of PE to source increases from 0.1 to 100, the depleted mass of PAHs increased from 0.4% to only 7%, and the equilibrium K<sub>s</sub> increased by one to two orders of magnitude for different PAHs. This elevation in K<sub>s</sub> caused by depletion was slightly higher for PAHs with relatively lower molecular weight: nearly two log unit increase for phenanthrene and pyrene vs. approximate one log unit increase for benzo(a)pyrene and dibenz(a,h)anthrancene. It was very likely that the higher mobility associated with the lower molecular weight PAHs rendered them more susceptible to extraction by passive samplers, resulting in a higher fraction of lighter PAHs desorbed from skeet than heavier PAHs. As more of the lighter PAHs were desorbed from skeet, the residual lighter PAHs became more recalcitrant to extraction, eventually reflected as a more drastic change in  $K_s$ . Such sorption hysteresis phenomena for HOCs has already been observed in charcoal materials and coal contaminated soils (Pignatello, 2009; Braida et al., 2003; Ahmed et al., 2015).

The different partitioning behaviors observed for different source materials as a response to depletion was particularly interesting. The fact that  $K_S$  does not change with increasing depletion from fuel oil and soot indicated a highly homogeneous sorption matrix with a uniform desorption characteristic in these materials. This observation for fuel oil was expected as the fuel oil No.6 used in this study is a dense and viscous phase,

mostly consisting of long-chained petroleum hydrocarbons. Previous studies have demonstrated that most of these petroleum hydrocarbons (including crude oil, light gas oil and gasoline) often exhibit linear sorption pattern for organic contaminants such as PCBs and PAHs (Jonker and Barendregt, 2006; Jonker et al., 2003b). This linear sorption is mostly dominated by PAH absorption and partitioning into the complex long-chain hydrocarbons. Unlike fuel oil, the lampblack soot used in this study was freshly spiked with PAH solvent matrix to increase the PAH contamination level, followed by solvent evaporation and one-month mixing on a roller to allow homogenization. As a typical type of black carbon, the sorption pattern for soot, as already discussed in Chapter 2, is often recognized as nonlinear, the equilibrium partitioning constant for which is mainly a function of the freely dissolved PAH concentrations (Cw) with which the soot is equilibrating (Zhu and Pignatello, 2005). Based on the measured results (Table 3), Cw did not change prominently (with relative standard deviation within 70%) despite that the mass of PAH extracted increased from 0.25% to 64%. Therefore, it seemed that the relative consistent C<sub>W</sub> has resulted in an insignificant fluctuation in Ks for soot at different levels of depletion. Moreover, different from fuel oil and soot, the increased PAH depletion from 0.4% to only 7% drastically increased the  $K_S$  for skeet by up to two order of magnitudes. The fact that the skeet particles had been weathered for decades in the field likely contributed to this phenomenon. The weathered coal-tar component of pitch is known to contain black carbon in the form of soot, coke, and cenospheres that get included in the tar during the production process (Khalil et al., 2006). As the tar is further processed and weathered in the field and degradable components are lost, the residual matrix becomes enriched in the black carbon residue. Therefore, it is reasonable to

assume that after decades of field weathering, the majority of the weakly-bond PAHs in skeet particles have been lost either via volatilization, degradation or migration into other sorption matrices (e.g. soil organic matter) in the environment. As the very small fraction of residual PAHs on low energy sites of skeet get depleted, the remaining PAHs that are occluded within become extremely recalcitrant to extraction, resulting in a higher and higher K<sub>s</sub>.

#### 3.3.3 Modeling PAH Partitioning Behavior in Skeet

Based on previous discussions, the desorption of PAHs in fuel oil is generally linear, and no significant effect of depletion on K<sub>s</sub> was observed for soot source materials either. However, for skeet source material, the nonlinearity in PAH partitioning can be generally described using the following two sorption domain model, derived from Luthy et al. (Luthy et al., 1997).

$$C_{\text{source}} = f_l K_l C_w + f_n K_n C_w^n \text{ (equation 3.2.5)}$$

In this modeling effort, the experimentally determined parameters, equilibrium  $C_W$  and  $C_{source}$  at different PE/source mass ratios were fitted to the model to solve for the unknown parameters:  $f_1$ ,  $f_n$  (mass fractions of linear and nonlinear sorption sites;  $f_1 + f_n=1$ ) and  $K_1$ ,  $K_n$  simultaneously using Polymath software. The returned results showed a negligible fraction of linear sorption domain for PAHs in skeet particles, where  $f_n / f_1$  is  $\approx 10$ , which indicated a dominating nonlinear sorption pattern for skeet particles. Thus, the linear sorption compartment in the above model can be neglected, and the equation becomes:

$$C_{skeet} = K_n C_w^n$$
 (equation 3.2.6)

The nonlinear partitioning behavior observed from skeet particles was modeled using above Freundlich equation and presented for four representative PAHs. As we can see in Figure 3, the measured  $C_{skeet}$  and  $C_w$  values fit well to the proposed Freundlich nonlinear model for all four PAHs, with R<sup>2</sup> values greater than 0.93. The observed nonlinearity coefficient n, is extremely small, ranging from 0.013 to 0.04, which is over one order of magnitude lower than the coefficients observed for typical black carbon materials (e.g. charcoal and activated carbon) reported in the literature (n ranges from 0.5 to 0.95)(Cornelissen et al., 2005a; Gomez-Eyles et al., 2013; Kleineidam et al., 2002b). This indicates a prominently higher nonlinearity than conventional black carbon materials. The detailed nonlinearity coefficients and partitioning constants for each individual PAHs (generated from modeling) were listed in Table 4.

## **3.4 Conclusion**

This is the first time desorption isotherm is being measured, and the results show that different PAH source materials react differently to desorbing conditions. All the past work on sorption nonlinearity was based on observations from adsorption isotherm studies. With the calculated nonlinearity coefficients, the skeet partitioning constant can be predicted at any given freely dissolved concentration using the proposed model. In risk assessment, sediment/soil partitioning constant is a key parameter to characterize the fate of the contaminants and to estimate the toxicity and exposure. Accounting for desorption nonlinearity when necessary should allow us improved ability to predict mass transfer and porewater reductions when soils are imposed to desorbing conditions such as during biodegradation or sorbent amendment. The modeling result from this study can benefit risk assessment by improving the accuracy of the prediction of PAH partitioning in weathered coal-tar contaminated sites over conventional OC/BC models.

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**Figure 1.** Paradigm of the passive depletion experiment setup. Four different mass ratios of polyethylene sampler to source materials (0.1, 1, 10, 100) were investigated.



- Phenanthrene
- Pyrene
- Benzo(a)pyrene
- Dibenz(a,h)anthracene

**Figure 2.** Effect of depletion on source material  $K_S$  for four representative PAHs (phenanthrene, pyrene, benzo(a)pyrene and dibenzo(a,h)anthracene). Error bar represents relative standard deviation, n=2.



**Figure 3.** Nonlinear sorption behavior observed in skeet particles for four representative PAHs (phenanthrene, pyrene, benzo(a)pyrene and dibenzo(a,h)anthracene).

source	mass of PE	mass of source	Mass ratio
material	(g)	material (g)	(PE /source)
skeet	0.01	0.1	0.1
skeet	0.01	0.01	1
skeet	0.1	0.001	10
skeet	0.5	0.005	100
soot	0.01	0.1	0.1
soot	0.01	0.01	1
soot	0.1	0.001	10
soot	0.5	0.005	100
fuel oil	0.01	0.1	0.1
fuel oil	0.01	0.01	1
fuel oil	0.1	0.001	10
fuel oil	0.5	0.005	100

Table 1. Masses of source materials and PE samplers used in passive depletion test.

**Table 2.** Percentage of total PAHs depleted from source materials at different mass ratios.

	PAH source materials		
mass ratio (PE /source)	skeet	soot	fuel oil
0.1	0.40%	0.25%	8.8%
1	1.0%	3.9%	41.7%
10	4.6%	31.3%	79.0%
100	7.2%	64.3%	N/A

N/A: percentage of total PAHs depleted higher than 100% was observed. The RSD for all measured depletion values were within 5%.

Table 3. Freel	y dissolved PAH	concentration	n (mg/L) at differen	<u>t mass ratio of PE to soot</u> .	
mass ratio	phenanthrene	pyrene	Benzo(a)pyrene	dibenz(a,h)anthracene	
0.1	1.9E-04	6.2E-05	2.2E-06	9.2E-08	
1	4.7E-04	1.6E-05	4.9E-06	5.0E-08	
10	9.4E-05	9.5E-05	6.3E-06	1.5E-07	
100	2.8E-05	1.4E-05	1.5E-06	4.2E-08	
The RSD for all measured values were within 10%.					

	Kn	n	R <sup>2</sup>
Phenanthrene	3179	0.017	0.95
Anthracene	4145	0.004	0.91
Fluoranthene	9889	0.015	0.92
Pyrene	9689	0.013	0.93
Benz(a)anthracene	5493	0.018	0.91
Chrysene	5806	0.022	0.93
Benzo(b)fluoranthene	5238	0.024	0.91
Benzo(k)fluoranthene	4962	0.024	0.93
Benzo(a)pyrene	7355	0.042	0.95
Indeno(1,2,3,-cd)pyrene	4462	0.021	0.94
Dibenz(a,h)anthracene	783	0.032	0.95
Benzo(g,h,i)perylene	3037	0.024	0.91

Table 4. Modeled nonlinearity coefficient (n) and partitioning coefficient (Kn) for skeet.

# Chapter 4: Predicting and Reducing PAH Dermal Bioavailability from Soils

# Abstract

Based on previous observation from Chapter 2, dermal uptake of PAHs by humans may also be predicted by the freely dissolved concentration exposed to the external skin surface. In this chapter, Fickian diffusion models were explored to explain and predict the dermal uptake of PAHs. In addition, the effectiveness of 2% biochar amendment was also investigated. The results showed that steady state diffusion model could adequately predict the PAH dermal flux for most of the soils, except for the charcoal amended ones, most likely due to the violation of the steady state assumption. The skin/ water partitioning coefficient for 16 parent PAHs was reported for the first time in this study. As for non-steady state diffusion model, the prediction of dermal flux was significantly improved over steady state model, with nearly all of the predictions falling within 1 log unit deviation. In terms of carbon amendment, decreased remedial performance was observed with increasing initial soil partitioning coefficient: the reduction in dermal bioavailability was greater in control and fuel oil spiked soils than skeet and soot spiked soils. Given the freely dissolved PAH concentration, one can utilize the proposed model as a screening tool for human risk assessment, to approximate the PAH dermal uptake from soils contaminated with various PAH source materials.

# 4.1 Introduction

PAH exposure to human beings from soils and quantification of bioavailability is more complicated due to the more complex human activities and exposure pathways, relative to ecological receptors. Inhalation of dust, direct skin contact with contaminated soil, consumption of contaminated vegetables, and accidental ingestion of contaminated soil are all common pathways for human beings exposure to PAH in soil (ATSDR, 1990), among which dermal contact and incidental soil ingestion are considered the most important exposure routes (Kadry et al., 1995; Ruby and Lowney, 2012). In terms of PAH dermal absorption, a series of studies have been performed on various subjects (e.g. monkey, rat, human skin) in the past using different PAH matrices including synthetic soils (with PAHs spiked into sand) and neat PAH compounds. The current EPA recommendation for estimating the dermal absorption of PAHs from soil is 13% of the total applied on the skin (EPA, 2004), which does not take into account the site specific PAH source materials, nor the soil sorption characteristics. Recent research by Spalt et al., 2009 has suggested that this recommendation value is overprotective and outdated, as it was derived from an in vivo study conducted two decades ago where neat PAH compounds and PAH spiked sand instead of actual soil matrix were investigated (Wester et al., 1990). However, it is now generally recognized that soil characteristics, composition (e.g. soil particle size, organic carbon content, black carbon content) and aging profile can impact the PAH fate and transport, as well as the exposure to the environment (Spalt et al., 2009). In the previous work from Chapter 2, we have evaluated the potential factors that may affect and control the dermal bioavailability from soil spiked with different PAH source materials, including solvent spike (control), fuel oil, soot and weathered coal tar particles. And interestingly, we have found that the PAH dermal uptake is not significantly correlated with total concentrations in soils, but is correlated to partitioning constants and freely dissolved concentrations. The results have

indicated that the freely dissolved concentrations may be used to predict PAH dermal uptake by humans in a similar way to estimating bioaccumulation in ecological receptors.

To quantify the bioavailable PAH fraction through skin absorption, an often used conventional approach involves a vertically continuous-flow Franz diffusion cell device, where the contaminated soil is applied on top of the skin and the skin is flushed from the bottom with an artificial cell fluid. This device usually requires artificial cell fluid circulation and flow control devices, where both the PAH fraction that has penetrated through skin into cell fluid, and that remaining in the skin layer must be considered, because the latter will continue to be absorbed into the human body (Reddy et al. 2000). Instead, we employed a simplified version of a diffusion cell based on earlier work done by Mayer et al., where instead of a cell fluid sink, a solid phase sink made of polydimethylsiloxane (PDMS) is used to quantitatively simulate the absorption of PAH into the sink (receptor fluid) of a Franz cell device following skin penetration (Figure 1). PDMS is a highly hydrophobic solid phase with strong PAH sorption capacity that can rapidly capture the PAHs and effectively occlude them within the solid phase, thus providing a near zero PAH concentration at the PDMS/skin interface. Due to the barrier effect of the stratum corneum, the role of active transport is limited (Scheuplein and Bronaugh 1983) and passive diffusion dominates this absorption process. Thus, PAH diffusion through epidermis is the key to understanding dermal bioavailability for short exposure duration. In this test, we have investigated whether PAH skin absorption can be described and predicted using Fickian diffusion models. During the past decades, in vitro dermal models have exhibited good correlation with in vivo tests and the former is considered the preferred approach due to its simplicity and low cost (Karadzovska et al.,

2013). Human cadaver skin is often considered the ideal test subject for *in vitro* tests. However, due to its high expenses, pig skin is alternatively employed as it is considered an appropriate substitute for human skin due to its similar histological and physiological characteristics and closely comparable permeability properties (Dick and Scott 1992, Simon and Maibach 2000, Meyer et al. 2003).

In terms of soil remediation, there are extensive successful applications for the use of strong geo-sorbents, such as biochar and activated carbon, to reduce the uptake and bioavailability of organic contaminants to ecological receptors (Gomez-Eyles et al., 2013). However, whether the success of such can be achieved in reducing human exposure is still unknown.

Therefore, this chapter has expanded the dermal uptake experiments onto more soils with a wider range of environmentally relevant concentrations, and attempted to explain and predict the PAH dermal uptake process using a Fickian diffusion model. The effectiveness of a novel remediation alternative, biochar amendment, on reducing PAH dermal bioavailability from soils spiked with typical, environmentally relevant PAH source materials (fuel oil, soot, and coal tar based skeet particles) and solvent matrices (control) was also investigated. In the end, the diffusion model performance and implications associated with biochar amendment have been discussed.

## 4.2 Materials and Methods

#### 4.2.1 Selection of Contaminated Soils

A selection of soils previously constructed in Chapter 2 were used in this study, which covered a wide range of PAH soil concentrations, PAH source materials and soil compositions, as listed in Table 1. Both low and high contamination levels were included for all four different sources. The only exception was that the 100 ppm target BaP concentration was not created for fuel oil spiked soils, as excessive addition of fuel oil could potentially change the property of the baseline soil, into a NAPL phase. Instead, 10 ppm target BaP concentration was selected for fuel oil. The solvent spike was used as control for other source material spikes, as the solvent was allowed to evaporate before soil homogenization. In addition, a subset of the soils was amended with 2% of charcoal, followed by mixing on a roller for one month. Then all the soils were transferred to a green-house for another 2 months of weathering with continuous wetting and drying cycles. The PAH concentrations in soils after weathering were previously determined in Chapter 2, as well as the freely dissolved PAH concentration through equilibrium partitioning using polyoxymethylene as a passive sampler. The soil partitioning coefficients were also calculated for each soil.

## 4.2.2 Measuring PAH Dermal Uptake

The pig skin of full thickness was purchased from a super market (Great Wall) located in Catonsville, Maryland. In this test, 5 cm<sup>2</sup> of full-thickness pig abdominal skin (of 2mm thickness) was excised using scissors and scalpel and transferred onto an evaporating dish. Soils spiked with different PAH source materials (solvent, fuel oil, soot, and skeet) were sieved down to less than 150  $\mu$ m (Yang et al., 1989), and 100 mg of each dry soil was evenly distributed onto the skin surface to reach a soil loading of 20 mg/cm<sup>2</sup> (Roy et al., 1998). After spreading the soil evenly, 10 drops of DI water were evenly placed across the skin surface to provide moisture as suggested by (Turkall et al., 2010), and was subsequently mixed gently with the soil using a spatula. During the exposure, a thin PDMS sheet with high PAH sorption capacity was placed underneath the skin to

capture any PAHs that could have potentially penetrated through the bottom layer of the skin after 16 hours' exposure. After that, the evaporating dish was sealed with parafilm and placed in the dark at 25 °C for 16 hours. However, we did not detect any PAHs in the PDMS sheet for any of the soils, which indicates the 2mm skin has a sorption capacity high enough to serve as a PAH sink for this particular case. The dermal uptake scheme is presented in Figure 2.

After 16 hours of exposure, the skin was rinsed with DI water and gently wiped with tissue paper to remove any soil residuals. Then the skin was sliced into small pieces and transferred into a 50 ml Teflon vial to undergo saponification using a method modified from Hyötyläinen et al. (Hyötyläinen et al., 2002). Briefly, 25 ml of 0.5M KOH in methanol/water 1:3 was added into the vial and PAH surrogates were spiked into the mixture. The vial was capped and placed into a water bath at 100 °C for four hours for saponification. After cooling, 20 ml of hexane was added into the vial and extracted for 30 minutes on an orbital shaker at 60 rpm. After extraction, the vial was centrifuged at 3000 rpm for 2 min and the hexane was transferred into another 60 ml vial using a glass pipette. The solvent extraction was repeated twice and the three aliquots were combined for further cleanup and PAH analysis.

#### 4.2.3 Measuring skin/water Partitioning Coefficient

Polyethylene (PE) strips with 25  $\mu$ m thickness that were preloaded with 16 parent PAHs have been used as a passive dosing source. Briefly, 1 ml of concentrated PAH standard mixture containing 16 parent PAHs, each of 2000  $\mu$ g/mL (from ULTRA Scientific Part Number: 801175052975), was spiked into 500 ml of methanol: water (3:1 v/v) solution. Pre-cleaned PE strips (soaked in acetone overnight) were added into the methanol: water solution for PAH impregnation. After two weeks, the PE strips were retrieved, rinsed and soaked with DI water for 24 hours to remove residual methanol in the sampler. Then, the PE samplers were transferred into 1 liter of 1000 mg/L sodium azide solution and allowed to equilibrate with the sodium azide solution for another two weeks. Then, abdominal pig skin was cut into thin slices (each weighing 1 g of approximately 1 mm thickness) and placed into the sodium azide solution to equilibrate with the PE on an orbital shaker at 100 rpm for five weeks. The addition of sodium azide was to prevent skin decay during the mixing. Additionally, the jar was wrapped with ice packs, which were replaced daily in order to lower the temperature and slow the skin decay. A stainless steel mesh was mounted on the surface of the solution to ensure that both skin and samplers were submerged during the mixing. The setup of this kinetic experiment is presented in Figure 3.

A subset of skin (of 1 g) and PE samplers (of 50 mg) were retrieved at different exposure times (3, 5, 7, 14, 21 and 35 days). The PE samplers were extracted and the skin strips were saponified using methods described in Chapter 2, and the PAH concentrations in aqueous phase and in the skin at each exposure time were determined. The corresponding skin/water partitioning coefficients at different exposure time were calculated as  $K_{Skin/Water} = C_{Skin}/C_W$ .

## 4.3 Results and Discussion

## 4.3.1 PAH Dermal Flux

The results of measured dermal flux are presented in Figure 4 for four representative PAHs with four different numbers of aromatic rings, including

phenanthrene, chrysene, benzo(a)pyrene and benzo(g,h,i)perylene. Soils with different PAH concentrations, compositions and source materials were included in the figure. As we can see from Figure 4, the measured dermal flux for individual PAH ranged over three orders of magnitude, among different soils. The PAHs with lower molecular weight and hydrophobicity (e.g. phenanthrene and chrysene) had much higher dermal flux than those with higher molecular weight and hydrophobicity (e.g. benzo(a)pyrene and benzo(g,h,i)perylene). Moreover, the most important observation from figure 4 is the overall positive correlation between dermal flux and freely dissolved concentrations, which is generally applicable to all the PAHs investigated across a large range in hydrophobicity. This positive correlation has been observed and discussed in Chapter 2, but only for soils with high contamination levels (100 ppm target BaP concentration). Through this figure, the application of this strong correlation has been demonstrated over lower environmentally relevant soil concentrations under different source materials. The directly measured freely dissolved PAH concentrations through passive samplers, has been extensively reported and proved to be predictive of PAH bioaccumulation in ecological receptors, such as benthic and soil invertebrates (Gomez-Eyles et al., 2011a). For these invertebrates, the exposure to PAH through skin contact with aqueous phase has been shown as a major exposure pathway, which share similarity in human dermal exposure to PAHs in soils. Based on the observation in Figure 4, the freely dissolved concentration has been included in the modeling effort to predict PAH dermal uptake by humans from soils.

The percent dermal absorption of PAHs from soils was also calculated for benzo(a)pyrene and compared with current EPA recommendation value of 13% in Figure

5. The percent dermal absorption varied greatly with source material and soil composition, and the EPA recommended bioavailability value was up to 100 times higher than the observed value in soot spiked soils . In general, soils spiked with solvent and fuel oil had much higher fraction of bioavailable PAH (1.9% to 9.6%) than soot and skeet spiked soils (0.1% to 1.7%), which could be explained by the higher soil  $K_D$  observed for the latter soils. The addition of 2% charcoal also reduced the bioavailable fraction by 40% to 70%. This comparison suggested that the use of default EPA recommended percent absorption value should be discouraged and the site specific PAH source materials, as well as the soil sorption characteristics have to be taken into account when assessing dermal bioavailability.

#### 4.3.2 Model Assumptions

Conceptually, the PAH dermal uptake can be considered as a combination of two transport processes, the desorption of PAH from soil particles into the aqueous phase that is in contact with the skin surface, and diffusion of PAH from the aqueous phase into the skin. Study in the past has suggested that the passive diffusion through stratum corneum is usually considered the rate-limiting step in dermal absorption process for most chemicals, including PAHs (Surber et al., 1990). The particles size of the soils used in this study was fine, of less than 150  $\mu$ m size. Therefore, the PAH desorption from soil into aqueous solution could be extremely fast, especially when the rapidly desorbing fraction of PAHs in soil dominated the desorption process. In this experiment, the volume of water that was spiked into the soil was small, of approximately 0.3 to 0.4 ml, which also facilitated the equilibrium between soil and aqueous phase. Based on the PAH hydrophobicity and previously measured soil K<sub>D</sub>, it was also estimated that negligible

desorption of PAHs (e.g. less than 1‰ for pyrene) from the soil particles was needed to reach equilibrium with this small volume of water. Moreover, previous study by Barnier et al. has reported fast desorption kinetics of the rapidly-desorbing PAH fractions from soils collected from both coking plant and manufactured gas plant, where 0.7% to 3% depletion of total PAHs was measured within the first hour of desorption (Barnier et al., 2014). Due to this fast desorption kinetics, it is therefore reasonable to assume that PAH aqueous concentration may approach equilibrium with the soil particles very rapidly. Whereas for the PAH partitioning between skin and aqueous phase, our following kinetic uptake experiment demonstrated that the equilibration time (of approximately 5 weeks) between these two phases was much longer than that between soil particles and aqueous phase (of hours). This comparison confirmed the model assumption that the diffusion of PAHs into the skin layer could be considered as the rate limiting step through the overall transport process. With these assumptions, diffusion equation based on Fick's first law was used as a starting point for the modeling effort:

$$J = \frac{D \Delta C_{\text{skin}}}{h} = \frac{D K_{\text{Skin}/\text{Water}} C_{\text{W}}}{h}; \quad \text{equation (1)}$$

where J is the steady state dermal flux; D is the PAH diffusivity in pig skin;  $\Delta C_{skin}$ , is the PAH concentration gradient in skin over, h, the skin thickness; C<sub>W</sub> is freely dissolved PAH concentration; and K<sub>Skin/Water</sub> is the skin/water partition coefficient. C<sub>skin</sub> can be rewritten as a multiplication of skin/water partitioning coefficient and the freely dissolved concentration at the very top skin surface.

And if we introduce:

$$K_p = \frac{\text{D K}_{\text{Skin/Water}}}{\text{h}}$$
 equation (2)

then:

$$J = K_p C_w$$
 equation (3)

with skin permeability Kp, the flux equation can be rewritten as equation 3. The skin permeability describes a chemical's potential for penetration and mobility through the skin layer, which has been extensively studied in the past. In this modeling effort, the Kp has been obtained using two different approaches:

1) Kp was derived from an empirical quantitative structure-permeation relationship from *in vitro* studies of a variety of skin subjects (e.g. human cadaver skin, animal skins including pig, rabbit, rat) reported by (Wen et al., 2007), as

$$Log K_P = 0.5752 log K_{OW} - 0.004475 MW - 2.64368 (R^2 = 0.7998)$$
 equation (4)

where Kp was estimated using PAH hydrophobicity and molecular weight.

2) the Kp was estimated by fitting experimental data from control (solvent spiked) soils with 1ppm BaP target concentration to equation 3, which was then used to predict dermal flux for the other soils.

## 4.3.3 Steady State Modeling Results

Figure 6 included the model predictions of dermal flux for eight PAHs with medium to heavy molecule weights: (benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3,-cd)pyrene, dibenz(a,h)anthracene and benzo(g,h,i)perylene), from all of the test soils. As we can see from Figure 6A, the use of literature Kp value led to an over-prediction of dermal flux by one to two orders of magnitude, for most of the soils, despite the PAH source materials, and soil concentrations. This over-prediction is likely a result of the variation ( $R^2 = 0.5$ ) in the empirical skin permeability used in the modeling. This literature was not exclusively

derived for PAHs, but over a wide range of organic contaminants. On one hand, the literature Kp was derived from diffusion cell studies with typical skin thicknesses ranging from 0.1 to 0.5 mm, which was much thinner than the skin used in this study and mostly consist of epidermis. On the other hand, the literature K<sub>P</sub> used in this prediction was not exclusively developed for PAHs, but for over a hundred chemicals. The residuals of the empirical regression (equation 4) may have led to the deviation observed in figure 6A. Figure 6B showed the prediction of dermal flux using permeability that was calibrated from one of the control soils (solvent soil with 1ppm target BaP). Compared to Figure A, the prediction using experimentally fitted Kp is greatly improved, with over 80 % of the predictions falling within one order of magnitude deviation. The only exception is that for some of the charcoal amended soils, the model under-predicted the flux by over one order of magnitude.

The steady state diffusion modeling assumes an ideal condition where the PAH concentration in skin layer is at equilibrium with the aqueous concentration throughout the exposure, which however, may not be the real case. As PAHs diffused into skin from aqueous phase, the concentration at different skin thicknesses increased with time. The time needed for the skin to reach steady state with the overlying aqueous concentration is often referred as lag time in dermal absorption studies. Depending on the properties (e.g. molar volume, hydrophobicity) of chemicals of interest, as well as the matrix in contact with the skin, the lag time can vary greatly (Vecchia B.E., 2002). However, in this steady state modeling, the lag time was not taken into account. In addition, it was suspected that the lag time for this dermal study might exceed 16 hours, as the PDMS never picked up any PAHs during the exposure period, which was an indication that a complete steady

state condition has never been reached through the entire thickness. Theoretically, the model performance for PAHs with heavier molecular weight and the soils with higher  $K_D$  would be more susceptible to the effect of lag time. And based on Figure 6, the observed deviation from prediction was the greatest (approximately 1 to 2 orders of magnitude) in charcoal amended soils with the highest  $K_D$ , while most of the rest predictions fell within one log unit deviation. Therefore, it is hypothesized that the violation of the steady state assumption might have resulted in the deviation observed from Figure B. And to address this, a non-steady state diffusion model has been developed in an attempt to improve the modeling.

## 4.3.4 Non-Steady State Modeling

To characterize the change of PAH concentration in the skin over exposure time, Fick's second law was applied to explain and predict the non-steady state dermal diffusion, as shown in the following:

$$\frac{dC_{skin}}{dt} = D \frac{d^2 C_{skin}}{dx^2} \qquad \text{equation (5)}$$

Similar boundary and initial conditions to steady state diffusion were assumed in this case: there is negligible depletion of PAHs from soil during the diffusion; rapid equilibrium between soil particles and aqueous phase on the surface of the skin will be reached at the beginning of the exposure and there is no penetration of PAHs through the bottom of skin layer after 16 hours' exposure:

$$C_{skin} (x > 0, t = 0) = 0$$

$$C_{skin} (x = 0, t) = C_0 = C_W K_{S/W} \quad \text{equation (6)}$$

$$C_{skin} (x = h, t) = 0$$

With these assumptions, the analytical solution to above differential equation can be obtained from (Crank, 1975), which is :

$$C_{skin}(x, t) = C_0 \operatorname{erfc}\left(\frac{x}{2\sqrt{Dt}}\right)$$
 equation (7)

where  $C_{skin}$  (x, t) represents the PAH concentration in skin at any depth at any exposure time, and  $C_0$  represents the PAH concentration in skin surface that is in contact with aqueous phase:

$$C(x, t) = C_W K_{S/W} \operatorname{erfc} \left( \frac{x}{2\sqrt{Dt}} \right)$$
 equation (8)

As we can see in the equation, there are two unknown parameters, skin/water partitioning coefficient ( $K_{S/W}$ ) and PAH diffusivity in skin (D), that are needed in order to calculate the PAH concentration in skin. The diffusivity D describes the mobility of a chemical through skin, which is a function of the properties of both skin and chemical of interest. In 2002, Vecchia and Bunge (Vecchia B.E., 2002) published an estimation for skin diffusivity for a library of hydrophobic chemicals. The reported quantitative structure–activity relationship is as follows:

 $\log (D/h) = -2.408 + 0.098 \log K_{OW} - 0.005 MW$  equation (9)

The empirical regression predicts diffusivity for human skin, using the hydrophobicity (K<sub>ow</sub>) and molecular weight (MW). Given the skin thickness of 2 mm, the skin diffusion coefficient can be obtained for each PAH compound. The other parameter that needs to be solved for is the skin/water partitioning coefficient. The skin/water partitioning coefficient for PAHs has been seldom studied in the past, and the major focus of the dermal absorption studies in the past has been the percutaneous absorption of medicines and pharmaceuticals. Surber et al. had published an empirical equation in 1992, to estimate skin/water partitioning coefficients for highly hydrophobic

organic contaminants, which was derived using PCBs, phenols, DDT and benzo(a)pyrene (C. Surber, 1992). However, such estimation was developed based on dermal absorption studies for stratum corneum layer only. The epidermis layer beneath the stratum corneum is more hydrophobic and known to have a much higher sorption capacity for PAHs (Simon et al., 2000) than stratum corneum. And the histological and partitioning properties of epidermis are different from stratum corneum (Chen et al., 2013). In order to obtain an accurate partitioning coefficient,  $K_{Skin/Water}$ , a kinetic PAH uptake experiment was conducted.

#### 4.3.5. Skin/water Partitioning Coefficient

As shown in Figure 7, the freely dissolved PAH concentrations did not change significantly with exposure time, for four representative PAHs with varying log K<sub>ow</sub> (from 4.46 to 6.70). The observed freely dissolved concentrations reflect consistent PAH concentrations in PE samplers, which indicated that the passive sampler have successfully functioned as an infinite PAH source for the partitioning test. The detailed freely dissolved concentration results are listed in Table 2.

The measured skin/water partitioning coefficients for different PAHs at different exposure times are presented in Figure 8. According to the figure, it seems that for all four PAHs, the partitioning between aqueous phase and skin is approaching equilibrium after five weeks. However, it is difficult to conclude from the last data points if the equilibrium has been completely reached or not. In order to examine that, a first-order uptake model was proposed to predict the equilibrium partitioning coefficient, which was adapted from (Jager et al., 2000):

$$\frac{dC_{skin}}{dt} = k_{uptake}C_{w} - k_{desorption}C_{skin} \qquad equation (10)$$

where  $C_{Skin}$  and  $C_W$  are the PAH concentration in skin ( $\mu g/g$ ) and in aqueous phase ( $\mu g/m$ ) at any given time t; as discussed earlier  $C_W$  is constant;  $\mathbf{k}_{uptake}$  is the PAH uptake rate from water to skin and  $\mathbf{k}_{desorption}$  is the PAH desorption rate from skin to water. The analytical solution to the above equation is as follows:

$$C_{skin} = \frac{C_w k_{uptake}}{k_{desorption}} + c1e^{-k_{desorption}t} \qquad equation (11)$$

c1 is the integration constant; with the boundary condition of  $C_{Skin} = 0$  when t=0,

$$c1 = -\frac{C_w k_{uptake}}{k_{desorption}}$$

the final solution to the equation became:

$$K'_{skin} = \frac{C_{skin}}{C_w} = \frac{k_{uptake}}{k_{desorption}} (1 - e^{-k_{desorption}t}) \qquad \text{equation (12)}$$

where K'<sub>skin</sub> is the skin/water partitioning coefficient at any given time t. The equilibrium skin/water partitioning coefficient, K'<sub>skin</sub> at t approaching infinity, is represented as  $k_{uptake}/k_{desorption}$ . The measured partitioning coefficients were compared with results from model fitting, as presented in Figure 8.

The lines shown in Figure 8 were from fitting data to a first order uptake model. As is shown, the measured partitioning coefficients were consistent with the modeling results, and the partitioning was close to equilibrium after five weeks mixing. The equilibrium partitioning values were then extrapolated from the model fitting, calculated as  $k_{uptake}/k_{desorption}$ . The detailed partitioning results are presented in Table 4. The modeled equilibrium partitioning coefficients (Log K<sub>skin/w</sub>) ranged from 2.0 to 4.1 for 16 priority parent PAHs, which were also strongly correlated with PAH hydrophobicity (K<sub>ow</sub>) (R<sup>2</sup> = 0.87), as shown in Figure 9. The modeled equilibrium partitioning results were used in the non-steady state diffusion model to predict the PAH dermal uptake. This is the first report of experimentally determined skin/water partitioning coefficients for 16 parent PAHs. The equilibration time of five weeks between aqueous phase and skin observed from this study is supposedly to be much longer than that between soil particles and aqueous phase, of less than one hour, estimated based on literature results as described earlier. This comparison confirmed the model assumption that the diffusion of PAHs into the skin layer can be considered as the rate limiting step through the overall transport process.

## 4.3.7. Non-Steady State Modeling Results

With the modeled  $K_{s/w}$  and empirical skin diffusivity D, the skin concentration at any exposure time and at any depth can be calculated from equation 8. By integrating the skin concentration across the whole skin thickness, and normalizing to exposure time of 16 hours, the predicted dermal flux can be obtained, in  $\mu g/cm^2/hour$ :

$$J = \frac{\int_0^h C_W K_{SK/W} \operatorname{erfc}\left(\frac{x}{2\sqrt{Dt}}\right) dx}{t}$$

the model predicted dermal flux for each soil was compared with measured results, as shown in Figure 10.

It's clear to see that nearly all of predictions for soils with different source materials and soil concentrations fell within 1 log unit deviation of the measured results, except for a couple of charcoal amended soils. Overall, the prediction from non-steady state diffusion is much improved compared to the steady state flux prediction, especially for the charcoal amended soils. However, the proposed non-steady state model still slightly under-predicted dermal flux for a couple of charcoal amended soils. In the non-

steady state modeling, it was assumed that the soil particles would reach equilibrium very rapidly with the aqueous phase that was on contact with the skin surface. However, this assumption may not hold true for all the soils, especially for those amended with charcoal. The mass transfer of very hydrophobic compounds such as PAHs from soil particles into porous geo-sorbents through aqueous phase can be slow at static condition, due to the boundary layer resistance (Jalalizadeh and Ghosh, 2016). Energy is needed to promote the transport of PAH into the inner pores of the charcoal material. However, such energy was not provided during the dermal uptake experiment to ensure the partitioning equilibrium between soil particles and charcoal. The equilibrium aqueous concentration  $(C_W)$  that was used in the modeling was previously measured through a partitioning test under a 30-day rigorous mixing on an end-over-end shaker, where aqueous concentration has been demonstrated to be in equilibrium with the charcoal (Gomez-Eyles et al., 2013). The addition of such high sorption domain have resulted in a greatly reduced aqueous concentration during the partitioning test, which may not have been fully achieved in this dermal study. Therefore, the use of the equilibrium aqueous concentration for charcoal amended soils may have led to an under prediction of the dermal flux.

## 4.3.8. Effect of Charcoal on Reducing Dermal Uptake

The effectiveness of 2% charcoal amendment on reducing PAH dermal uptake from soils was investigated under different source materials. For each source material, the percent reductions of PAH dermal uptake from charcoal amended soils were calculated against their corresponding 1 ppm target BaP soils, where the dermal fluxes from amended and un-amended soils were first normalized to their respective soil concentrations. And as shown in Figure 11, depending on PAH source materials, addition of charcoal has various effects on reducing PAH dermal uptake. Four representative PAHs have been selected for demonstration. According to the figure, the effect of charcoal on reducing PAH dermal uptake did not change prominently with PAH hydrophobicity for solvent and fuel oil spiked soils. Detailed reduction results for individual PAHs are listed in Table 4. Also, no clear correspondence between dermal flux reduction and PAH hydrophobicity can be drawn for soot and skeet spiked soils. However, according to Table 4, the soot spiked soil had the greatest variability in observed reduction of dermal uptake, which ranges from 1% to 74%. As discussed in Chapter 2, the soot spiked soils had the highest heterogeneity in soil concentration. And the variability is mainly caused by normalizing dermal flux to soil concentration during reduction calculation.

As an alternative, the overall reduction of dermal flux for total PAHs (sum of 12 parent PAHs listed in Table 4) has been calculated. The effectiveness of charcoal amendment was generally strongest for solvent and fuel oil spiked soils, (with a reduction from 98-60%), followed by skeet and soot spiked soils, with reductions from 50-35%. Such trend is expected due to the extremely high soil  $K_D$  for soot and skeet spiked soils observed in Chapter 2 (e.g. log  $K_D$  for BaP of 6.5 and 5.8 for skeet and soot soils, respectively) than fuel oil spiked soils (e.g. log  $K_D$  for BaP of 5.2), and the control soil had the lowest sorption capacity for PAHs (e.g. log  $K_D$  for BaP of 4.9). So in general, the effectiveness of soil amendment decreased with increasing native soil  $K_D$ .

As discussed earlier, it is suspected that the charcoal may not be in equilibrium with the soil particles, which indicates that the PAHs can still be in the process of
diffusing into the inner pores of the charcoal, resulting in a continuously reduced freely dissolved concentration in the charcoal/soil slurry mixture, thus a continuously increased remedial performance. It has been demonstrated from several field studies that *in situ* sequestration and immobilization treatment of hydrophobic organic compounds, such as PAHs and PCBs using strong geo-sorbents such as AC and biochar often becomes more effective over time due to the progressive mass transfer (Kupryianchyk et al., 2012; Patmont et al., 2015). Therefore, in reality, the effectiveness of charcoal amendment is expected to be improved (with increased reduction of dermal bioavailability relative to our observation) over application time.

## 4.4 Conclusions

To conclude, with steady state dermal diffusion, the use of literature permeability values failed to adequately predict dermal flux within one log unit deviation, the permeability calibrated from 1ppm control soil greatly improved the prediction for most of the soils, except for charcoal amended soil. This is very likely due to the violation of the non-steady state assumption. As for non-steady state diffusion, the skin/ water partitioning coefficient for 16 parent PAHs have been reported for the first time from this study, the log  $K_{S/W}$  ranged from 2 to 4. The prediction of dermal flux using non-steady state diffusion is significantly improved over steady state model, with nearly all of the predictions falling within 1 log unit deviation across five orders of magnitude in exposure concentration. With site specific PAH porewater concentrations measured directly from passive samplers, one can utilize this unsteady state model as a screening tool for human risk assessment, to approximate the PAH dermal uptake from soils based on the soil

partitioning characteristics. In the end, the effectiveness of a relatively novel soil remediation approach, the application of charcoal amendment, has been investigated. Decreased remedial performance (reduction of dermal bioavailability) was observed with increasing soil  $K_D$ : the bioavailability reduction was greater in control and fuel oil spiked soils than skeet and soot spiked soils. However, in reality, the effectiveness of charcoal amendment is expected to improve with application time as the added charcoal equilibrates with the native PAHs.

# 4.5 Reference

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**Figure 1.** Typical Franz diffusion chamber (A) and experimental setup for PAH dermal diffusion (B). A beaker of water was supplied in (B) to provide moisture for the skin diffusion. Picture of Franz cell was obtained from <u>http://www.speciation.net</u>



**Figure 2.** Dermal uptake scheme. Full thickness (2 mm) pig skin was used as a surrogate for human skin. PDMS sheet of 1 mm was placed underneath the skin layer.



**Figure 3.** Experimental setup for skin/water partitioning test. Thin skin slices were equilibrated with PE samplers that were pre-loaded with PAHs for five weeks. Sodium azide (100mg/L) was added to prevent the skin decay and stainless steel mesh was mounted to ensure immersion of PE and skin in the solution.



**Figure 4.** Correlation between measured PAH dermal flux and freely dissolved concentrations for four representative PAHs from all soils constructed with different source materials.



**Figure 5.** Measured PAH dermal bioavailability (% of total dose) of benzo(a)pyrene from soils with different source materials and soil concentrations vs. EPA recommended bioavailability value of 13% (red line).



**Figure 6.** Predictions of dermal flux for 8 PAHs using steady state diffusion model with Kp derived under two different scenarios: A, literature Kp and B, Kp calibrated against date from control soil.



**Figure 7.** Freely dissolved PAH concentrations ( $\mu$ g/L) measured by PE samplers at different exposure times for four representative PAHs (phenanthrene, pyrene, benzo(a)pyrene, benzo(g,h,i)perylene). Error bar represents standard error, n = 2.



**Figure 8.** Measured skin/water partitioning coefficients (solid circles) at different exposure time for 4 representative PAHs (phenanthrene, pyrene, benzo(a)pyrene and benzo(g,h,i)perylene) vs. modeled partitioning coefficients (lines). Error bar represents standard error, n = 2



Figure 9. Correlation between PAH  $K_{OW}$  and equilibrium skin/water partitioning coefficients K skin/w from model fitting.



**Figure 10.** Predicted dermal flux using non-steady state modeling vs. measured dermal flux for all soils with different source materials, PAH concentration and composition. PAHs included in the figure: benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3,-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene.



**Figure 11.** Effect of charcoal addition (2% by mass) on reducing PAH dermal uptake from soil under different source materials (represented by different colors) for four individual representative PAHs, including phenanthrene, pyrene, benzo(a)pyrene and dibenz(a,h)anthracene, as well as total PAHs. Error bar represents standard error, n = 3.

PAH sources	Target BaP concentration						
	1ppm	1ppm	10ppm	100 ppm			
PAH solvent	BSS	BSS + 2% charcoal		BSS			
Fuel oil	BSS	BSS + 2% charcoal	BSS				
Soot	BSS	BSS + 2% charcoal		BSS			
Skeet	BSS	BSS + 2% charcoal		BSS			

**Table 1.** List of soils investigated in the dermal study

**Table 2.** Freely dissolved PAH concentrations ( $\mu$ g/L) (mean value, n = 2) measured at different exposure time in skin/water partitioning experiment. Measured concentration values were within 10% deviation.

Cw (ug/L)	day 1	day 3	day 5	day 7	day 14	day 22	day 35
Naphthalene	2.20	2.66	1.84	1.06	1.39	1.40	1.37
Acenaphthylene	1.19	0.89	0.63	0.67	0.51	0.63	0.49
Acenaphthene	1.33	1.13	0.91	0.94	0.78	0.88	0.93
Fluorene	0.98	0.83	0.68	0.73	0.60	0.64	0.77
Phenanthrene	0.51	0.47	0.42	0.45	0.52	0.54	0.66
Anthracene	0.75	0.68	0.61	0.67	0.71	0.72	0.58
Fluoranthene	0.18	0.18	0.16	0.18	0.17	0.18	0.19
Pyrene	0.17	0.16	0.15	0.16	0.16	0.19	0.18
Benz(a)anthracene	0.08	0.08	0.07	0.08	0.08	0.11	0.07
Chrysene	0.06	0.06	0.06	0.06	0.07	0.09	0.07
Benzo(b)fluoranthene	0.03	0.03	0.02	0.03	0.03	0.04	0.04
Benzo(k)fluoranthene	0.03	0.03	0.02	0.03	0.03	0.04	0.04
Benzo(a)pyrene	0.05	0.05	0.05	0.05	0.05	0.06	0.07
Indeno(1,2,3,-cd)pyrene	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Dibenz(a,h)anthracene	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Benzo(g,h,i)perylene	0.04	0.04	0.04	0.04	0.04	0.04	0.04

**Table 3.** Experimentally determined log K'skin (L/kg) (mean value, n = 2) at different exposure time vs. modeled log Kskin (L/kg) at equilibrium. Measured log K'skin values were within 0.2 log unit deviation.

Exposure time (days)	1	3	5	7	14	22	35	Modeled Kskin @ equilibrium
Naphthalene	1.7	1.8	2.0	2.2	2.1	1.9	2.0	2.04
Acenaphthylene	2.2	2.5	2.7	2.7	2.8	2.7	2.8	2.78
Acenaphthene	2.2	2.5	2.7	2.7	2.9	2.9	2.9	2.90
Fluorene	2.3	2.5	2.8	2.7	2.9	3.0	3.0	2.98
Phenanthrene	2.3	2.6	2.8	2.8	2.8	2.9	3.0	2.96
Anthracene	2.3	2.6	2.8	2.8	2.9	3.0	3.3	3.43
Fluoranthene	2.6	2.6	3.1	3.1	3.1	3.4	3.6	4.27
Pyrene	2.7	2.8	3.1	3.1	3.2	3.4	3.6	4.03
Benz(a)anthracene	2.9	3.1	3.3	3.3	3.4	3.5	3.9	3.70
Chrysene	3.0	3.2	3.5	3.5	3.5	3.5	3.9	3.62
Benzo(b)fluoranthene	3.2	3.3	3.6	3.5	3.7	3.7	3.9	3.84
Benzo(k)fluoranthene	3.2	3.4	3.7	3.6	3.7	3.7	3.9	3.77
Benzo(a)pyrene	3.2	3.3	3.6	3.6	3.7	3.8	3.9	3.83
Indeno(1,2,3,-cd)pyrene	3.0	3.5	3.7	3.8	3.7	3.8	4.1	4.06
Dibenz(a,h)anthracene	3.0	3.4	3.6	3.7	3.7	3.8	4.1	4.11
Benzo(g,h,i)perylene	2.9	3.2	3.5	3.5	3.6	3.7	3.9	3.94

Table 4. Percent reduction of PAH dermal uptake for individual PAHs from soils w	vith
different source materials.	

percent reduction	solvent	fuel oil	soot	skeet
Phenanthrene	99%	79%	74%	26%
Anthracene	99%	31%	52%	41%
Fluoranthene	95%	31%	55%	10%
Pyrene	99%	58%	25%	67%
Benz(a)anthracene	91%	36%	1%	34%
Chrysene	90%	54%	73%	54%
Benzo(b)fluoranthene	95%	65%	24%	65%
Benzo(k)fluoranthene	96%	79%	63%	78%
Benzo(a)pyrene	87%	68%	33%	50%
Indeno(1,2,3,-cd)pyrene	91%	77%	6%	31%
Dibenz(a,h)anthracene	98%	85%	33%	67%
Benzo(g,h,i)perylene	96%	75%	NA	44%

# Chapter 5: Effect of Source Materials on PAH Bioaccessibility Assessed by Physiologically Based Extraction Tests

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## Abstract

Incidental soil ingestion by humans is considered the primary pathway for systemic exposure to PAHs in soils. In this study, a simulated physiologically-based extraction test (PBET) was used to assess the PAH bioaccessibility from a library of soils previously constructed with different typical PAH source materials (fuel oil, soot and coal tar based skeet), soil organic matter, and minerals to achieve different PAH concentrations. Source material had a strong impact on PAH bioaccessibility: soils spiked with soot and skeet generally exhibited the lowest bioaccessibility, followed by fuel oil and solvent spiked soils. Among all soil compositions, the presence of 2% charcoal had the greatest effect on reducing PAH bioaccessibility, with the biggest reduction (of approximately 80%) observed for soils spiked with solvent and fuel oil. Across all soils constructed, soil partitioning coefficients (KD) were observed to be negatively correlated (p<0.01) with PAH bioaccessibility, with R2 ranging from 0.65 to 0.74. We demonstrate that a partitioning based model that accounts for the sorption capacity of the simulated gut fluid is able to reasonably predict PBET extraction for most soils across 5 orders of magnitude in PAH mass extracted. Further investigation may be necessary to validate these results using in-vivo studies.

# **5.1 Introduction**

Human exposure to polycyclic aromatic hydrocarbons (PAHs) in soils can be through incidental soil ingestion, direct dermal contact, and inhalation of particles. Based on the current EPA regulatory paradigm (USEPA, 2012), incidental soil ingestion is considered the primary pathway for systemic exposure to chemicals in contaminated soils. Bioavailability of PAHs to human and ecological receptors can be markedly diminished when PAHs are associated with soils and sediments, and this has been reported in many studies since 1980. Ortega-Calvo et al. proposed ways to incorporate bioavailability assessments into prospective risk assessment for ecological exposures (Ortega-Calvo et al., 2015). As summarized in the study by Ruby et al., the bioavailability of PAHs in soils to human receptors has also been extensively researched (Ruby et al., 2016).

It is recognized that the total oral dose of PAHs from soils does not necessarily represent the bioavailable fraction, which is limited to those PAHs that are solubilized in the gastrointestinal (GI) tract and eventually absorbed into human systemic circulation. There are a number of animal models developed to predict the oral PAH bioavailability from soils to humans (Ruby et al., 2016). The broad application of *in vivo* models to understand the bioavailability of PAHs from soil is limited due to the complexity of conducting animal studies, their high cost, and animal ethics. Therefore, *in vitro* extraction tests have attracted attention during the past two decades as possible alternatives to developing data that are useful for understanding bioavailability. In vitro models attempt to predict PAH bioavailability by measuring the PAH fraction that is solubilised from soil in bench-top extraction tests. Many of these systems to date have

been "physiologically-based," using extraction fluids that simulate the conditions of the gastrointestinal tract. The fraction that is liberated from the soil in these "in vitro" systems is referred to as the "bioaccessible" fraction, or "in vitro bioaccessibility (IVBA)". Several recent studies have focused on optimizing the simulated GI conditions (e.g. agitation, pH, incubation time) and understanding how different components of GI fluid (e.g. bile, lipids, etc.) affect PAH bioaccessibility. (Cave et al., 2010; Gouliarmou and Mayer, 2012; Lu et al., 2009; Oomen et al., 2004; Tao et al., 2010) In addition to the gastrointestinal environments, it is also reported that soil properties such as organic matter content can also affect PAH bioaccessibility.(Vasiluk et al., 2007) However, the role of PAH interaction with different geochemical components in soil in influencing PAH bioaccessibility is still not fully understood.

PAHs are released into the soil within different matrices (e.g. coal tar, soot or fuel oil) and results from previous studies have demonstrated how different forms of carbon in soil influence PAH bioavailability to ecological receptors.(Accardi-Dey and Gschwend, 2001; Cornelissen et al., 2006; Cornelissen et al., 2005a; Jonker and Koelmans, 2002; Khalil et al., 2006) Previous research in our laboratory has shown that both PAH source materials and soil geochemical composition controls partitioning of PAHs into the aqueous phase, with the difference in partitioning constants of up to two orders of magnitude among different PAH sources (Xia et al., 2016). Since the digestive process in the mammalian GI tract also involves an aqueous medium and PAHs need to desorb from the soil matrix and partition into the gastrointestinal fluid, sorption to soil is very likely to influence bioavailability. However, the digestive process in a vertebrate animal is more complex than a simple partitioning into water. For example, facilitated transport involving micelle formation can enhance the dissolution of poorly water soluble PAHs increasing their bioaccessibility.(Oomen et al., 2004) Therefore, how the enhanced sorption of PAHs in source materials and soil components influences uptake in the human gut environment is also yet to be fully raveled.

This research expands on our prior work to assess partitioning behavior of PAHs from soil with different PAH sources and soil characteristics, and assesses whether that aqueous partitioning behavior predicts the results from a physiologically-based extraction test of the same soils. To provide insights with regard to the influence of PAH source and different soil characteristics, this work evaluates PAH bioaccessibility from a library of soils previously constructed with typical PAH source materials (fuel oil, soot, coal tar based skeet particles), different forms of organic matter (peat moss and humus), different PAH concentrations (across four orders of magnitude) and different soil components. Modeling approaches using partitioning theory (e.g. freely dissolved PAH concentration and partition constants) are explored to explain and predict the range of observed PAH bioaccessibility in soils.

## **5.2 Materials and Methods**

## 5.2.1. PAH Contaminated Soils.

Details for construction of PAH contaminated soils and weathering process were described in Xia et al.(Xia et al., 2016). Briefly, a "baseline synthetic soil" (BSS) containing 10 percent peat moss, 20 percent kaolin clay, and 70 percent silica sand was constructed. Then various soil compositions and source materials (weathered coal tarbased skeet particles, lampblack soot particles, and fuel oil) were introduced into the BSS to obtain a wide range of PAH concentrations and soil matrix (Table S1). All of the components were sieved to < 150  $\mu$ m prior to soil construction. The control soil (without source materials) was created by spiking PAH stock solution in dichloromethane into the BSS and allowing solvent dissipation afterwards. All constructed soils were homogenized, and then weathered at the U.S. Army Edgewood Chemical Biological Center for 8 weeks (ECBC, Aberdeen Proving Ground, MD).

#### 5.2.2. Soil PAH Concentrations and Partition Coefficients.

PAH concentrations in weathered soils were measured and partitioning coefficients (K<sub>D</sub>) for each source material and soil were also determined through aqueous equilibrium experiments using polyoxymethylene passive samplers as described in (Xia et al., 2016).

# 5.2.3. Simulated Gastrointestinal Fluid.

The in vitro extraction method used in this study was modified from the one reported in (Ruby et al., 2002). The soluble lipid source was changed from 6 mL/L of oleic acid to 3 mL/L of sunflower oil because the latter contains more diverse lipid types, which favors micelle formation; and a lower lipid concentration was used to discourage

the formation emulsions during solvent extraction. Porcine bile was used in place of bovine bile due to its greater similarity to human bile (Oomen et al., 2004), and the bile concentration was reduced from 4 g/L to 2 g/L as the latter has been shown to be equally effective at mobilizing PAHs from soil (Tao et al., 2010). For the physiologically-based extraction, the simulated stomach fluid consisted of 0.95 L of ASTM Type II deionized water with 30 mL of trace-metal grade concentrated ( $\geq$  65 percent) HCl (Fisher Scientific, USA), 30.0 g of glycine (Acros Organics, USA), 1.0 g of porcine pepsin (Sigma, USA), 5 g of Bovine Serum Albumin (BSA, Fraction V; Fisher Scientific, USA), 3 mL of sunflower oil (Spectrum Chemical Mfg., USA), and 2.5 g of porcine mucin (Type III; Sigma, USA) (Figure S3). After the addition of all the above ingredients, the volume of the solution was brought to 1 L and the pH of the fluid was adjusted to 1.50 ± 0.05 by drop-wise addition of concentrated HCl. Transition to the intestinal phase was then simulated by altering the pH of the artificial stomach fluid to 6.5, and the addition of porcine bile and porcine pancreatin extract as described below.

# 5.2.4. Physiologically-Based Extraction Testing (PBET).

The PBET used in this study included two phases: simulated stomach extraction and simulated small intestine extraction. For the extraction process, 0.4 g of soil (airdried and sieved to <150  $\mu$ m) was dosed into a centrifuge tube with a Teflon-lined screw cap, followed by addition of 40 mL of simulated stomach fluid (soil/liquid ratio 1:100) (James et al., 2011). The tube was then placed on an end-over-end extraction device in a water bath at 37 °C for 1 hour to simulate the gastric conditions. Then the pH of the digestive fluid was adjusted to 6.5±0.5 by drop-wise addition of concentrated NaOH into each tube, followed by addition of 80 mg of porcine bile extract (Sigma, USA) and 20 mg of porcine pancreatin extract (Sigma, USA) to achieve the simulated conditions of the small intestine. After a four-hour extraction with end-over-end rotation at 37  $^{\circ}$ C, the tube was centrifuged at 1500 x g for 10 minutes to separate the extraction fluid from the soil particles (Van de Wiele et al., 2004). Thirty mL of the supernatant was then transferred into a clean 60 mL glass vial for determination of PAH concentration.

## 5.2.5. Method Development for PAH Recovery from PBET.

Preliminary recovery efficiency testing revealed poor recovery of PAH out of the GI fluid when spiking the GI fluid before the 5 hour PBET process. An extensive method optimization was therefore performed, and details are provided in the SI. The optimum method involved three long sequential withdrawals of PAHs from the PBET fluid (16 h, 24 h, 24 h) using 20 ml of hexane at 60 rpm agitation (to avoid the formation of emulsions). After each agitation, the solution was allowed to stand for 1 hour for complete separation of the solvent phase. Hexane solvent withdrawals were pooled and dried by shaking with 10g of anhydrous sodium sulfate overnight, before silica gel cleanup (EPA Method 3630C). Solvents were then analyzed using an Agilent GC (Model 6890) with a mass spectrometer detector following EPA method 8270. Figure S3 provides a flow diagram outlining the entire process, including the physiologically-based extractions in both simulated gastric and intestinal fluids, and sample preparation to isolate and analyze the PAHs from the physiologically-based fluids.

# 5.2.6. Bioaccessibility Calculation.

The PAH bioaccessibility from soil was calculated as ratio of the mass of PAH extracted by the PBET fluid to the total mass of PAH measured in soil after weathering:

bioaccessibility (%) = 
$$\frac{M_{pAH in GI fluid}}{M_{pAH in soil}}$$
 eq(1)

## 5.2.7. Measuring GI Fluid Partitioning Constant.

The  $K_{GI-5h}$  for the PBET system used in this study was experimentally determined using polyethylene passive samplers pre-impregnated with PAHs as PAH source, as well as a tool for determining freely dissolved aqueous PAH concentration after the 5 hours mixing. The passive samplers were then removed and simulated GI fluid was extracted with hexane three times to quantify the PAH concentration in the fluid (see supporting information for method details).

$$K_{GI-5h} = \frac{C_{GI}}{C_W}$$
 eq (2)

where  $C_{GI}$  (in  $\mu g/g$ ) and  $C_W$  (in  $\mu g/mL$ ) are the PAH concentrations in GI fluid and aqueous phase measured from the partitioning test.

## 5.2.8. Quality Control.

Reagent and procedural blanks (10 percent frequency) were included with sample analysis and all measurements were blank corrected using the arithmetic means of all procedure blanks. PBET extractions were performed in triplicate for each soil sample. The gastrointestinal extraction method recoveries (10 percent frequency) were determined by spiking 100 µl of PAH matrix solution (mixture of 16 PAHs, Ultra Scientific, USA) in dichloromethane into the digestion fluid in replacement of soil samples at the beginning of the PBET procedure. The recoveries of 16 PAHs from PBET procedure ranged from 70% to 125%. A certified reference soil (Resource Technology Corporation, CRM141-50) was also analyzed together with study soils (5 percent frequency) to assess the consistency of the gastrointestinal extraction procedure. The variability (relative standard deviation) of PAH bioaccessibility from the certified

reference soil ranged from 30% to 50%. Four internal standards (1-fluoronaphthalene, pterphenyl-d14, benzo(a)pyrene-d12 and dibenz(a,h)anthracene-d14) were used for PAHs analysis and deuterated phenanthrene was used as a surrogate standard to monitor losses in the whole PBET extraction and cleanup procedures. The overall surrogate recoveries varied from 70% to 130 %.

#### 5.3. Results and Discussion

#### 5.3.1. Effect of Source Material on Bioaccessibility.

PBETs were performed on soils constructed with different PAH source materials, concentrations, and soil matrix. Blank BSS without addition of PAH source of any form was also included and no significant amount of PAHs was detected in the simulated gastrointestinal fluid extract. As shown in Figure 1, PAH source material had a great impact on the bioaccessibility of PAHs from soils. This effect was observed irrespective of the PAH concentrations. The soils presented in Figure 1 were all baseline soils spiked with different PAH source materials at a targeted concentration of 1mg/kg of B(a)P. The fuel oil and solvent spiked (control) soils generally had the highest bioaccessible PAH fraction (from 60% to 100%), followed by soot spiked soils (30% to 40%) and then skeet spiked soils (20%). The observed high dissolution of PAHs into the gastrointestinal fluid for solvent-spiked soils was expected due to the lack of strong sorbing domains either in the soil or the PAH source. The observed PAH bioaccessibility from fuel oil spiked soils was high and similar to solvent spiked soils. For soils spiked with either PAHs in solvent or fuel oil, the added PAHs are readily solubilized into the PBET fluid. GI fluid components such as bile salts are known to have surfactant properties, which can

decrease the surface tension of the hydrophobic phases (e.g. lipids, organic matters, NAPLs) and favor the mobilization of the bound PAHs (Oomen et al., 2004; Tao et al., 2010). This is not surprising as the role of in vivo GI fluid is to solubilize fats and oils as an important component of the digestion process.

Soils spiked with skeet and soot as the PAH source showed the lowest bioaccessibility of PAHs. Both skeet and soot exhibit strong sorption for PAHs in partitioning studies (Figure S4) (Xia et al., 2016). The extremely high PAH sorption capacity (K<sub>D</sub>) associated with lamp-black soot used in this study has been frequently reported (Jonker and Koelmans, 2002)(Cornelissen et al., 2005b), which explains the low PAH bioaccessibility observed from soot spiked soils. Similarly low bioaccessibilities (15-50%) of B(a)P bound to soot have been reported by others, even when attempting to enhance the PBET using an extended colon-phase or adding infinite sinks such as a silicone rod or sheet to the GI fluid (Gouliarmou et al., 2013; Oomen et al., 2004; Zhang et al., 2015). Recent in vivo bioavailability study from Robert et al. has also observed much lower relative bioavailability from soot spiked soils than other source materials (Roberts et al., 2016).

Like soot, high PAH sorption capacity has also been often observed for coal tar derived pitch materials (Ghosh and Hawthorne, 2010; Khalil et al., 2006), which explains the low PAH bioaccessibility from skeet spiked soils. Other studies have reported similarly low bioaccessibility (15-41%) for soils from a former tar and pitch works site, using a similar bioaccessibility method (Lorenzi et al., 2012). The skeet particles used in this study were made of a mixture of crushed limestone (70 percent) and coal tar (30 percent; used as a binder). The fact that the skeet target fragments has been through decades of field weathering might contribute to its enhanced  $K_D$  and reduced bioaccessibility. Additionally, in our previous study we observed skeet  $K_D$  increased 2-3 orders of magnitude further in a highly nonlinear fashion as more PAHs desorbed from the skeet particles. Therefore, as PAHs are released from the skeet into GI fluid, it is likely that the bioaccessibility of the PAHs remaining in the skeet will be increasingly lower.

The soils with 0.1mg/kg target B(a)P exhibited similar bioaccessibility trend across different source materials compared to 1 ppm soils, except the PAH bioaccessibility from the solvent spiked soils was lower, and the difference between soot and skeet spiked soils diminished (Figure S5). When the soil concentration increased to 10 mg/kg target B(a)P, the PAH bioaccessibility of the fuel oil soil dropped compared to observations at lower concentrations (Figure S6). It is possible that at the highest dose of oil, there is an excess of oil that remains un-solubilized by the simulated GI fluid (exceeding the ability of micelles to solubilize the oil, thus resulting in residual fuel oil in the system that serve as a "sink" for the PAHs); or there may be some form of interaction with components of the PBET that restricted the release of PAHs from fuel oil at the highest oil dose tested. At higher target concentration of B(a)P (of 10 mg/kg) in soils, the soot soil also displaced skeet soil as the most recalcitrant to PBET extraction (Figure S6). Exactly the same trend was also found in soils with target concentration of 100 mg/kg B(a)P (Figure S7). Similar results were observed in our previous partitioning studies, higher K<sub>D</sub> was observed for the skeet-spiked soils compared to the soot-spiked soils at 1 mg/kg target B(a)P, but this trend was reversed at the highest concentration (of 100 mg/kg target B(a)P) where the strongest partitioning was observed for the soot-spiked

soils. This observed reverse in relative  $K_D$  values could be explained by the larger amount of soot relative to skeet present in the constructed soils at high target concentrations (50% soot by weight, 2.2% skeet by weight) and the previously measured high  $K_D$  for soot (Xia et al., 2016).

#### 5.3.2. Effect of Concentration on Bioaccessibility.

During soil construction, the amount of PAH source materials introduced to BSS varied based on the target PAH concentration: approximately one order of magnitude higher dosage of source material was used to obtain soil PAH concentration of one order of magnitude higher. For control soils, increased amount of solvent spiking should not have any impact on the overall soil sorption characteristics because the added solvent was lost via evaporation during soil homogenization and soil weathering. However, as shown in Figure 2, for the solvent-spiked soils there was an apparent trend of an initial increase in bioaccessibility with increasing PAH concentration, followed by an eventual decrease at the highest spiked target concentration of 100 mg/kg B(a)P. The initial increase in bioaccessibility with PAH concentration may be expected based on typically observed nonlinearity in adsorption of PAHs where high energy sites on soil particles were filled up preferentially at low concentrations, while low energy sites (more easily extractable) being populated at higher concentration. In our previous work we observed a weak nonlinearlity for the sorption of B(a)P to sand and clay components, but not in peat.<sup>15</sup> Additionally, the decrease in bioaccessibility at the highest concentration for the solventspiked soil may indicate capacity limitation of the fixed volume of simulated GI fluid. When investigating B(a)P bioaccessibility at different concentrations, a study by Sips et al (AJAM et al., 2001) also found a clear levelling off in percent bioaccessibility in soils

spiked with concentrations above 100 mg/kg BaP. Gouliarmou et al.(Gouliarmou et al., 2013; Gouliarmou and Mayer, 2012) identified the capacity issue as one of the drawbacks of not having a PAH sink in the in-vitro test simulating uptake through the intestinal membrane. They suggested the inclusion of a sorptive sink in the form of silicone rods and demonstrated that the extraction capacity measured in their simulation system was increased by orders of magnitude compared to a fixed volume of GI fluid alone. A different sorptive phase (sunflower oil) was also included in our PBET system. However, such enhanced extractability from a sorptive sink does not necessarily guarantee an improved predictability of the in vitro model, as no valid comparisons were made with results from in-vivo studies to confirm such need in a PBET system in order to be predictive of bioavailability as measured in animals.

A decreased bioaccessibility with increasing concentration is observed as a consistent trend for the fuel oil and soot spiked soils. This was most likely due to the enhanced soil sorption capacity for PAHs resulting from the added black carbon source materials. For these soils, the soil composition was altered as a result of increased source material addition that remained in the soil. For example, in soot spiked soils, with the target B(a)P concentration elevated from 0.1 to 100 mg/kg, the fraction of soot in soil was increased from 0.05% to 50%, which would theoretically give rise to an increase of soil sorption capacity by three orders of magnitude from soot alone. At such high level of soot in the soil, it is also possible that the soot carbon interacted with the PBET components, such as sorbing the lipid fraction or other key constituents that are critical for the formation of PAH solubilizing micelles. A recent in vivo study has also reported reduced PAH relative bioavailability with increased soil concentration for soot spiked

soils (Roberts et al., 2016). However, for skeet spiked soils, the measured bioaccessibility was not significantly different among different soil concentrations for most PAHs investigated, which was expected as the K<sub>D</sub> values for skeet spiked soils were shown to be very consistent among different soil concentrations in our previous study (Xia et al., 2016).

## 5.3.3. Effect of Soil Composition on Bioaccessibility.

The effect of different soil compositions on PAH bioaccessibility was primarily evaluated on solvent and fuel oil spiked soils. As shown for solvent spiked soils in Figure 3, the baseline soil and soil with reduced clay content (2%) showed the highest PAH bioaccessibility. Based on previous sorption isotherm study (Figure S4), clay had slightly higher  $K_D$  than sand, thus decreased clay content (from 20% to 2%) would result in reduced overall soil sorption capacity and may therefore result in increased PAH bioaccessibility in solvent spiked soils. The soil with kaolin clay replaced by montmorillonite had lower PAH bioaccessibility than baseline soils, especially for 4-5 ringed PAHs. This observation can be explained by the increased  $K_D$  in soils with montmorillonite relative to baseline soils observed in our previous study (Figure S8). PAH bioaccessibility was generally lower in soils with humus instead of peat despite having a slightly lower K<sub>D</sub> (Figure S8), suggesting soil K<sub>D</sub> is not the only factor controlling bioaccessibility. Interestingly, soils with peat content reduced to 1% had much lower PAH bioaccessibility than baseline soils in both solvent (Figure 3) and fuel oil (Figure S9) spiked soils. These results are contrary to what would be expected based on how organic carbon influences PAH sorption and bioaccessibility in soils. However,

the result is consistent with finding from our partitioning study, where the soils with reduced peat content generally had a higher or equivalent K<sub>D</sub> relative to baseline soils.

#### 5.3.4. Effect of black carbon on bioaccessibility.

To evaluate the effect of elevated native black carbon content on PAH bioaccessibility, soils with all four PAH source materials were altered to include 2% charcoal. Different impacts from charcoal were observed for soils with different PAH sources (Figure 4). The effect of charcoal addition was the greatest in the solvent and fuel oil spiked soils with up to 80% reduction in PAH bioaccessibility where the native soil K<sub>D</sub> was the lowest. The presence of charcoal had less impact on reducing bioaccessibility for soot spiked soils, and the least impact (around 50% overall reduction) was observed in skeet spiked soils where the native soil  $K_D$  was the highest. These observations were in line with the soil  $K_D$  results reported in our previous study: charcoal dramatically decreased the bioaccessibility of PAHs in all soils, and the effect weakened as the initial soil K<sub>D</sub> increased (Xia et al., 2016). Also, the effect of charcoal was very consistent among different PAH compounds. This prominent effect of charcoal on reducing bioaccessibility observed from this study was in good agreement with the results from an in vivo animal (rat) study conducted by (Roberts et al., 2016), where over 67% reduction in relative bioavailability was observed in all of their charcoal amended soils. These results confirm that the use of black carbon amendments that have been previously shown to reduce PAH bioavailability to ecological receptors in soils (Gomez-Eyles et al., 2011) and sediments (Ghosh et al., 2011), can also reduce PAH dissolution in simulated conditions of the human gut and are therefore likely to reduce PAH bioavailability to humans following ingestion of contaminated soils.

## 5.3.5. Relation between Soil K<sub>D</sub> and Bioaccessibility.

Given the apparent relationship between  $K_D$  and bioaccessibility observed above, three PAHs with different number of aromatic rings (chrysene, benzo(a)pyrene and benzo(g,h,i)perylene) were selected for a comparison of the relation between measured  $K_D$  and bioaccessibility. As shown in Figure 5, across all soil samples evaluated, PAH soil partitioning constants were significantly (p<0.01) negatively correlated with PAH bioaccessibility for all three PAHs, with R<sup>2</sup> ranging from 0.65 to 0.74. The lowest bioaccessibility was observed for benzo(g,h,i)perylene which has the highest molecular weight and also highest  $K_D$  among PAHs. While separate power correlations are shown for each compound in Figure 5, the differences between PAHs can be resolved by accounting for PAH hydrophobicity by dividing  $K_D$  with PAH partitioning in gut fluid ( $K_{GI-5h}$ ) as shown in Figure S12. This strong correlation was observed over a wide range of soil properties (such as soil compositions, source materials), as well as broad range of aqueous and soil concentrations encompassing 4 orders of magnitude.

While the solubilization of PAHs into the simulated GI fluid involves much more complex transport processes than simple mass transfer to an aqueous phase, there appears to be a strong relationship between bioaccessibility and PAH partitioning in soils. The effect of partitioning on bioaccessibility has been rarely studied in the past, and to our knowledge this is the second demonstration (after a study by James et al.(James et al., 2016)) that physicochemical processes in soil (e.g. partitioning constants) can impact how PAHs are released in the gut environment. The study by James et al.<sup>28</sup> observed negative linear correlation between soil fugacity capacity and bioavailability estimated through an in vivo swine study.(James et al., 2016) However, in their study, most of the

observed correlations were weak and deteriorated with PAH hydrophobicity, with the coefficients of determination ( $\mathbb{R}^2$ ) decreasing from 0.72 to 0.13, from benzo(a)anthracene to benzo(k)fluoranthene (James et al., 2016). This weak correlation might be caused by the large variance in the detected bioavailability of PAHs in swine blood, with standard error spanning up to 2 orders of magnitude, compared to the standard errors of less than 0.3 observed from our PBET model that provides a more simplified and controllable environment than the GI tract of a live animal. One thing to note is that, although both soil fugacity and bioavailability can be strongly affected by soil partition constants, the effect on freely dissolved concentration and in vitro extraction are distinct. While the high partition constants for skeet relative to solvent-spiked soil resulted in a freely dissolved concentration of 2 orders of magnitude lower, the corresponding bioaccessibility for skeet-spiked soils was reduced by a factor of only 4, (20% vs. 80%). A plausible link between  $K_D$  and bioaccessibility in simulated gut fluid is the partitioning of the PAHs in the gut fluid matrix as described below.

## 5.3.6. A partitioning based model for PBET.

A mechanistic partitioning model was developed to explain the PAH dissolution from soil into simulated GI fluid, where a two-step sequential transport process was assumed: desorption of PAHs from soil into the aqueous phase, and subsequent absorption of PAH from the aqueous phase into simulated GI media (e.g. enzymes, lipids). Due to the fine size of the soil particles (<150  $\mu$ m) dosed into GI fluid and the vigorous mixing, it was assumed that PAH desorption from the soil would occur rapidly and partitioning into aqueous phase would be close to equilibrium in the first transport process. For the second transport process, all simulated GI solutes could be treated as one

amorphous entity with an overall aqueous partition constant, K<sub>GI-5h</sub>, which was assumed to be constant for a PBET system with specified GI composition and environment. It is noteworthy that K<sub>GI-5h</sub> describes the pseudo-equilibrium partitioning of PAHs between the simulated GI fluid and the freely dissolved aqueous phase after 5 hours of mixing (1) hour for gastric phase and 4 hours for intestinal phase), which is not a function of soil concentration or soil compositions, but solely PAH properties for a specified PBET system. The experimentally determined log  $K_{GI-5h}$  was strongly positively (R<sup>2</sup>=0.95) correlated with PAH hydrophobicity (log K<sub>OW</sub>), which ranged from 1.95 (for naphthalene) to 5.50 (for dibenz(a,h)anthracene) (Figure S10). The experimentally determined K<sub>GI-5h</sub> was then compared with estimated K<sub>GI-5h</sub> assuming sunflower oil was the dominant sorption domain in the simulated GI mixture (estimated  $K_{GI-5h} = f_{oil} * K_{oil}$ , where  $f_{oil}$  was the mass fraction of sunflower oil and  $K_{oil}$  was approximated as  $K_{OW}$ ). The precision of the K<sub>GI-5h</sub> measurement was confirmed by the good consistency with estimated K<sub>GI-5h</sub>, where less than 1 log unit deviation was observed for each of the 16 PAHs tested (Figure S11 and Table S2).

The mass of PAHs extracted by PBET was modeled based on mass balance of PAHs:

$$M_{S}C_{S}^{0}=M_{S}K_{D}C_{W}+M_{GI}K_{GI-Sh}C_{W} \quad eq (3).$$

where  $M_S$  and  $M_{GI}$  (both in grams) are the masses of soil particles and gut fluid mixture used in our PBET;  $C_S^0$  is the initial PAH concentration in the soil and  $C_W$  is the PAH aqueous concentration after 5 hour's mixing;  $K_D$  is the equilibrium soil partitioning constant. Thus,

mass of PAHs extracted by PBET = 
$$M_{GI}K_{GI-5h}C_W = M_{GI}K_{GI-5h}\frac{M_SC_S^0}{M_SK_D + M_{GI}K_{GI-5h}}$$
 eq (4).

Model predicted PBET extraction from equation 4 was compared with measured results in Figure 6, for 8 parent PAHs with the highest molecular weights. The model performance varied with PAH source material. The model provided good prediction of PAH extractability by PBET for all the solvent and fuel oil spiked soils, regardless of the soil concentrations or compositions (Figure 6A). Decent model prediction is also observed for most soot spiked soils, except for the one with 100 ppm target B(a)P concentration, which has the highest soil K<sub>D</sub> compared to the rest of soot soils. No prominent effects of soil concentrations, soil compositions, nor PAH hydrophobicity (K<sub>ow</sub>) on model performance were observed for soils spiked with these three source materials. Skeet spiked soils also showed a good prediction of PBET extraction except for a few cases, especially for the three highest molecular weight PAHs, at high concentration, and in the presence of charcoal (Figure 6B). One possibility is that for high K<sub>D</sub> materials, entrainment of fine particles of PAH source materials in the GI fluid or co-entrainment of the source particles with GI-fluid colloids and inadequate separation by centrifugation, may be elevating apparent assessment of the extractability. The biological relevance is uncertain of any high-K<sub>D</sub> fines entrained in PBET.

Overall, these results indicate that a simple partitioning based model can reasonably predict PBET extraction across 5 orders of magnitude in PAH mass extracted. The benefits of using the partitioning based model are: 1) soils can be screened for relative bioaccessibility based on an aqueous partitioning test which is much simpler to perform compared to a PBET, 2) using the model, several scenarios can be tested including altering the soil to PBET mass ratios, altering the fraction of lipids, and introduction of sorptive sink if necessary. Further work is needed to address how each of the GI fluid ingredients (e.g. surfactants, lipids and enzymes) may interact with the various soil components and PAH source materials and ultimately, in-vivo studies may be necessary for validation.

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**Figure 1.** Effect of PAH source materials (control, fuel oil, soot and skeet particles) on percentage of PAHs extracted by gastrointestinal fluid from soils with 1mg/kg target B(a)P (n= 3, error bars represent standard error).



**Figure 2.** Effect of soil PAH concentration (as target B(a)P) on percentage of PAHs extracted by gastrointestinal fluid from soils with different source materials (n= 3, error bars represent standard error, asterisk represents significant difference among groups ( $\alpha$ =0.05)).



**Figure 3.** Effect of soil compositions on percentage of PAHs extracted by gastrointestinal fluid from solvent spiked soils with 1mg/kg target B(a)P (n= 3, error bars represent standard error).



**Figure 4**. Reduction of PAH bioaccessibility after charcoal addition from soils spiked with different source materials to achieve 1mg/kg target B(a)P.



**Figure 5.** Correlation between measured soil  $K_D$  and PAH bioaccessibility extracted by gastrointestinal fluid for four, five and six ringed PAHs (chrysene, benzo(a)pyrene and benzo(g,h,i)perylene, respectively) from all 30 soil samples. Error bars (n=3) represent standard error.



**Figure 6.** Comparison between measured and model predicted PAH extraction by PBET for 8 PAHs in soils constructed with different source materials and different target PAH concentrations. PAHs presented in the figure: benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3,-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene. A: includes all solvent, fuel oil and soot spiked soils; B: skeet-spiked soils.

# Chapter 6: Effect of Source Material and Bio-char Amendment on PAH Uptake in Earthworms

# Abstract

Water exposure and soil ingestion are two major exposure routes for earthworms to PAHs in soils. However, due to its low water saturation and high heterogeneity, soil may not always be at equilibrium with the porewater and the earthworms even after 30 days' exposure. Therefore, the equilibrium partitioning approach for estimating PAH bioaccumulation in earthworm may not be applicable to soil environment. Although source materials are known to have a strong impact on soil K<sub>D</sub>, how this further affects PAH uptake in soil invertebrates via each exposure route is still unknown. To improve the understanding of bio-uptake of PAHs in soil invertebrates, a mechanistic kinetic model encompassing both exposure routes has been developed in an attempt to provide more accurate prediction of PAH bioaccumulation under both short-term and long-term exposure scenarios. The results show that the overall PAH uptake in earthworm is significantly correlated with soil  $K_D$ , which is a function of source material and soil composition.  $K_D$ controls both freely dissolved PAH concentration and assimilation efficiency of PAHs into worm tissue through soil ingestion. The variation in bioaccumulation factors measured from different soils indicated that soil concentration was not an indicator for PAH bioaccumulation in worms. The relatively consistent bioconcentration factors measured across soils with different source materials indicated that freely dissolved PAH concentration might be a more appropriate predictor for bioaccumulation. The kinetic biouptake model inclusive of both water exposure and soil ingestion was able to provide acceptable predictions (of within one log unit deviation) for nearly all of the soils.

## **6.1 Introduction**

Soil invertebrates are exposed to HOCs through two primary exposure routes: 1) ingestion of contaminated soils and 2) dermal uptake of HOCs through aqueous phase (Erstfeld and Snow-Ashbrook, 1999; Peijnenburg et al., 2012; Lee et al., 2015). The bioaccumulation of HOCs in soil invertebrates plays a crucial part in risk assessment as it forms the base of the terrestrial food web that is primarily responsible for the transfer of soil-bound contaminants to the upper trophic levels (Navarro et al., 2016). It is generally recognized that soil partitioning coefficient  $K_D$  is one key parameter that controls the bioaccumulation of HOCs in organism (Zhu et al., 2009). This K<sub>D</sub> is affected by a variety of factors (e.g. source material, geochemical properties of the soil) as discussed in Chapter 2 (Koelmans et al., 2006), where it has also been demonstrated that conventional OC/BC models fail when distinctly different source materials are present, whose sorption characteristics cannot be simply described by either OC or BC (Xia et al., 2016). While the role of source materials in soil K<sub>D</sub> has been investigated in Chapter 2, how source materials affect PAH uptake in soil invertebrates via each exposure route is still unknown. In terms of water exposure route, theoretically, the source materials can alter the freely dissolved PAH concentration and therefore control the uptake through skin diffusion. However, such hypothesis has never been tested under different source materials in soils, and the contribution of water exposure to overall uptake has never been investigated for different source materials. As for soil ingestion route, it is still unclear whether the PAHs in the soils would be completely solubilized for uptake during the digestion phase inside the intestines of a soil invertebrate, especially when extremely high sorption domains such as soot or skeet are present. Lu et al. observed reduced assimilation efficiencies for different PAH

compounds through gut digestion of a benthic oligochaete when exposed to coal tar impacted field sediments (Lu et al., 2004). It is possible that the assimilation efficiency of PAHs would also vary in a soil invertebrate when exposed to soils with various source matrix. Therefore, in this chapter, how source materials would affect the bio-uptake of PAHs in soil invertebrates through oral and dermal exposure pathways has been investigated.

In traditional risk assessment, the bioaccumulation of HOCs are usually assessed using an equilibrium partitioning approach (Burns, 1996; McCulloch, 1996; Ololade, 2010). Such estimation often uses generic bioaccumulation factors from the literature, and derives the bioconcentration in organism at equilibrium condition from either freely dissolved concentrations or total concentrations in soil. While it has been successfully applied to sediments, this approach may not be directly extrapolated to soil environment (Reichenberg and Mayer, 2006). The critical assumption of equilibrium between aqueous phase, soil particles, and organisms may not always be achieved and may require much longer contact time as soils are not saturated and are often more heterogeneous than sediment (Gestel, 1995). Thus the application of equilibrium approach to soils can result in significant variability in predictions of bioaccumulation for higher molecular weight PAHs using freely dissolved concentration (Ma et al. 1998).

To improve the understanding of bio-uptake of PAHs in soil invertebrates, a mechanistic kinetic model encompassing both exposure pathways and not restricted to equilibrium partitioning was developed in an attempt to provide more accurate prediction of PAH bioaccumulation for both short-term and long-term exposure scenarios. The reliability of this model was tested over a broad range of soils with different compositions and contamination sources. Bioaccumulation studies were performed on a selection of artificially constructed soils from previous study. Earthworm (*E. fetida*) was selected as our test organism species because it has been frequently used in bioaccumulation and toxicity tests for numerous HOCs (Jager et al. 2003, Bergknut, Sehlin et al. 2007) due to its ubiquity and critical role in the terrestrial food web, soil health, and plant and crop growth (Bergknut et al. 2007). The model performance was tested under different source materials. A subset of the soils was amended with 2% bio-char, which underwent the same bioaccumulation test as the un-amended soils, to investigate the effectiveness of this novel soil remediation approach. The remediation performance, assessed as reduction in PAH bio-uptake, was discussed in the end.

## **6.2 Materials and Methods**

## 6.2.1. Soil Selection

In this task, a subset of formulated soils with a variety of contents of geo-sorbents such as organic carbon and black carbon from previous study was investigated (see Table 1). The PAH concentration in soil after weathering was measured and reported in Chapter 2. Soil samples were equilibrated with deionized and sterile water for 30 days and freely dissolved PAH concentrations were measured using polyoxymethylene (POM) passive samplers (Hawthorne et al., 2011). Detailed methods can be found in Chapter 2. Three typical PAH source materials including fuel oil No. 6, lampblack soot and coal tar weathered skeet particles were investigated. Target soil concentration of 1mg/kg BaP for different soils was used for this experiment to rule out the effect of different soil concentration on PAH bio-uptake. The actual soil concentrations measured after

weathering were lower than the target concentration, likely due to volatilization and degradation during weathering. The difference between actual and target concentrations varied with different soils. However, all the test soils still had very comparable soil concentrations (within the same order of magnitude) as shown in Table 1.

## 6.2.2. Bioaccumulation Study

PAH uptake in soil invertebrates (E. fetida, obtained from Aquatic Research Organisms, Hampton, New Hampshire) was measured in three replicates following the method described in (Gomez-Eyles et al., 2011). Briefly, 200 g of homogenized soil was placed in a glass beaker. Moisture of the soils was maintained at around 60% of soil water holding capacity by adding DI water into the soil to provide optimum earthworm growth conditions. The weights of the soil beakers were recorded each day before and after addition of DI water. The pH of the study soils was adjusted to  $7\pm1$  by adding Ca(OH)<sub>2</sub> powder at the beginning of the test. To do this, a subset of soil samples was mixed with DI water to create a slurry, and the pH of which was measured and the dosage of Ca(OH)<sub>2</sub> was calculated for each soil. After that, 10 g of adult earthworms were introduced into each beaker and kept in a dark room at  $20\pm2$  °C. The beakers were then sealed with nylon mesh and secured with rubber bands to prevent earthworms from escaping during the exposure. Since the exposure duration was long (30 days) and most soils were artificially constructed with relatively low organic matters (with measured TOC contents less than 8%), as recommended by OECD guidance (OECD 2010), additional organic matter had to be added to ensure the survival of the worms. Therefore, before the addition of earthworms, 1.4 g of dry cow manure (ground into powder) was mixed into each soil sample. After that, a weekly rate of 7 mg of dried manure per g soil (dry weight) was dosed on the soil surface.

After 30 days, the earthworms were withdrawn from the soils, rinsed with deionized water and depurated for 5 hours on moist filter paper following ASTM method. The weight of the worms from each beaker was measured, and the tissue samples were stored at -4 °C until analysis.

#### 6.2.3. Measuring PAH in Worm Tissue

The frozen wet worm tissue was cut into small pieces and transferred into a 50 ml Teflon vial to undergo saponification for 4 hours using a method modified from (Hyötyläinen et al., 2002). After saponification, 20 ml of hexane solvent was added into the vial and mixed on an orbital shaker for 30 minutes at 60 rpm. After solvent extraction, the vial was centrifuged at 3000 rpm for 2 mins and the hexane was transferred into another 60 ml vial using a glass pipette. The solvent extraction was repeated twice and the three aliquots were combined for further PAH cleanup and analysis.

#### 6.2.4. Measuring Assimilation efficiency of PAHs in worm

A separate, short (3 hour) bioaccumulation study was carried out to measure the assimilation efficiency of PAHs in E. *fetida* through ingestion of selected soils following method modified from (Lu et al., 2004). Four artificial soils spiked with different source materials (solvent, fuel oil, soot and skeet) with the highest target BaP concentrations (100 ppm) were used in this test. The selection of the high contamination level was due to the concern of the potential detection limit issue for analysis of PAHs in worm tissue after short exposure in the assimilation efficiency experiment. Briefly, 100 g of dry soil was added into a 400 ml beaker, and DI water was added to the soil to its 60% water holding capacity. Twenty-four adult *E. fetida* were added into each beaker and exposed to the soil for 3 hours (about one gut transit time) (Jager et al., 2003), then the worms were removed

from the soils and divided equally into two groups: an ingestion group and a depuration group. Worms in the ingestion group were immediately analyzed for PAHs following methods mentioned in previous section, and worms in the depuration group were placed on clean soil and allowed to purge the gut for 5 hours for complete egestion of residual soil. After that, the worms in depuration group were processed and analyzed for PAHs. The concentration in worm was corrected against background PAH levels in worm tissue. The assimilation efficiency for each soil has been calculated as the ratio of residual PAH concentration in worms at complete egestion to the total PAH ingested from soil, as:

$$\alpha = \frac{\text{residual PAH in worms at complete egestion}}{\text{total PAH initially ingested from soil}}$$

The measured assimilation efficiency was discussed under different source materials. The relationship between  $\alpha$ , PAH hydrophobicity and soil partitioning coefficient K<sub>D</sub> was investigated.

#### 6.3 Results and Discussion

#### 6.3.1 PAH Bioaccumulation in Earthworms

The PAH bioaccumulation in earthworms from each soil was expressed as bioaccumulation factors, where PAH concentration in tissue was normalized to PAH concentration in soil, as:

$$BAF = \frac{PAH \text{ concentration in worm tissue } (\mu g/g)}{PAH \text{ concentration in soil } (mg/kg)}$$

The bioaccumulation factors of two representative PAHs, pyrene and benzo(a)pyrene were presented in Figure 1, for soils contaminated with different source materials (with 1ppm target BaP concentration).

As we can see from Figure 1, PAH source materials had a great effect on bioaccumulation in earthworms. The great variation observed in BAF values from different soils indicated that soil concentration was not predictive of PAH bioaccumulation in earthworm across different source materials. PAH uptake in worms was exponentially correlated with soil  $K_D$  (p < 0.03,  $R^2 > 0.94$ ): the highest PAH bioaccumulation was observed in control soils (solvent spike) which had the lowest soil K<sub>D</sub>, followed by fuel oil, soot, and skeet soils. As discussed in Chapter 2, PAH source materials can control the overall soil sorption capacity for PAHs, and alter the freely dissolved concentrations. This observation can be explained as water exposure is a major pathway for overall PAH uptake in earthworms. Also, the high soil  $K_D$  may limit desorption of PAHs from soil particles into the digestion fluid in the gut of an earthworm. The strongly negative correlation between soil K<sub>D</sub> and PAH extraction by simulated human gut fluid has already been demonstrated and discussed in Chapter 5. Therefore, similarly, it is reasonable to hypothesize that such trend may also exist in the intestinal environment of a soil invertebrate. This hypothesis has been addressed in the following sections.

#### 6.3.2 Effect of Source Material on PAH Assimilation into Worms through Ingestion

The assimilation efficiency of PAHs in earthworm was investigated for all four source materials. Two representative PAHs, pyrene and benzo(a)pyrene are presented in Figure 2. The assimilation efficiency describes the fraction of PAHs that are absorbed into worm tissue from the soil, which is controlled by the dissolution of PAHs from soil and subsequent assimilation into the gut tissue. As shown in the figure, the source materials also had a great impact on PAH assimilation efficiency: gut assimilation efficiency was strongly linearly correlated with log soil  $K_D$  (p < 0.02,  $R^2 > 0.97$ ) for both PAHs

investigated. The soils with highest  $K_D$  (skeet spiked soils) had the lowest gut assimilation efficiency. The difference in assimilation efficiency observed among different source materials was prominent: for pyrene, the assimilation efficiency ranged from 5% for skeet spiked soils to 110% for solvent spiked soils; and for benzo(a)pyrene, a wide range of assimilation efficiency from 5% to 80% was also observed. The variance in assimilation efficiency has demonstrated that PAHs may not always be completely extracted/solubilized during intestinal digestion in earthworms, and the soil sorption characteristics have a dominating impact on the assimilation of PAHs from soils. This phenomenon was observed for sediment invertebrates and clams in previous work (McLeod et al. 2004; Fadaei et al. 2015), but has not been shown previously for earthworms. In order to further explore the trend between soil  $K_D$  and assimilation efficiency for 8 medium to heavy molecule weights were plotted against soil  $K_D$  as shown in Figure 3.

Interestingly, we can see that for solvent, fuel oil, and soot spiked soils, the PAH assimilation efficiency generally decreased with increasing soil  $K_D$  and their trends can be grouped and described by one same slope, for all of the 8 PAHs investigated. An overall correlation between soil  $K_D$  and PAH assimilation efficiency in earthworms was derived for these three source materials, with a  $R^2 = 0.65$ , which indicated that the assimilation efficiency can be approximated using soil  $K_D$  (in log unit), regardless of the type of source materials, or the PAH hydrophobicity. This correlation demonstrated the dominating role of soil  $K_D$  in PAH uptake in earthworm through ingestion. However, for skeet spiked soils, no significant change in PAH assimilation efficiency was observed for different PAHs with different soil  $K_D$ : a consistently low assimilation efficiency of less than 4% was observed

for all the PAHs. One explanation for this phenomena could be the extremely high nonlinear  $K_D$  associated with skeet spiked soils. In Chapter 3, we have discussed the effect of PAH depletion from source material on partitioning, and observed that for fuel oil and soot, the PAH partitioning behavior remained unchanged as PAH is being depleted from the materials; however, for skeet, the sorption affinity for PAHs drastically increased by two orders of magnitude with a depletion of only 7% by mass. When PAHs are being desorbed from skeet particles during intestinal soil digestion with worm body, the PAH affinity to skeet becomes exponentially higher, which eventually overwhelmed the extraction ability from the intestinal digestion fluid in earthworm. Consequently, only the small fraction of fast desorbing PAHs have been released from the skeet. The escalated  $K_D$  in skeet spiked soils during soil digestion may be the cause of the consistently low assimilation efficiencies observed for all 8 PAHs.

#### 6.3.3 PAH Bioconcentration Factor (BCF)

Bioconcentration factor, BCF, is a similar term to bioaccumulation factor, but defined as the ratio of PAH concentration in earthworm lipid to the freely dissolved concentration. BCF for three representative PAHs, pyrene, BaP, and benzo(g,h,i)perylene were calculated for soils of 1 mg/kg target BaP concentration with different source materials. As shown in Figure 4, the BCFs for pyrene were comparable among different source materials, with log BCF ranging from 3.2 to 3.6; however, for benzo(g,h,i)perylene, the BCF ranges from 5.0 to 6.0. This might indicate that for lower molecular weight PAHs, freely dissolved concentration could potentially be used to predict PAH bioaccumulation in earthworm regardless of the contamination sources. As PAH hydrophobicity increased, the variance in measured BCF increased: for example the standard deviation in log BCF for

pyrene measured 0.19, which increased to 0.35 for benzo(a)pyrene, and 0.46 for benzo(g,h,i)perylene. For higher molecular weight PAHs, the measured BCFs were higher in solvent spiked soils, which could be due to that the control soil with lower K<sub>D</sub> equilibrated with the aqueous phase and earthworm more quickly than other soils. And in a similar way, the slightly lower BCFs observed in soot and skeet spiked soils might be due to the disequilibrium in bioaccumulation in earthworm. However, compared to BAF values, BCFs were in general more consistent across different soils, which indicated that freely dissolved PAH concentration instead of soil concentration is a much more accurate predictor for bioaccumulation.

To investigate how well freely dissolved concentration alone could predict for bioaccumulations, an equilibrium model was tested. The prediction of individual PAH concentrations in earthworm was estimated as the combination of the freely dissolved aqueous concentration (( $C_w$ ) measured by passive samplers) and literature BCF values:  $C_{worm} = C_w * BCF$ . BCF values were assumed to equal K<sub>ow</sub> value for individual PAHs (Jonker et al., 2007). As is shown in Figure 5, the equilibrium model generally underpredicted bioaccumulation by half to two orders of magnitude. No obvious trend in prediction was observed for different source materials.

The probable disequilibrium among soil, earthworm, and aqueous phase suggests that a more comprehensive kinetic model that involves both exposure pathways should be developed to improve the prediction of PAH bioaccumulation in earthworm under unsteady state scenario.

## 6.3.4 Effect of Charcoal on Reducing PAH Uptake in Earthworm

The effect of charcoal amendment was investigated at soil concentration of 1 mg/kg target BaP. As shown in Figure 6, the application of 2% charcoal had various effects on reducing PAH bioaccumulation depending on the source material. Middle to heavy molecular weight PAHs (from pyrene to benz(g,h,i)perylene) were selected for this demonstration, as contamination issue was encountered with some of the lighter PAHs. For almost all the PAHs investigated, the solvent spiked soils had the highest reduction in PAH bioaccumulation from charcoal addition, ranged from 94% to 98%. As shown in Figure 7, this effect was consistent among PAHs with different hydrophilicities in solvent spiked soils. The greatest effect of charcoal observed for solvent spiked soils is expected because the control soil had no external sorption matrix compared to other types of soils, thus having the lowest soil K<sub>D</sub>. The overall sorption capacity of the control soil was more prominently affected by the addition of the very strong geo-sorbent.

The soot and skeet spiked soils had less reduction in PAH bioaccumulation than solvent soils. The detailed reduction for each PAH and each source material was presented in Figure 7. For soot and skeet spiked soils, the charcoal had comparable effects on reducing PAH bioaccumulation, with reduction ranging from around 50% to 90% for different PAHs. No significant change in reduction was observed with increasing PAH hydrophobicity for these two source materials. The least effect of charcoal was observed in fuel oil spiked soils, with the reduction of bioaccumulation ranging from 50% to 80%. As for fuel oil spiked soils, based on our previous measurement on soil K<sub>D</sub> from Chapter 2, theoretically, reduced impact from charcoal should have been observed for fuel oil spiked soils, followed by soot and skeet spiked soils, as the latter two had

the highest measured soil K<sub>D</sub>. The least reduction in fuel oil spiked soil was likely resulted from the fouling of charcoal by fuel oil. The fuel oil matrix contains many hydrocarbons other than PAHs that can sorb onto the charcoal surface. The similarly abated remedial performances in reducing PAH freely dissolved concentration and PAH bioaccessibility in simulated human GI environment due to fouling have also been observed and discussed in Chapter 2 and Chapter 5, respectively.

#### 6.3.5 Modeling PAH Bio-uptake in Earthworm

In order to improve the prediction for PAH bioaccumulation in earthworm, a firstorder kinetic uptake model inclusive of both dermal absorption and dietary ingestion pathways modified from (Sun et al., 2009) was used:

$$dC_{e}/dt = k_{der}C_{w} + IR \alpha C_{soil} - k_{e}C_{e} \qquad (1)$$

where  $k_{der}$  (L kg<sup>-1</sup>d<sup>-1</sup>) and  $k_e$  (d<sup>-1</sup>) were the dermal uptake and overall elimination rates of PAHs in earthworm, which follow a first-order kinetics. C<sub>w</sub> (mg/L) was the freely dissolved PAH concentration and C<sub>soil</sub> (mg PAH /kg soil) was the soil concentration; IR was the daily soil ingestion rate (mg feces mg tissue <sup>-1</sup> d<sup>-1</sup>);  $\alpha$ , the assimilation efficiency of PAH from soil. Assuming that soil concentration, aqueous concentration, soil ingestion rate, PAH assimilation efficiency, dermal uptake rate and PAH elimination rate within earthworm were constant for a given PAH compound and soil type during the exposure, the integrated form yields:

$$C_e = A(1-exp^{-k_e t}), A = (k_{der}C_w + IR \alpha C_{soil})/k_e$$
 (2)

 $k_{der}$ ,  $k_e$  and IR were experimentally determined and adopted from (Jager et al., 2003; Jager et al., 2000; Lu et al., 2004; Ma et al., 1998; Matscheko et al., 2002) (Table 2). The soil ingestion rate IR was assumed constant and independent of soil types. The PAH

assimilation efficiency in *E. fetida* from soils was measured experimentally. The dermal uptake rate constant (Jager et al., 2000) and the elimination rate constant were dependent on the organism type and PAH hydrophobicity. The exposure time, t, as 30 (days) in this study. With these experimentally determined and empirical parameters, the PAH bioaccumulation in earthworms at any exposure duration was estimated using above equation.

### 6.3.6 Kinetic Bio-uptake Modeling Results

The predicted PAH bioaccumulation in earthworm using equation 2 was compared with observed results for four PAHs: phenanthrene, pyrene, benzo(a)pyrene and benzo(g,h,i)perylene in Figure 8. The overall prediction for all 8 soils fell within one log unit deviation of observed PAH bioaccumulation. However, the model performance varied with PAH hydrophobicity: the predictions of bioaccumulation for lighter PAHs such as phenanthrene and pyrene were generally more accurate than the heavier ones such as benzo(a)pyrene and benzo(g,h,i)perylene: the bioaccumulation of the latter was mostly over-predicted by our proposed model. One assumption used in the modeling was that the PAH aqueous concentration, C<sub>w</sub>, would reach fast equilibrium with the soil particles at the beginning of the exposure. However, such assumption could be violated for PAH with high molecular weights, such as benzo(a)pyrene and benzo(g,h,i)perylene. And this violation would deteriorate with increasing K<sub>ow</sub> and soil partitioning coefficient, as more time would be required for heavier PAHs to reach equilibrium with soil with higher sorption capacity. Therefore, the use of equilibrium freely dissolved concentration in the modeling could partially contribute to the over-prediction. As shown in Figure 9, the contributions to overall PAH bioaccumulation from dermal/oral exposure route differed with PAH hydrophobicity and sorption characteristics. As for heavier PAHs like benzo(a)pyrene, PAH uptake through soil ingestion was a dominating exposure route, with over 70% of the total bioaccumulation coming from oral route, than lighter PAHs like phenanthrene. For lighter PAHs, the dermal exposure routes overweighed oral ingestion route, due to the higher solubility and mobility of lighter PAHs. However, in general, the addition of charcoal increased the weight of oral ingestion in overall bioaccumulation regardless of the PAH hydrophobicity. According to Figure 8, most of the over-predictions observed were for heavier PAHs such as benzo(a)pyrene and benzo(g,h,i)perylene. Considering the dominating role of oral exposure route for these PAHs, one could suspect that the residual variance from the assimilation efficiency correlation, as well as the literature ingestion rate used in the kinetic model are possibly the major cause to the over-prediction.

Figure 10 shows the prediction of PAH bioaccumulation in earthworm from soils spiked with different source materials. Although no significant trend of prediction was observed across different source materials, the predictions for un-amended soils were generally more accurate than amended soils: nearly half of the charcoal amended soils exhibited an over-prediction of bioaccumulation by half order of magnitude. Again, this may partially be explained by the use of equilibrium aqueous concentration for charcoal amended soils in the modeling, which would lead to a biased estimation of PAH uptake through water exposure. The literature ingestion rate and the assimilation correlation used in the oral exposure route of the modeling could also be another cause. Despite the slight over-estimation for some of the charcoal amended soils, the overall model prediction was still reasonable, within one log unit deviation. Moreover, in order to improve the prediction, the PAH desorption kinetics from soil into aqueous phase, especially for those amended with charcoal, needed to be fully characterized and taken into account in the modeling.

# 6.4 Conclusion

The variation in BAF values measured from different soils indicated that soil concentration is not an appropriate indicator for PAH bioaccumulation in worms. The PAH uptake in earthworm was demonstrated to be significantly correlated with soil  $K_D$ , which also controls the assimilation efficiency of PAHs into worm tissue through soil ingestion. The relatively consistent BCF values observed across soils with different source materials indicated that freely dissolved PAH concentration is a more appropriate predictor for bioaccumulation than soil concentration. A kinetic bio-uptake model inclusive of both water exposure and soil ingestion was developed for the first time to explain and predict the PAH uptake in *E. fetida*. The model provided acceptable prediction (of within one log unit deviation) for nearly all of the soils investigated. Moreover, the predictions for lower molecular weight PAHs as well as un-amended soils were more accurate than those for higher molecular weight PAHs and charcoal amended soils, possibly due to the violation of equilibrium model assumption in the latter. The application of charcoal had various effects on soils with different source materials, with a general trend of higher reduction observed in soils with lower K<sub>D</sub>. The only exception was observed for fuel oil spiked soils where the potential fouling of charcoal by fuel oil could have resulted in an abated performance in reducing PAH bioaccumulation in worms.

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**Figure 1.** Effect of source material on PAH bioaccumulation in earthworms, for baseline soils spiked with different source materials with target BaP concentration of 1 mg/kg. Error bar represents standard error, n = 3.



**Figure 2.** Effect of source material on PAH assimilation efficiency (n = 1) in earthworms, for baseline soils spiked with different source materials with target BaP concentration of 1 mg/kg.



**Figure 3.** Correlation between measured soil  $K_D$  and PAH assimilation efficiency (n = 1) in earthworm in soils spiked with source materials.



**Figure 4.** Measured bioconcentration factors in earworms for three representative PAHs (pyrene, benzo(a)pyrene) and benzo(g,h,i)perylene) from soils contaminated with different source materials with target BaP concentration of 1 mg/kg. Error bar represents standard error, n = 3.



**Figure 5.** Observed PAH bioaccumulation in earthworm vs. predicted bioaccumulation using freely dissolved concentration measured by passive samplers ( $C_w$ ) and literature BCF values:  $C_{worm} = C_w * BCF$  (Jonker et al., 2007) in soils with (solid shapes) and without (open shapes) biochar amendment.



**Figure 6.** Effect of 2% biochar amendment on reducing PAH uptake in earthworms. PAH bioaccumulation is expressed as bioaccumulation factors and all the soils investigated have target BaP concentration of 1 mg/kg. Error bar represents standard error, n = 3.



**Figure 7.** Effect of 2% charcoal on reducing PAH uptake in earthworms after 30 days' bioaccumulation for 8 middle to heavy molecular weight PAHs from soils with target BaP concentration of 1 mg/kg. Error bar represents standard error, n = 3.



**Figure 8.** Comparison between predicted PAH uptake in earthworms in soils with target BaP of 1 mg/kg for four representative PAHs using kinetic model and observed PAH bioaccumulation after 30 days' exposure.



**Figure 9.** Comparison of fractions of PAH bioaccumulation through dermal and oral exposure routes for phenanthrene and benzo(a)pyrene from soils with different source materials.



**Figure 10.** Comparison between predicted PAH uptake in earthworms in soils with target BaP of 1 mg/kg using kinetic model and observed PAH bioaccumulation after 30 days' exposure. Filled shapes represent soils amended with bioachar.
PAH sources	Target BaP concentration (mg/kg)	Measured BaP concentration (mg/kg)	Carbon type and content
Solvent	1	0.23	10 % peat
Solvent	1	0.32	10 % peat + 2% charcoal
Soot	1	0.75	10 % peat
Soot	1	0.25	10 % peat + 2% charcoal
Fuel Oil	1	0.12	10 % peat
Fuel Oil	1	0.46	10 % peat + 2% charcoal
Skeet	1	0.85	10 % peat
Skeet	1	0.89	10 % peat + 2% charcoal
Control	0	0	10 % peat

**Table 1.** List of soils used for earthworm bioaccumulation study.

**Table 2.** Parameters used in the kinetic bio-uptake model.

paramters	description	Unit	reference source
Csoil	PAH concentration in soil	mg/kg	this study
Cw	PAH concentration in aqueous phase	mg/L	this study
ke	elimination rate constant	d-1	Jager et al., 2000
$k_{der}$	uptake rate constant through water exposure	L/(kg·d)	Jager et al., 2000
IR	soil ingestion rate	mg/(kg·d)	Jager et al., 2003
α	assimilation efficiency	%	this study
t	exposure time	d	this study

### Chapter 7: Research Summary and Recommendations

#### 7.1 Conclusions and Implications toward Motivating Research Questions

The soil partitioning coefficient is a key parameter that controls the fate and transport of PAHs in the environment, as well as its exposure to different receptors. While past work has primarily focused on soil organic geochemistry, such as type and abundance of black carbon, to explain PAH bioavailability in soils and sediments, very little work exists that has focused on the role of different PAH sources to soil. This Ph.D. research aimed to better understand the effect of PAH source materials on the overall soil partitioning coefficient and its subsequent effect on bioavailability to human and ecological receptors through various exposure routes, which may eventually benefit risk assessment. The study also evaluated how PAHs associated with different source materials respond to attempts of bioavailability reduction through charcoal amendment, which may also provide valuable information for future remediation design in risk management. Conclusions and implications were made toward the five motivating research questions, as described below.

# 1) How does PAH partitioning behavior change with different source materials and depletion from the source material?

The PAH partitioning in different source materials has been initially discussed in Chapter 2, and the change in partitioning as a response to depletion has been further discussed in Chapter 3. While there is general understanding that PAH bioavailability in soil can be influenced by many factors including PAH sources and soil properties, the relative importance of each of these factors and how they interact with each other is not well understood. To address this knowledge gap, a set of weathered artificial soils was constructed to have a better control of the different factors likely to affect PAH bioavailability. PAH sorption to the different PAH sources and soil components were measured in isotherm studies and different modeling approaches were tested to explain the PAH partitioning behavior. The results showed that PAH sources to soil play an important role in PAH partitioning, followed by soil composition. The effectiveness of biochar amendment on reducing freely dissolved PAH concentrations was also affected by source materials: lower effectiveness was generally observed for soils spiked with soot and skeet that had higher sorption capacity for PAHs. The results highlighted the importance of using weathered field soils with native PAH source materials for bioavailability assessment of soil, which challenged traditional approaches for PAH toxicity and bioavailability studies with animals and human cadaver skin that have relied largely on solvent spiked soils, not accounting for PAH source material effects.

In Chapter 3, the hypothesis has been raised that the depletion of PAHs in source materials may also affect its partitioning. This chapter extended the investigation of the effect of PAH depletion in partitioning from skeet onto other sources including fuel oil No. 6 and lampblack soot. PE samplers were used as a passive extraction device to facilitate PAH depletion from source material, and different PE to source material mass ratios were employed to allow varying degrees of PAH depletion. The results showed that different source materials responded differently to PAH depletion. As for fuel oil and soot, increased PAH depletion from source materials did not significantly affect equilibrium partitioning coefficient. However, for skeet, with only 7% PAH depleted, the equilibrium partitioning coefficient increased by up to two orders of magnitude. The nonlinear partitioning behavior for skeet could be described by a Freundlich equation. While nonlinear partitioning based on adsorption isotherms is well documents for black carbon materials, the issue of

nonlinear desorption kinetics from source materials has not been previously studied. The proposed model can be used to predict the skeet partitioning coefficients under a depletion environment. While the partitioning behavior for skeet material is assumed to be constant, which could be greatly underestimated; this model may benefit risk assessment by improving the accuracy of prediction of coal-tar pitch contaminated soil/sediment partitioning coefficient by orders of magnitude than conventional OC or OC+BC models. The proposed model can also be applied to sedimentary conditions.

# 2) How do source materials affect dermal uptake of PAHs, and can this process be explained by a partitioning and diffusion model?

The importance of source materials in partitioning has been illustrated in Chapter 2 and 3, however, whether source materials can affect PAH bioavailability to humans still remains unknown. In Chapter 2, an in-vitro dermal uptake study was initiated to illustrate the effect of source material and PAH partitioning on this key exposure pathway for humans. It has been discovered that PAH absorption rates are greatly influenced by PAH source materials in soil, which ranged over an order of magnitude. Such variation could not be explained based on PAH concentration differences in soil, but found to be significantly correlated with freely dissolved PAH concentration. Thus, it was demonstrated for the first time that dermal uptake is directly related to aqueous concentration in soil and not the total concentration, which suggest that dermal uptake studies need to relate the uptake to aqueous concentration or partitioning instead of total concentrations.

Since the freely dissolved PAH concentration in soils has been shown to be good indicator of PAH bioaccumulation in soil invertebrates, theoretically dermal uptake of PAHs by humans may also be predicted by the freely dissolved concentration exposed to

the external skin surface. In Chapter 4, a Fickian diffusion model was explored to explain and predict the dermal uptake by humans. As discussed in Chapter 4, steady state diffusion model can adequately predict the PAH dermal flux for most of the soils, except for the charcoal amended ones, most likely due to the violation of the steady state assumption. The skin/ water partitioning coefficient for 16 parent PAHs have been reported for the first time in this study. As for non-steady state diffusion model, the prediction of dermal flux is significantly improved, with nearly all of the predictions falling within 1 log unit deviation. In the end, the effectiveness of a relatively novel soil remediation approach, the application of charcoal amendment, has been investigated. In general, decreased remedial performance was observed with increasing soil partitioning coefficient: the reduction in dermal bioavailability was greater in control and fuel oil spiked soils than skeet and soot spiked soils. Given the freely dissolved PAH concentration, one can utilize the proposed model as a screening tool for human risk assessment, to approximate the PAH dermal uptake from soils contaminated various PAH source materials. The current bioavailability value of 13 % recommended by EPA for dermal exposure was demonstrated to be up to 100 times higher than our observed results in Chapter 4. One reason for the wide use of this outdated and overly conservative value is the lack of established methods that can take into account site specific information into risk calculation. Fortunately, the diffusion models from Chapter 4 may provide a solution, where one can utilize the directly measured freely dissolved concentration by passive sampling to approximate the PAH dermal bioavailability to humans within a factor 10, regardless of the PAH contamination source, soil concentration, or composition. These model may greatly improve the accuracy of the site specific risk assessment in terms of dermal exposure over the default value that does not account for site-specific conditions. Besides, the in vitro diffusion method using pig skin may provide an economical, simple and valuable alternative (relative to *in vivo* animal studies) for estimating dermal bioavailability of PAHs to human beings from soil, which can also be extended to other HOCs. Traditional dermal test often utilizes Franz cell diffusion devices, which are very complicated and costly to implement. This in vitro diffusion method proposed in Chapter 4 is less expensive and much simpler, which may be more applicable in future dermal absorption studies.

## 3) How do source materials affect PAH desorption in the GI environment, and can this process be explained by a modified partitioning model?

Incidental soil ingestion is considered the other primary pathway for systemic exposure to chemicals in contaminated soils. It is recognized that the total oral dose of PAHs from soils does not necessarily represent the bioavailable fraction, which is limited to those PAHs solubilized in the gastrointestinal (GI) tract. In this chapter, how the enhanced sorption of PAHs in source materials and soil components influences the solubilization of PAHs in the human gut environment is discussed. This research also expands on the prior work to assess whether that aqueous partitioning behavior can predict the results from a physiologically-based extraction test of the same soils. The results have shown that source material had a strong impact on PAH bioaccessibility: soils spiked with soot and skeet generally exhibited the lowest bioaccessibility, followed by fuel oil and solvent spiked soils. Among all soil compositions, the presence of 2% charcoal had the greatest effect on reducing PAH bioaccessibility, with the biggest reduction (of approximately 80%) observed for soils spiked with solvent and fuel oil. No fouling of charcoal by fuel oil was observed during PBET, likely a result of the elevated temperature,

the surfactant effect from bile, and the lipase in the system which mobilized and solubilized the fuel oil. Across all soils constructed, soil partitioning coefficients (K<sub>D</sub>) were observed to be negatively correlated (p<0.01) with PAH bioaccessibility, with R<sup>2</sup> ranging from 0.65 to 0.74. Overall, the modeling results indicate that a simple partitioning based model can reasonably predict PBET extraction across 5 orders of magnitude in PAH mass extracted. The benefits of using the partitioning based model are: 1) soils can be screened for relative bioaccessibility based on an aqueous partitioning test which is much simpler than performing PBET, 2) using the model, several scenarios can be tested including altering the soil to PBET mass ratios, altering the fraction of lipids, and introduction of sorptive sink if necessary. The current EPA suggested bioavailability value for incidental soil ingestion is 100%, which was proven to be overly conservative based on the comparison against site specific results from Chapter 5. The partitioning based model developed in this chapter not only provides explanation for the PAH uptake mechanisms within human digestive systems, but also provides a solution to address the uncertainty and accuracy issue with the current default EPA value. With passive sampling being more and more widely used in today's risk assessment, one can utilize the site specific partitioning measurement ( $K_D$ ) by passive sampling as the input for the partitioning based model, to approximate the PAH bioavailability to humans following soil ingestion, within a factor of 3 to 10, depending on specific contamination sources. The model is simple and readily applicable provided the site specific K<sub>D</sub> value and does not require further additional measurements from the site, which may greatly improve the accuracy and build confidence in human risk assessment for oral exposure. Additionally, the partitioning theory and its model developed in this

chapter may also provide explanation for the uptake of other HOCs, such as pesticides and PCBs by humans following soil ingestion.

## 4) How do PAH source materials and soil compositions affect PAH uptake in earthworms, and can this uptake process be predicted using a biouptake model?

Water exposure and soil ingestion are two major exposure routes for earthworm to PAHs in soils. While the role of source materials in soil K<sub>D</sub> has been investigated in Chapter 2, how source materials affect PAH uptake in soil invertebrates via each exposure route is still unknown. The equilibrium partitioning approach for estimating PAH bioaccumulation in earthworm may not be applicable to soil environment due to its low water saturation and high heterogeneity. To improve the understanding of bio-uptake of PAHs in soil invertebrates, a mechanistic kinetic model encompassing both exposure routes has been developed in an attempt to provide more accurate prediction of PAH bioaccumulation under both short-term and long-term exposure scenarios. In conclusion, the overall PAH uptake in earthworm is demonstrated to be significantly correlated with soil partitioning coefficient, which is a function of source material and soil compositions that control both freely dissolved PAH concentration and assimilation efficiency of PAHs into worm tissue through soil ingestion. The variation in BAF values measured from different soils indicated that soil concentration is not an indicator for PAH bioaccumulation in worms. The relatively consistent BCF values observed across soils with different source materials indicated that freely dissolved PAH concentration may be a more appropriate predictor for bioaccumulation. A kinetic bio-uptake model inclusive of both water exposure and soil ingestion was developed for the first time to explain and predict the PAH uptake in *E. fetida*. The model has provided acceptable predictions (of within one log unit deviation)

for nearly all of the soils. Such model is superior to conventional equilibrium approach as it not only accounts for both exposure pathways, but also addresses the unsteady state bioaccumulation scenarios, which is common for heterogeneous media like soil. Since *E. fetida* has been frequently used as an indicator of soil health and toxicity, an accurate prediction of bioaccumulation of HOCs in earthworms is crucial in risk assessment for ecological receptors. The proposed model in this chapter can benefit the risk assessment by improving the accuracy of the prediction of bioaccumulation of HOCs in earthworms.

### 5) *How does PAH bioavailability change in response to application of biochar amendment, and does the remediation efficiency vary with different source materials?*

Soil amendment using strong geo-sorbents like AC and biochar has been a hot topic in the past decade due to its proven effectiveness in reducing bioavailability to ecological receptors in both sediment and soil environments for a variety of contaminants. While these novel amendment approaches represent promising site remediation strategies for ecological receptors, it is still unclear if these treatments would be effective in reducing the risk to humans through dermal and oral exposure pathways. Also, the impact of PAH sources (e.g. fuel oil, soot, coal tar, creosote) and soil composition on bioavailability reduction potential needs to be investigated in order to provide more realistic remediation designs and anticipation of performance. The reductions in bioavailabilities to different receptors via various exposure routes in response to charcoal amendment have been discussed in Chapter 4, 5 and 6 separately. Based on conclusions from the previous chapters, the soil partitioning coefficient and freely dissolved concentrations are the nexus to bioavailabilities in spite of the exposure pathways or receptors. The application of charcoal reduces bioavailabilities to different receptors by altering the overall soil partitioning coefficient and the freely dissolved concentrations. Overall, charcoal amendment can have various effects on soils with different source materials, with a general trend such that higher reduction in bioavailabilities from charcoal amendment was always observed in soils with lower partitioning coefficient. Although, occasional abated performance has been observed for fuel oil spiked soils where the potential fouling of charcoal by fuel oil is suspected as the cause. In general, charcoal amendment showed promising effectiveness in mitigating various forms of exposures to PAH in soils: 70- 95% reduction in PAH bioaccumulation in earthworms, 50-80% reduction in PAH oral bioaccessibility to humans and 40-98% reduction in PAH dermal uptake by humans. These results can provide valuable information for potential field scale remediation strategies associated with HOCs, and for post-remediation risk management. Depending on the specific contamination source and desired remediation goals, an estimate of the required dosage of biochar can be approximated based on the observations from this chapter. Importantly, despite the relatively lower effectiveness observed in some soils, strong geo-sorbents like AC and biochar are expected to have further capacity for additional sorption given more application time in the field. In the end, as suggested from this work, a successful remediation design using biochar or AC amendment will require site specific characterization of soil/sediment partitioning.

#### **7.2 Recommendations for Future Work**

1) One underlying assumption for the diffusion model in Chapter 3 is that the soil particles would reach instant equilibrium with aqueous phase and that the soil could serve as an infinite source for PAHs. The modeling results have suggested possible violation

under the instant equilibrium assumption between soil and aqueous phase. In order to improve the model, a more accurate description of the freely dissolved concentration profile during dermal diffusion is required. And to do so, the PAH desorption kinetics from different soils into aqueous phase has to be characterized and accounted for. Although the inclusion of such desorption compartment into the existing diffusion model may improve the comprehensiveness of the model and introduce new parameters (such as, soil porosity, soil density, PAH diffusivity in water), one should be careful and find the balance between the model functionality/performance and the inevitable errors introduced by the many new parameters.

2) Another aspect in improving the diffusion model is through improving the accuracy of the existing model parameters. For example, the PAH diffusivity through skin used in the model was derived from an empirical quantitative structure–activity relationship, which was not specifically derived for PAHs. Since diffusivity is a critical parameter in the model, it may be worthwhile to experimentally determine the skin diffusivity for individual PAHs in future work.

3) The skin subject selected in the dermal study has the merits of easy accessibility, low cost and similarities in histological and physiological characteristics with humans. However, in order to gain wider acceptance and application of this novel measurement and modeling approach, it is essential that the proposed model be validated against results from experiments with human cadaver skin. A successful validation of such will greatly benefit the field and make dermal diffusion study more applicable in more laboratories.

4) In Chapter 5, the proposed partition model gave decent predictions of PBET extractions for most of the soils, except for skeet spiked soils. Although our modeling

results have indicated that partitioning may be the dominating mechanism for this transport process, the simulated GI tract is a complex environment with other complicated interactions (such as surfactant effect) involved. In order to fully understand the underprediction associated with skeet spiked soils, and to improve the model prediction for PBET extraction, future work is needed to address how each of the GI fluid ingredients (e.g. surfactants, lipids and enzymes) may interact with the various soil components and PAH source materials.

5) Just like pig skin, PBET is also a conventional estimation approach for oral bioavailability. Many different physiologically-based modeling systems have been developed to simulate the human GI extraction processes and few have been successfully validated against *in vivo* studies. The proposed PBET system in this work is superior to other simulations in ways that it has much higher precision and can be reasonably explained and predicted using partitioning theory. However, in order for it to gain wider acceptance, further work is needed to initiate *in-vivo - in vitro* validations using the same soils.

### Appendix:

#### **Chapter 2: Supplemental Information**

#### Soil Weathering.

Weathering of the soils used in the present study was conducted at the U.S. Army Edgewood Chemical Biological Center (Roman Kuperman, ECBC, Aberdeen Proving Ground, MD), and consisted of two-months of hydrating and air-drying cycles at ambient environmental conditions in a greenhouse.(Kuperman et al., 2005) Briefly, each of the spiked soils (1 kg) was spread from 2.5 to 4 cm thick in an open glass container and hydrated with ASTM type I water to 60 percent of the soil's water holding capacity. The container with hydrated soil was weighed and placed in a greenhouse before the drying process. After one week, the container was reweighed to determine moisture loss and rehydrated to the original weight and thoroughly mixed. This process continued on a weekly basis for eight weeks, after which the soils were air-dried, disaggregated and sieved to < 150  $\mu$ m, and stored at 4 °C before use.



**Figure S1.** Average PAH concentrations in source materials before introduction into soils (n=3, error bars represent standard error).



**Figure S2.** Effect of soil composition on PAH partitioning in solvent and fuel oil spiked weathered soils with 1mg/kg target BaP (n= 3, error bars represent standard error).



Figure S3. K<sub>D</sub> in BSS spiked with different source materials targeted to achieve 0.1 mg/kg target BaP concentration (n= 3, error bars represent standard error).



Figure S4. K<sub>D</sub> in BSS spiked with different source materials targeted to achieve 10 mg/kg target BaP concentration (n= 3, error bars represent standard error).



**Figure S5.**  $K_D$  in BSS spiked with different source materials targeted to achieve 100 mg/kg target BaP concentration (n= 3, error bars represent standard error).

Effect of Aging on Soil K<sub>D</sub>.

To investigate the effect of aging on PAH partitioning, a separate aqueous equilibrium study was carried out on soils that were freshly constructed with no weathering and K<sub>D</sub> values were compared to that of weathered soils (Figure S6). The weathering process had the largest impact on lighter PAHs especially for fuel oil and soot where additional PAHs were added to the source materials. For example, soot and fuel oil soil K<sub>D</sub>s for phenanthrene and anthracene increased after weathering by a factor of 6 compared to a factor of 1.5 for B(a)P. A very small change in K<sub>D</sub> was observed for pyrene and higher molecular weight PAHs for soils with fuel oil, soot, or skeet as PAH source materials. Overall, the 8-week accelerated weathering process seem to have a much smaller impact on PAH partitioning relative to other factors like the type of PAH source material. Previous studies have reported a strong effect of aging on PAH bioavailability (Tang et al., 1998), but those studies involved fresh spiking of soils with PAHs in solvent that are likely to be impacted more strongly by weathering processes.



**Figure S6.** Comparison of  $K_D$  between freshly constructed and weathered (two months) soils with 1 mg/kg target BaP (n= 3, error bars represent standard error).



Figure S7. Ratio of K<sub>D</sub> in weathered soils with/without 2% charcoal under different source materials of 1mg/kg target B(a)P (n= 3, error bars represent standard deviation).



Figure S8. Ratio of K<sub>D</sub> in fuel oil spiked soils with/without 2% charcoal addition at different PAH concentrations (as Target BaP).

#### Modeling details for Figure 3 (OC & OC+BC models)

The single domain model ( $K_D = f_{OC}*K_{OC}$ ) only accounts for sorption into natural organic carbon (OC) and the dual domain model ( $K_D = f_{OC}*K_{OC} + f_{BC}*K_{BC}*C_w$ <sup>n-1</sup>) accounts for both linear sorption to OC and non-linear sorption to black carbon (BC).  $f_{OC}$  and  $f_{BC}$  are the measured TOC and BC content in the soils (Table S4);  $K_{OC}$  (from Xia G. S. 1998) and  $K_{BC}$  (from Koelmans et al.2006) are generic organic carbon and black carbon partitioning constants for PAHs from the literature; n is the nonlinearity coefficient and Cw is the measured aqueous PAH concentration at equilibrium. The coal tar model ( $K_D = f_{OC}*K_{coal tar}$ ) assumes that all the carbons (OC+BC), especially in the skeet and soot soils, have the same sorption capacity as coal tar ( $K_{coaltar}$ , from Arp et al. 2014; and the foc is the measured TOC content).



**Figure S9.** Model predicted  $K_D$  against observed  $K_D$  in fuel oil spiked soils at different PAH concentrations (as target BaP).



**Figure S10.** Comparison of  $K_D$  of BaP in solvent spiked and fuel oil spiked soils with identical soil compositions and PAH concentration (as target BaP) (n= 3, error bars represent standard error).



**Figure S11.** Increase of measured Log  $K_{skeet}$  for four different PAHs (phenanthrene, pyrene, benzo(a)pyrene, and benzo(g,h,i)perylene) with increased mass ratio of POM/Skeet (0.125, 5, 50, 200, 500) in a well-mixed system (n=2).



**Figure S12.** Comparison between predicted  $K_D$  from sorption model and observed  $K_D$  for skeet spiked soils using  $K_{SKEET}$  values orders of magnitude higher than measured (shown for PHE, PYR, B(a)P and B(ghi)P; log-transformed data). Blue diamonds represent PHE; black triangles represent PYR; orange squares represent B(a)P and green circles represent B(ghi)P; solid fills for each shape represent soils with addition of 2% charcoal.

РАН	Concentration in Stock
	(mg/l)
Naphthalene	10
Acenaphthylene	0
Acenaphthene	18
Fluorene	12
Phenanthrene	280
Anthracene	177
Fluoranthene	626
Pyrene	461
Benz(a)anthracene	367
Chrysene	359
Benzo(b)fluoranthene	414
Benzo(k)fluoranthene	173
Benzo(a)pyrene	1000
Indeno(1,2,3,-cd)pyrene	297
Dibenz(a,h)anthracene	94
Benzo(g,h,i)perylene	357

**Table S1.** PAH concentrations (mg/L) in stock spiking solution. All measurements have relative standard deviation of less than 1%, n = 2.

 Table S2.
 Summary of PAH concentrations (as BaP) in experimental soils after 8 weeks of weathering (n=3).

	BaP concentrations in weathered soils					
PAH Sources	Synthetic soil	Synthetic soil- 2 percent charcoal fines	Synthetic soil- peat content reduced to 1 percent	Synthetic soil- kaolinite content reduced to 2 percent	Synthetic soil- kaolinite replaced with montmorillonite	Synthetic soil- peat replaced with humus
Solvent spike	0.05, 0.12, 2.6, and 18.1 mg/kg BaP	0.02 and 0.32 mg/kg BaP	0.42 mg/kg BaP	0.15 mg/kg BaP	0.26 mg/kg BaP	0.27 mg/kg BaP
Soot	0.04, 0.75, 5.1, and 58.9 mg/kg BaP	0.41 mg/kg BaP	-	-	-	-
Skeet Particles	0.11, 0.85, 0.93 13.2 mg/kg BaP	0.89 mg/kg BaP	-	-	-	-
Fuel Oil	0.1, 0.11, 0.09 and 3.7mg/kg BaP	0.1, 0.46, and 9.7 mg/kg BaP	1.0 mg/kg BaP	0.59 mg/kg BaP	-	-

	Kpeat	KCLAY	KSAND	Kcharcoal (at Cw =1 ng/L)	K <sub>FUEL</sub>	K <sub>FUEL</sub> Raoult's law estimate (MW=800 g/mole)	Ksoot	Kskeet (with minimal depletion)
PAH Compound								
Phenanthrene	3.82	NA	NA	7.03	5.41	5.27	5.44	5.13
Pyrene	4.82	2.00	1.55	7.67	6.35	6.27	6.52	6.00
B(a)P	6.51	3.91	3.45	8.77	8.54	8.29	9.27	7.85
Benzo(g,h,i)perylene	6.70	3.70	3.48	8.57	9.32	9.12	9.14	8.66
NA: measurement error.	All meas	urements h	ave standa	rd errors of less thar	n 0.1 log un	t.		

 Table S3. Measured PAH partition coefficients for soil components and source materials.

	Target BaP	Composition	Total Organic	Black	
1741 Source	concentration	composition	Carbon (%)	Carbon (%)	
	(mg/kg)		average ± stdv	average ±	
	(			stdv	
Control	0	BSS	2.63 ± 0.01	0.36 ± 0.03	
Control	0	BSS+2% charcoal	2.84 ± 0.01	$0.41 \pm 0$	
Solvent	0.1	BSS	2.37 ± 0.02	$0.24 \pm 0.02$	
Solvent	1	BSS	2.45 ± 0.01	$0.31 \pm 0.01$	
Solvent	10	BSS	2.39 ±0.01	$0.44 \pm 0.01$	
Solvent	100	BSS	$2.48 \pm 0.01$	0.34 ± 0	
Solvent	0.1	BSS+2% charcoal	2.51 ± 0.01	$0.34 \pm 0.01$	
Solvent	1	BSS+2% charcoal	2.55 ± 0.01	$0.38 \pm 0.03$	
Solvent	10	BSS+2% charcoal	$2.51 \pm 0.02$	$0.48 \pm 0.01$	
Solvent	1	BSS+1% peat	0.33 ± 0	$0.02 \pm 0.01$	
Solvent	1	BSS+2% clay	2.73 ± 0.12	$0.35 \pm 0.03$	
Solvent	1	BSS+ clay	$3.01 \pm 0.05$	$0.26 \pm 0.01$	
		replaced by			
		Montmorillonite			
Solvent	1	BSS+ peat	$0.42 \pm 0.01$	$0.02 \pm 0.01$	
Soot	0.1	BSS	2.09 ± 0.01	$0.33 \pm 0.04$	
Soot	1	BSS	2.39 ± 0	$0.77 \pm 0.02$	
Soot	10	BSS	5.37 ± 0.05	0.98 ± 0.06	
Soot	100	BSS	43.00 ± 0	34.52 ± 1.26	
Soot	1	BSS+2% charcoal	3.50 ± 0	3.29 ± 0.20	
Skeet	0.1	BSS	2.98 ± 0	0.46 ± 0.06	
Skeet	1	BSS	3.01 ± 0.01	0.38 ± 0.03	
Skeet	1	BSS_duplicate	3.14 ± 0.05	$0.38 \pm 0.01$	
Skeet	10	BSS	2.80 ± 0.05	$0.40 \pm 0.01$	
Skeet	100	BSS	3.59 ± 0.03	0.62 ± 0.06	
Skeet	1	BSS+2% charcoal	3.16 ± 0.02	0.52 ± 0.02	
Fuel oil	0.1	BSS	2.94 ± 0.06	$0.21 \pm 0.01$	
Fuel oil	1	BSS	2.72 ± 0.06	0.35 ± 0.04	
Fuel oil	1	BSS_duplicate	2.89 ± 0.04	$0.42 \pm 0.01$	
Fuel oil	10	BSS	$4.85 \pm 0.01$	$0.64 \pm 0.03$	
Fuel oil	0.1	BSS+2% charcoal	$2.61 \pm 0.04$	$0.29 \pm 0.05$	
Fuel oil	1	BSS+2% charcoal	2.95 ± 0.03	$0.38 \pm 0.01$	
Fuel oil	10	BSS+2% charcoal	$4.46 \pm 0.04$	$0.26 \pm 0.03$	
Fuel oil	1	BSS+1% peat	0.51 ± 0	$0.06 \pm 0.01$	
Fuel oil	1	BSS+2% clay	2.95 ± 0.82	0.52 ± 0.12	
Note: BSS consists of 10% peat moss, 20% kaolin clay and 70% sand.					

**Table S4.** Total organic carbon and black carbon contents in experimental soils after 8 weeks of weathering (n=3, error bars represent standard deviation).

		PAH mass fraction in
PAH Source	Target BaP concentration(mg/kg)	peat based on equilibrium partitioning phenanthrene
Skeet	1	96%
Skeet	10	69%
Skeet	100	18%
Soot	0.1	83%
Soot	1	32%
Soot	10	4.60%
Soot	100	0.30%
		benzo(g,h,i)perylene
Skeet	0.1	97%
Skeet	1	83%
Skeet	10	33%
Skeet	100	4.70%
Soot	0.1	42%
Soot	1	6.80%
Soot	10	0.72%
Soot	100	0.05%

**Table S5.** Mass fraction of PAH redistribution into peat under equilibrium (between source materials and soil components) and non-equilibrium conditions.

PAH Source	Target BaP concentration	Composition	Mass of soil (g)	Mass of POM (g)
	(mg/kg)			
Control	0	BSS	40.2	1.003
Control	0	BSS+2% charcoal	40.1	0.947
Solvent	0.1	BSS	40.1	0.025
Solvent	1	BSS	40.6	0.026
Solvent	10	BSS	40.3	0.028
Solvent	100	BSS	40.1	0.027
Solvent	0.1	BSS+2% charcoal	40.0	4.953
Solvent	1	BSS+2% charcoal	6.1	1.007
Solvent	10	BSS+2% charcoal	6.0	0.205
Solvent	1	BSS+1% peat	40.3	0.026
Solvent	1	BSS+2% clay	40.1	0.027
Solvent	1	BSS+ clay replaced by Montmorillonite	39.9	0.026
Solvent	1	BSS+ peat replaced by Humus	28.4	0.025
Soot	0.1	BSS	41.8	0.532
Soot	1	BSS	7.1	0.510
Soot	10	BSS	7.0	0.502
Soot	100	BSS	7.2	0.510
Soot	1	BSS+2% charcoal	42.4	1.980
Skeet	0.1	BSS	39.8	0.025
Skeet	1	BSS	40.3	0.026
Skeet	1	BSS_duplicate	39.6	0.025
Skeet	10	BSS	39.7	0.026
Skeet	100	BSS	39.8	0.205
Skeet	1	BSS+2% charcoal	6.0	1.058
Fuel oil	0.1	BSS	39.8	0.026
Fuel oil	1	BSS	39.9	0.025
Fuel oil	1	BSS_duplicate	39.6	0.025
Fuel oil	10	BSS	40.5	0.026
Fuel oil	0.1	BSS+2% charcoal	40.1	5.013
Fuel oil	1	BSS+2% charcoal	5.9	1.031
Fuel oil	10	BSS+2% charcoal	5.9	0.208
Fuel oil	1	BSS+1% peat	40.3	0.027
Fuel oil	1	BSS+2% clay	40.5	0.025
Note: BSS co	onsists of 10% peat m	oss, 20% kaolin clay and	70% sand	

Table S6.	Average masses	of POM and	soil used fo	or the equilibrium	partitioning
experiment	t.				

Table S7. Masses of POM and soil components used for sorption isotherm study.

Corresponding solvent spiked soil target B(a)P concentration	Mass of component (g)	Mass of POM (g)
0.1 mg/kg	0.52	50
1 mg/kg	0.052	9.68
10 mg/kg	0.055	5.56
100 mg/kg	0.021	5.61
0.1 mg/kg	0.53	0.22
1 mg/kg	0.2	0.11
10 mg/kg	0.11	0.051
100 mg/kg	0.023	0.012
0.1 mg/kg	0.51	50.1
1 mg/kg	0.053	5.04
10 mg/kg	0.053	4.98
100 mg/kg	0.049	4.93
0.1 mg/kg	0.89	0.0034
1 mg/kg	0.85	0.0027
10 mg/kg	0.74	0.0026
100 mg/kg	0.88	0.0023
	Corresponding solvent spiked soil target B(a)P concentration 0.1 mg/kg 1 mg/kg 10 mg/kg 100 mg/kg 0.1 mg/kg 10 mg/kg 100 mg/kg 0.1 mg/kg 10 mg/kg 100 mg/kg 100 mg/kg 100 mg/kg 100 mg/kg 100 mg/kg 100 mg/kg 100 mg/kg	Corresponding solvent spiked soil target B(a)P         Mass of component (g)           concentration         0.52           1 mg/kg         0.52           1 mg/kg         0.055           10 mg/kg         0.021           0.1 mg/kg         0.2           10 mg/kg         0.2           10 mg/kg         0.2           10 mg/kg         0.11           00 mg/kg         0.023           0.1 mg/kg         0.53           10 mg/kg         0.51           10 mg/kg         0.53           10 mg/kg         0.53           10 mg/kg         0.53           1.1 mg/kg         0.53           1.0 mg/kg         0.649           0.1 mg/kg         0.89           1 mg/kg         0.85           1.0 mg/kg         0.74           1.00 mg/kg         0.74

#### **Chapter 5: Supplemental Information**

#### Method Development for PAH Solvent Extraction from PBET.

Low PAH recoveries were observed when spiking PAHs into the gut fluid before the 5 hour PBET process. An extensive method optimization was therefore performed that included an investigation into the use of different contact times and mixing speeds. Bioaccessible PAHs in the supernatant were then extracted three times by hexane solvent on an orbital shaker and its extraction efficiency from PBET into solvent system (for PAH analysis by GC-MS) was investigated: a mixture of PAHs in dichloromethane was first spiked into the simulated digestion fluid followed by hexane addition, and the combined hexane extract was analyzed after different contact times at different mixing speeds. We found out that solvent contact with the digestion fluid at a high rotation speed (100 rpm) increased the formation of an irreversible emulsion, which inhibited the separation of the hexane phase and decreased the extraction efficiency (Figure S1). In order to reduce the emulsion, several centrifugation and emulsion breaking techniques (e.g. salination) were performed but turned out unsuccessful. Eventually, reduced rotation speeds were tested and an optimum of 60 rpm was found to be the highest agitation speed without risk of emulsifying. In addition, the solvent contact durations were also evaluated and improved recovery efficiencies were observed with increased contact time, especially for 4-6 ringed PAHs (with higher carcinogenic potency) (Figure S2). A sequential extraction time of 16h + 24h + 24h was selected for this study based on this evaluation.



**Figure S1.** Formation of emulsion during solvent extraction of digestion fluid at different agitation speeds (60 rpm and 100 rpm). Reversible emulsion was observed at agitation of 60 rpm and irreversible emulsion was observed at agitation speed of 100 rpm.



**Figure S2.** Recoveries of mass of PAHs in solvent extraction of digestion fluid at different contact times (n=3, error bars represent standard error).



Figure S3. Flow diagram for physiologically-based extraction test.



**Figure S4.** Comparison of measured partitioning coefficients (log  $K_D$  [L/kg]) for soil components and source materials for PHE, PYR, B(a)P and B(ghi)P). The dark red square represents  $K_D$  for skeet measured with minimal depletion (at POM/skeet mass ratio of 0.125) while, the orange squares represent  $K_D$  measured with increasing depletion of PAHs from skeet (by increasing POM/skeet mass ratio). For soot and fuel oil,  $K_D$  was measured at only one POM/source mass ratio.


**Figure S5.** Effect of PAH source materials (control, fuel oil, soot and skeet particles) on percentage of PAHs extracted by gastrointestinal fluid from soils with 0.1 mg/kg target B(a)P (n= 3, error bars represent standard error).



**Figure S6.** Effect of PAH source materials (control, fuel oil, soot and skeet particles) on percentage of PAHs extracted by gastrointestinal fluid from soils with 10 mg/kg target B(a)P (n= 3, error bars represent standard error).



**Figure S7.** Effect of PAH source materials (control, soot and skeet particles) on percentage of PAHs extracted by gastrointestinal fluid from soils with 100 mg/kg target B(a)P (n= 3, error bars represent standard error).



**Figure S8.** Effect of soil composition on PAH partitioning in solvent and fuel oil spiked weathered soils with 1mg/kg target BaP (n= 3, error bars represent standard error).



**Figure S9.** Effect of soil compositions on percentage of PAHs extracted by gastrointestinal fluid from fuel oil spiked soils with 1 mg/kg target B(a)P (n= 3, error bars represent standard error).

# Determination of K<sub>GI</sub>.

Briefly, 45 mg of precleaned polyethylene (PE) passive sampler was placed into PAH impregnation solution (methanol/water 3:1 by volume) in a 1L jar and allowed to equilibrate on an orbital shaker for 28 days following Fernandez et al., Fernandez et al., 2009) The PAH impregnated-sampler was then soaked in deionized water to remove residual methanol in the sampler. After that, the passive sampler was placed in 40 ml of deionized water in a 60ml vial and the system was allowed to equilibrate for 28 days. After 28 days, 1.2g of glycine, 0.04g of pepsin, 0.2 g of BSA, 0.12ml of sunflower oil and 0.1g of porcine mucin were added into the vial and pH was adjusted to  $1.50 \pm 0.05$ by dropwise addition of concentration HCl. The vial was then placed into the end-overend rotator in the water bath at 37°C for an hour, followed by another 4 hours mixing after addition of 80ml of porcine bile and 20 mg of porcine pancreatin extract and pH alteration to 6.5. At the end of the mixing, the passive sampler was removed from the GI fluid and wiped with wet tissue paper to remove residual GI solute from the sampler. The simulated GI fluid and the passive sampler were then extracted separately overnight three times using 30 ml of hexane. Pooled extract for each media were used to determine PAH concentrations in both passive sampler (CPE) and GI solute (CGI). The aqueous PAH concentration in the system was calculated assuming  $C_W = C_{PE}/K_{PE}$ , using  $K_{PE}$  values previously reported in Choi et al. (Choi et al., 2013).



**Figure S10.** Experimentally determined  $K_{GI-5h}$  as a function of PAH hydrophobicity ( $K_{OW}$ ) for 16 parent PAHs. Error bar represents standard error, n = 2.



**Figure S11.** Comparison between experimentally determined  $K_{GI-5h}$  and estimated  $K_{GI-5h}$  assuming sunflower oil is the dominant sorption domain in the simulated GI mixture (estimated  $K_{GI-5h} = f_{oil} * K_{oil}$ , where  $f_{oil}$  is the mass fraction of sun flower oil and  $K_{oil}$  is approximated as  $K_{OW}$ ). Error bar represents standard error, n = 2.



**Figure S12.** Overall correlation between  $K_D / K_{GI-5h}$  and PAH bioaccessibility evaluated based on five PAHs with a range of hydrophobicities (chrysene, benz(a)anthracene, benzo(a)pyrene, benzo(g,h,i)perylene and dibenz[a,h]anthracene) from all 30 soil samples. Error bars (n=3) represent standard error.

PAH Sources	Baseline Synthetic Soil (BSS)	BSS-2 % charcoal	BSS-peat reduced to 1 %	BSS – kaolin reduced to 2 %	BSS - kaolin replaced with montmorillonite	BSS-peat replaced with humus
Solvent	0.1,1,10,100 mg/kg BaP	0.1,1,10 mg/kg BaP	1mg/kg BaP	1 mg/kg BaP	1mg/kg BaP	1mg/kg BaP
Soot	0.1,1,10,100 mg/kg BaP	1mg/kg BaP	-	-	-	-
Skeet Particles	0.1,1,10,100 mg/kg BaP	1mg/kg BaP	-	-	-	-
Fuel Oil	0.1,1,10 mg/kg BaP	0.1, 1,10 mg/kg BaP	1mg/kg BaP	1mg/kg BaP	-	-
<sup>a</sup> Concentrations shown above are target values before weathering						

**Table S1.** Compositions of experimental soils and their respective PAH concentrations (as target B(a)P levels).

**Table S2.** Comparison between experimentally determined  $K_{GI-5h}$  and estimated  $K_{GI-5h}$  assuming sunflower oil is the dominant sorption domain in the simulated GI mixture (estimated  $K_{GI-5h} = f_{oil} * K_{oil}$ , where  $f_{oil}$  is the mass fraction of sun flower oil and  $K_{oil}$  is approximated as  $K_{OW}$ ). Measured soil log  $K_{GI}$  had relative standard deviation of less than 2%.

	estimated log K <sub>GI</sub>	measured log K <sub>GI</sub>
Naphthalene	2.10	1.95
Acenaphthylene	2.87	2.88
Acenaphthene	2.72	3.03
Fluorene	2.98	3.18
Phenanthrene	3.26	3.50
Anthracene	3.25	3.58
Fluoranthene	3.96	4.10
Pyrene	3.68	4.21
Benz(a)anthracene	4.56	4.76
Chrysene	4.61	4.83
Benzo(b)fluoranthene	4.58	5.33
Benzo(k)fluoranthene	4.91	5.29
Benzo(a)pyrene	4.93	5.31
Indeno(1,2,3,-cd)pyrene	5.50	5.49
Dibenz(a,h)anthracene	5.60	5.50
Benzo(g,h,i)perylene	5.50	5.33

<b>Table S3.</b> Measured soil K <sub>D</sub> of three representative PAHs for skeet spiked soils with
different concentrations and compositions. Measured soil log KD had relative standard
deviation of less than 10%.

source	target concentration						
material	(ppm)	soil composition	soil log K <sub>D</sub>				
pyrene							
Skeet	0.1	BSS	5.03				
Skeet	1	BSS	5.09				
Skeet	10	BSS	6.13				
Skeet	100	BSS	5.81				
Skeet	1	BSS+2% charcoal	6.26				
benzo[a]pyrene							
Skeet	0.1	BSS	6.67				
Skeet	1	BSS	6.61				
Skeet	10	BSS	7.06				
Skeet	100	BSS	6.82				
Skeet	1	BSS+2% charcoal	7.76				
benzo[ghi]perylene							
Skeet	0.1	BSS	6.97				
Skeet	1	BSS	7.07				
Skeet	10	BSS	7.71				
Skeet	Skeet 100		7.49				
Skeet	1	BSS+2% charcoal	8.05				

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