FINAL REPORT

PAH Interactions with Soil and Effects on Bioaccessibility and Bioavailability to Humans

SERDP Project ER-1743

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Gordon Sweet
Exponent, Inc.

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Lowney, Yvette W.; Ruby, Michael V.; Bunge, Annette; Gomez-Eyles, Jose; Ghosh, Upal; Kissel, John; Peckham, Trevor., Roberts, Stephen; Shirai, Jeffrey; Xia, Huan; Menzie, Charles.

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Exponent Inc., Boulder, CO
Integral Consulting, Louisville, CO
University of Florida, Gainesville, FL
University of Maryland, Baltimore, MD
University of Washington, Seattle, WA
Colorado School of Mines, Golden Co

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14. ABSTRACT

This work was conducted in response to SERDP's 2010 Statement of Need 10-04: *Mechanisms of Contaminant Interaction with Soil Components and its Impact on the Bioavailability of Contaminants.* The project evaluated the interactions between polycyclic aromatic hydrocarbons and soils, and how these interactions control the oral and dermal bioavailability of PAHs in soil to humans. The study provides results that can inform assessments and risk management considerations for DoD sites at which PAH-contaminated soils are driving clean-up decisions.

The objectives included: Task 1) Identify which PAH sources, exposure pathways, and individual PAHs are driving risk assessments and remedial decisions to focus research where it can be most effective; Task 2) Develop an understanding of the mechanisms by which PAHs are sequestered in soil, so the magnitude of bioavailability adjustments can be predicted, and to elucidate factors that control the dissolution of PAHs from soil; Task 3) Develop an animal model that provides quantitative measures of the relative oral bioavailability of PAHs in soil, and generate a database from this animal model to understand bioavailability across a diversity of soil types and contaminant sources; Task 4) Evaluate use of simple *in vitro* extraction tests to predict *in vivo* measures of relative bioavailability; and, Task 5) Assess the effect of soil-chemical interactions on the dermal absorption of PAHs.

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ACRONYMS

AUC	area under the curve
BaP	benzo[a]pyrene
BC	black carbon
DoD	Department of Defense
EPA	U.S. Environmental Protection Agency
GI	gastrointestinal
IVIVC	in vitro to in vivo correlation
PAH	polycyclic aromatic hydrocarbon
PBET	physiologically-based extraction test
RBA	relative oral bioavailability
ROD	Record of Decision
RPF	relative potency factor
UMBC	University of Maryland, Baltimore Campus
UFl	University of Florida in Gainesville

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Abstract

Objectives

The work reported herein was conducted in response to SERDP's 2010 Statement of Need 10-04: *Mechanisms of Contaminant Interaction with Soil Components and its Impact on the Bioavailability of Contaminants*. The project was a multi-disciplinary evaluation to characterize the interactions between polycyclic aromatic hydrocarbon (PAHs) and soils and how these interactions control the oral and dermal bioavailability of PAHs in soil to humans. The study provides results that can inform assessments and risk management considerations for Department of Defense (DoD) sites where PAH-contaminated soils are driving clean-up decisions.

Several specific tasks were included in the broad research conducted under this effort. The objectives of the different aspects of the project included: Task 1) Identify which specific PAH sources, exposure pathways, and individual PAHs are driving risk assessments and remedial decisions to focus research where it can be most effective; Task 2) Develop an understanding of the mechanisms by which PAHs are sequestered in soil, so the magnitude of bioavailability adjustments can be predicted, and elucidate the factors that control the dissolution of PAHs from soil; Task 3) Develop an animal model that provides quantitative measures of the relative oral bioavailability (RBA) of PAHs in soil and generate a database of information from this animal model to understand bioavailability across a diversity of soil types and contaminant sources; Task 4) Evaluate potential use of simple *in vitro* extraction tests to predict *in vivo* measures of relative bioavailability (as indicated by the *in vivo* model); and, Task 5) Assess the effect of soil-chemical interactions on the dermal absorption of PAHs.

Technical Approach

To allow for a rigorous and controlled evaluation of the effects of PAH concentration, soil compositions, and PAH source materials on the chemistry and bioavailability of PAHs from soil (studied in Tasks 2–5), a series of artificial soils were constructed with a range of PAH concentrations, soil compositions, and different PAH source materials of specific relevance to DoD sites (skeet fragments, soot, and fuel oil). The ability to control factors that might affect soil-chemical interactions was identified as more important to the project goals than a less controlled study design using contaminated field soils. These constructed soils were subjected to several weeks of artificial "weathering" to capture some of the effects of weathering that occur in the natural environment.

Task 1 entailed reviewing publicly available information on relevant exposure pathways for PAHs in soils and combining that with information available in the database of Records of Decision (RODs) for DoD sites. This review identified the specific PAHs driving remedial decisions as well as the emerging regulatory approaches for assessing toxicity of PAH mixtures. Led by Dr. Upal Ghosh at the University of Maryland, Baltimore Campus (UMBC), Task 2 involved an evaluation of the partitioning behavior of PAHs from the library of soils created for the project. Under the direction of Dr. Stephen Roberts at the University of Florida in Gainesville, FL, Task 3 involved the development of an *in vivo* model for measuring bioavailability of oral administration of PAH-contaminated soil using laboratory rats. After several pilot investigations using various species and strains of laboratory animals, as well as different measurement endpoints for bioavailability, it was determined that the research would require using soils contaminated with radiolabeled PAHs to provide analytical detection limits that were adequately sensitive to evaluate soil contamination levels of relevance to remedial decision-making. For the *in vivo* animal model with the rat exposed orally to radiolabeled compounds in soils, the measurement endpoint was the Area Under the Curve (AUC) of benzo[a]pyrene (BaP) and metabolites entering the blood. Relative bioavailability was determined by comparing the resultant AUC for a particular administration with soil to the AUC for soluble BaP administered in food.

Task 4 entailed investigation of the bioaccessibility of PAHs from soil under simulated physiological conditions. Studies reporting on laboratory extraction methods to predict the bioavailability of PAHs from soils to humans have been undertaken by several researchers, but efforts to date have been hampered by lack of a database of meaningful bioavailability data from a validated animal research model. In this project, an initial evaluation of the soils constructed at UMBC (described above) was conducted using a physiologically-based extraction test (PBET) modified from methods available in the published literature. The second phase of *in vitro* method evaluation, using the soils dosed to animals, was conducted at the University of Florida. The assessments included evaluation of a simplified physiologically-based extraction method, simple solvent extractions, and also investigated the use of a solid-phase sink to see if that approach would simplify the analytical efforts. The evaluation of dermal absorption of BaP from soil under Task 5 was conducted within the laboratory of Dr. John Kissel at the University of Washington, with collaboration from Dr. Annette Bunge from the Colorado School of Mines. This component of the research evaluated the dermal absorption of BaP from four soils. The soils were selected to represent different conditions and included the soil used in the study reported by Wester (1990) that forms the basis of the U.S. Environmental Protection Agency's (EPA's) current recommendation of 13% dermal absorption of BaP from soil. Testing was also performed to assess the dermal absorption of BaP from a solvent vehicle (acetone) to serve as a basis of comparison for understanding the effects of soil-chemical binding on absorption characteristics. In this task, ^{14}C BaP was weathered into soils using weekly wet-dry cycles. Absorption through human epidermis was assessed using an *in vitro* study design and application of a fine fraction of the soil.

Results

The results of each project task are summarized below.

Task 1: The primary human health risk drivers for PAH-contaminated soils are the larger PAHs (i.e., four- to six-ring) associated with cancer endpoints. Thus, BaP, benz[a]anthracene, benzo[b]fluoranthene, indeno[1,2,3-cd]pyrene, and dibenz[a,h]anthracene will likely be risk drivers for human health risk assessment at DoD sites.

Task 2: The source of PAH contamination is the primary factor controlling the portioning behavior of PAHs from soil. PAHs that enter soil as part of a matrix that is rich in black carbon (BC), such as within soot or coal tar pitch, are much less bioavailable than PAHs that are spiked to soil in the laboratory or that enter soil within fuel oil. Mineral characteristics of the soil (e.g., type of content of clay, presence of humic acids) have much less influence on the binding of PAHs. Conversely, the addition of charcoal to the soil results in higher binding within the soil matrix, possibly pointing to opportunities for *in situ* remedial opportunities to address PAH-contaminated soils.

Task 3: The *in vivo* evaluation of the RBA of PAHs yielded result that supported the finding that PAH sources are the most important factor controlling bioavailability. PAHs introduced to soil in fuel oil demonstrated higher bioavailability than soils contaminated with PAHs in solvent or soot. Over all the soils tested, RBA values ranged from 65% to 100% (for BaP concentrations of 1–100 mg/kg). At the highest concentration tested (100 mg/kg BaP) soot demonstrated lower bioavailability of BaP than soils contaminated with PAHs in solvent or PAHs in fuel oil, with RBA of 24% for soot-spiked soils, and RBAs of 55% and 100% for solvent-spiked soils and fuel oil-spiked soils, respectively. In all cases, adding charcoal to the soil before weathering resulted in a significant (three- to four-fold) decrease in measured RBA. The use of the radiolabel also afforded the ability to understand some of the nature of initial binding of PAHs to soil during the weathering process and the limitations of some analytical methods to capture total PAH content of soils.

Task 4: The extraction of soils using a PBET method indicated that the partitioning behavior of PAHs in soil observed as part of Task 2 is correlated with PAH dissolution under physiological conditions. While this is promising for possible application of PBET, there remain complexities associated with the PBET system, requiring specific method development to ensure quantitative recovery of PAHs from the PBET solution. Results from bioaccessibility testing of the same soils dosed to rats to identify RBA show good promise for the use of *in vitro* methods to predict bioavailability as measured in rats: results with a simplified PBET were relatively reproducible

for a given soil, and *in vitro* to *in vivo* correlation (IVIVC) demonstrated an $R^2 = 0.57$. A simple solvent extraction of soils using n-butanol had a higher IVIVC, with an $R^2 = 0.74$. Extraction with EPA Method 3550C was not a good predictor of RBA as measured in rats ($R^2 = 0.43$), resulting in over predictions for some soils and under predictions for others.

Task 5: Dermal absorption of BaP from soil was examined as flux of the compound into or through the skin over time. Absorption of BaP from soil was significantly lower than absorption of BaP applied to the skin in solvent. The absorption was independent of soil type or concentration of BaP in the soil over the limited range of soil types and concentrations evaluated. Absorption was proportional to the duration of contact between the soil and the skin surface. Additionally, the mass of BaP recovered in the skin after washing to remove soil was proportional to soil concentration and independent of time, possibly suggesting that soil residue remained on the surface of the skin even after washing. Results indicate that the concentration range used for this part of the research may have saturated the binding ability of the study soils. This would result in an overestimate of the dermal bioavailability of BaP in soil, suggesting the need for additional work. Based on these results and other tasks, it is reasonable to expect that the nature of the source of PAHs to soils will be an important factor influencing dermal exposure and absorption.

Lessons Learned

Overall, the broadest conclusions that can be drawn from the research conducted under SERDP Project ER-1743 include:

- The source material in which PAHs are introduced to soil is very important in determining the nature of the soil-chemical interactions between the PAHs and the soil components and controls the dissolution of PAHs from contaminated soils, both in laboratory chemical characterization efforts and within mammalian systems when tested *in vivo*. PAHs introduced to soil in carbon-rich sources such as soot and coal tar-based skeet are sequestered in the soil in a more stable form and recalcitrant to extraction. For example, in animal testing the RBA of BaP was close to 100% when introduced to soil in fuel oil but as low as 23% when introduced in soot.

- The composition of soils can also affect the dissolution or bioavailability of PAHs from soil, particularly the black carbon content, which enhances the binding of PAHs within the soil. Our evaluations indicate that the presence of charcoal substantially reduces PAH bioavailability; RBA from soils with added charcoal was less than one third of the RBA observed in paired soils without added charcoal.

- Within the concentration range of environmental relevance used in this study (0.1 to 100 ppm BaP), PAH concentration was much less influential on partitioning and bioavailability than source of PAHs.

- Based on paired testing of the RBA of BaP from soils *in vivo* with a rat model and extraction testing of the same soils, both PBETs and simple solvent extractions show promise as methods for predicting the RBA of BaP.

- EPA Method 3550C, commonly used to characterize the concentration of PAHs in soils from contaminated sites, does not extract the total amount of PAHs from some soils. Our investigations *in vivo* suggest the gastrointestinal tract of intact animals can be more efficient than EPA Method 3550C at extracting PAHs from some soils but not always.

- Percutaneous absorption of BaP from soil was significantly lower than absorption of BaP applied to the skin in solvent, and absorption was independent of soil type or concentration of BaP in the soil over the limited range of soil types and concentrations evaluated. Absorption was proportional to the duration of contact between the soil and the skin surface. Additionally, the mass of BaP recovered in the skin after washing to

remove soil was proportional to soil concentration and independent of time, suggesting that soil residue remained on the surface of the skin even after washing. This preliminary effort was conducted with four field soils that were spiked with BaP in the lab, suggesting studies that incorporate considerations of different sources of PAHs to soil may be important for understanding site-specific exposures.

- This research utilized a series of artificial soils constructed with a range of PAH concentrations, soil compositions, and different PAH source materials of specific relevance to DoD sites (skeet fragments, soot, and fuel oil). Although unable to provide information on the bioavailability of PAHs from any particular site, the ability to control these factors allowed the research to identify key soil-chemical interactions likely to affect bioavailability at any site. The results indicate site-specific factors such as PAH source and some soil characteristics are important to understanding potential exposures to PAH at a contaminated site.

1 Introduction and Summary

The work reported herein was conducted in response to SERDP's 2010 Statement of Need 10-04: *Mechanisms of Contaminant Interaction with Soil Components and its Impact on the Bioavailability of Contaminants*. The project was a multi-disciplinary evaluation to characterize the interactions between polycyclic aromatic hydrocarbons (PAHs) and soils and how these interactions control the oral and dermal bioavailability of PAHs in soil to humans. This research was identified as being relevant to SERDP because PAHs have emerged as one of the most important contaminants driving risk estimates and remedial decisions for soils at Department of Defense (DoD) sites. Understanding the oral bioavailability and dermal absorption of PAHs from soil allows for more accurate assessment of potential human health risks from exposure to contaminated soils, and therefore the information can affect decisions regarding the need for site cleanup and affect risk-based soil cleanup goals for PAHs.

Several specific tasks were included within the broad research conducted under this effort. The different aspects of the project included the following tasks:

1) Identify which specific PAH sources, exposure pathways, and individual PAHs are driving risk assessments and remedial decisions to focus research where it can be most effective. This was conducted by reviewing information on relevant exposure pathways for PAHs from the published literature and publicly available technical reports, synthesis of information in the database of Records of Decision (RODs) available from the U.S. Environmental Protection Agency (EPA) to indicate which specific PAHs are driving remedial decisions, and tracking emerging regulatory approaches for assessing toxicity of PAH mixtures.

2) Develop an understanding of the mechanisms by which PAHs are sequestered in soil, so that the magnitude of bioavailability adjustments can be predicted, and of the factors that control the dissolution of PAHs from soil. This work was largely conducted in the laboratory of Dr. Upal Ghosh at the University of Maryland, Baltimore Campus (UMBC), and included an evaluation of the partitioning behavior of PAHs from soils. To allow for a rigorous and controlled evaluation of the effect of PAH concentration, soil compositions, and PAH source materials on the soil-chemical interactions of PAHs, a series of artificial soils were constructed with a range of PAH concentrations, soil compositions, and different PAH source materials of specific relevance to DoD sites (skeet fragments, soot, and fuel oil). The ability to control all these factors that might affect the soil-chemical interactions was identified as more important to the project goals than a less-controlled study design using contaminated field soils. The results of this work indicate that the source of PAH contamination is the primary factor controlling the portioning behavior of PAHs from soil. PAHs that enter soil as part of a matrix that is rich in black carbon (BC), such as soot or coal tar pitch, are much more recalcitrant than PAHs spiked to soil in the laboratory or that enter soil in fuel oil. The findings of this task indicated that across the factors evaluated, mineral characteristics (e.g., type of content of clay, presence of humic acids) has much less influence on

the binding of PAHs. Conversely, the addition of charcoal to the soil resulted in higher binding within the soil matrix, possibly pointing to opportunities for *in situ* remedial opportunities to address PAH-contaminated soils.

3) Develop an animal model that provides quantitative measures of the relative oral bioavailability (RBA) of PAHs in soil and generate a database of information from this animal model to understand bioavailability across a diversity of soil types and contaminant sources. The *in vivo* model development and soil research was conducted in the laboratory of Dr. Stephen Roberts at the University of Florida in Gainesville, FL. After several pilot investigations using various species and strains of laboratory animals, as well as different measurement endpoints for bioavailability, it was determined the research would require using soils contaminated with radiolabeled PAHs to provide analytical detection limits that were adequately sensitive to evaluate soil contamination levels of relevance to remedial decision-making. The results of the *in vivo* evaluation of the RBA of PAHs are consistent with the evaluation of soil-chemical interactions in terms of identifying that PAH sources are the most important factor controlling bioavailability. PAHs introduced to soil in fuel oil demonstrated higher bioavailability than soils contaminated with PAHs in solvent or soot (RBA values ranged from 65% to 100% for benzo[a]pyrene [BaP] concentrations of 1–100 mg/kg). At the highest concentration tested (100 mg/kg BaP), soot demonstrated lower bioavailability than soils contaminated with PAHs in solvent or PAHs in fuel oil, with RBA of 24% for soot-spiked soils and RBAs of 55% and 100% for solvent-spiked soils and fuel oil-spiked soils, respectively. In all cases, adding charcoal to the soil before weathering resulted in a significant (three- to four-fold) decrease in measured RBA. The use of the radiolabel also afforded the ability to understand some of the nature of the initial binding of PAHs to soil during the weathering process and the limitations of some analytical methods to capture total PAH content of soils. This is an important consideration in assessing the bioavailability of PAHs from soils.

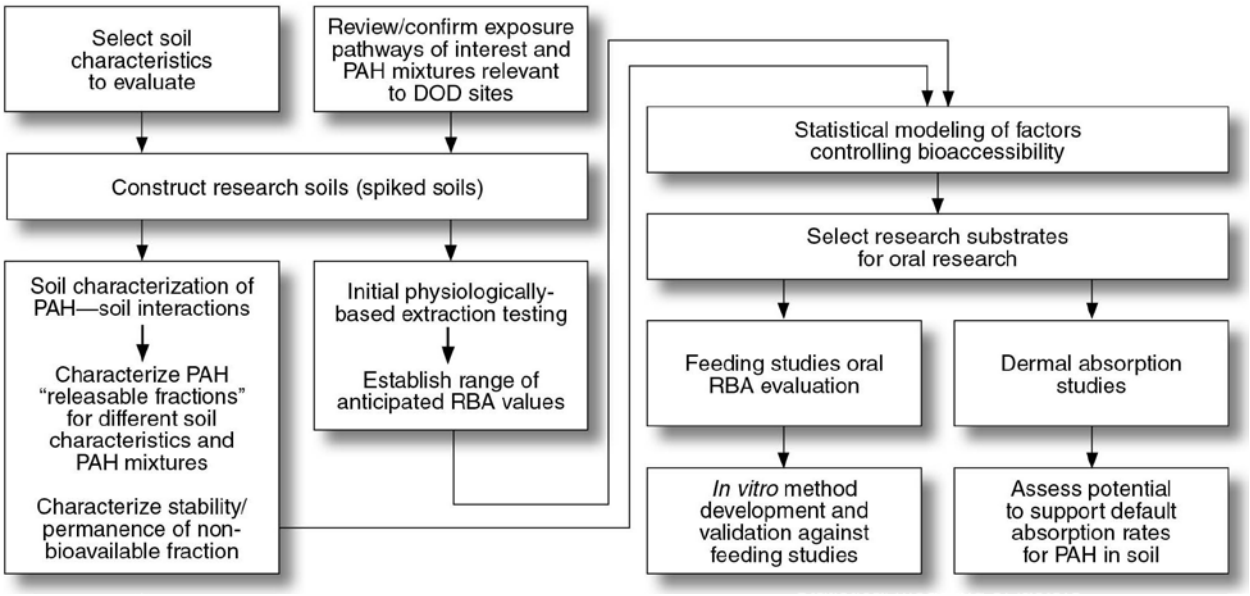
4) Evaluate potential use of simple *in vitro* extraction tests to predict *in vivo* measures of relative bioavailability (as indicated by the *in vivo* model). Studies reporting on laboratory extraction methods to predict the bioavailability of PAHs from soils to humans have been undertaken by several researchers, but efforts to date have been hampered by lack of a database of meaningful bioavailability data from a validated animal research model. Under the U.S. regulatory paradigm, validation against animal models is generally required before *in vitro* methods can be used to generate data to support adjustments in human health risk assessments. In this project, an initial evaluation of the soils constructed at UMBC (described above) was conducted using a physiologically-based extraction test (PBET) modified from methods available in the published literature. This initial evaluation identified the correlation of partitioning behavior of PAHs in soil with PAH dissolution under physiological conditions. The evaluation also revealed complexities associated with the PBET system, requiring specific method development to ensure quantitative recovery of PAHs from the PBET solution. Because the animal bioavailability data generated in this project utilized radiolabeled soils evaluated at the University of Florida, the second phase of *in*

vitro method evaluation, using the soils dosed to animals, was conducted at the University of Florida. The assessments included evaluation of a simplified physiologically-based extraction method (simplified in response to insights gleaned from the PBET work conducted at UMBC), simple solvent extractions, and also investigated the use of a solid-phase sink to see if that approach would simplify the analytical efforts. Results show good promise for the use of *in vitro* methods to predict bioavailability as measured in rats: results with a simplified PBET were relatively reproducible for a given soil, and *in vitro* to *in vivo* correlation (IVIVC) demonstrated an $R^2 = 0.57$. A simple solvent extraction of soils using n-butanol had a higher IVIVC, with an $R^2 = 0.74$. Extraction with EPA Method 3550C was not a good predictor of RBA as measured in rats ($R^2 = 0.43$), resulting in over predictions for some soils and under predictions for others.

5) Assess the effect of soil-chemical interactions on the dermal absorption of PAHs. Conducted in the laboratory of Dr. John Kissel at the University of Washington, with collaboration from Dr. Annette Bunge from the Colorado School of Mines, this component of the research evaluated the dermal absorption of BaP from four soils. The soils were selected to represent different conditions and included the soil used in the study reported by Wester (1990) that forms the basis of EPA's current recommendation of 13% dermal absorption of BaP from soil. Testing was also performed to assess the dermal absorption of BaP from a solvent vehicle (acetone) to serve as a basis of comparison for understanding the effects of soil-chemical binding on absorption characteristics. In this task, ^{14}C BaP was weathered into soils using weekly wet-dry cycles. Absorption through human epidermis was assessed using an *in vitro* study design and application of a fine fraction of the soil. Results were reported in terms of BaP flux into or through the skin over time. Results indicate that absorption from soil was significantly lower than absorption of BaP applied to the skin in solvent, and absorption was independent of soil type or concentration of BaP in the soil over the range of soil types and concentrations evaluated. Absorption was proportional to the duration of contact between the soil and the skin surface. Additionally, the mass of BaP recovered in the skin after washing to remove soil was proportional to soil concentration and independent of time, possibly suggesting soil residue remained on the surface of the skin even after washing. Results suggest the concentration range studied in this research, although low, may have saturated the binding ability of the study soils, suggesting study that incorporates considerations of different sources of PAHs to soil may be important to understanding site-specific exposures.

Below is a schematic of the different components of the research undertaken in this project followed by a more detailed "abstract" of each research component. Attached appendices provide details of the research methods, generated data, results, and conclusion. Appendices also include references and copies of presentations at professional conferences that occurred over the duration of the research.

PAH Bioavailability from Soils—Schematic of Project Tasks



Overall, the broadest conclusions that can be drawn from this research are that:

- The use of a series of artificial soils for this research means the results are unable to provide information on the specific bioavailability of PAHs from any particular site, though the ability to control these factors allowed the research to identify key soil-chemical interactions likely to affect bioavailability at any site. This research utilized a series of soils constructed with a range of PAH concentrations, soil compositions, and different PAH source materials of specific relevance to DoD sites (skeet fragments, soot, and fuel oil). The results indicate site-specific factors, especially PAH source, can have a significant influence on the bioavailability of PAHs from soil and so are important to understanding potential exposures to PAHs at a contaminated site.

- The source material in which PAHs are introduced to soil is very important in determining the nature of the soil-chemical interactions between the PAHs and the soil components and also controls the dissolution of PAHs from contaminated soils, both in laboratory chemical characterization efforts and in mammalian systems when tested *in vivo*. PAHs introduced to soil in carbon-rich sources such as soot and coal tar-based skeet are sequestered in the soil in a more stable form and recalcitrant to extraction. For example, in animal testing the RBA of BaP was close to 100% when introduced to soil in fuel oil but as low as 23% when introduced in soot.

- The composition of soils can also affect the dissolution or bioavailability of PAHs from soil, particularly the BC content, which enhances the binding of PAHs within the soil. Our evaluations indicate the presence of charcoal substantially reduces PAH bioavailability; RBA from soils with added charcoal was less one-third of the RBA observed in paired soils without added charcoal.

This suggests further investigation may be warranted to better understand the possibility of BC amendments to reduce bioavailability and bioaccessibility of PAHs from PAH-impacted soils.

- Based on paired testing of the RBA of BaP from soils *in vivo* with a rat model and extraction testing of the same soils, both PBETs and simple solvent extractions show promise as methods for predicting the RBA of BaP.
- EPA Method 3550C, commonly used to characterize the concentration of PAHs in soils from contaminated sites, does not extract the total amount of PAHs from some soils. Our investigations *in vivo* suggest the gastrointestinal tract of intact animals can be more efficient at extracting PAHs from some soils but not always.

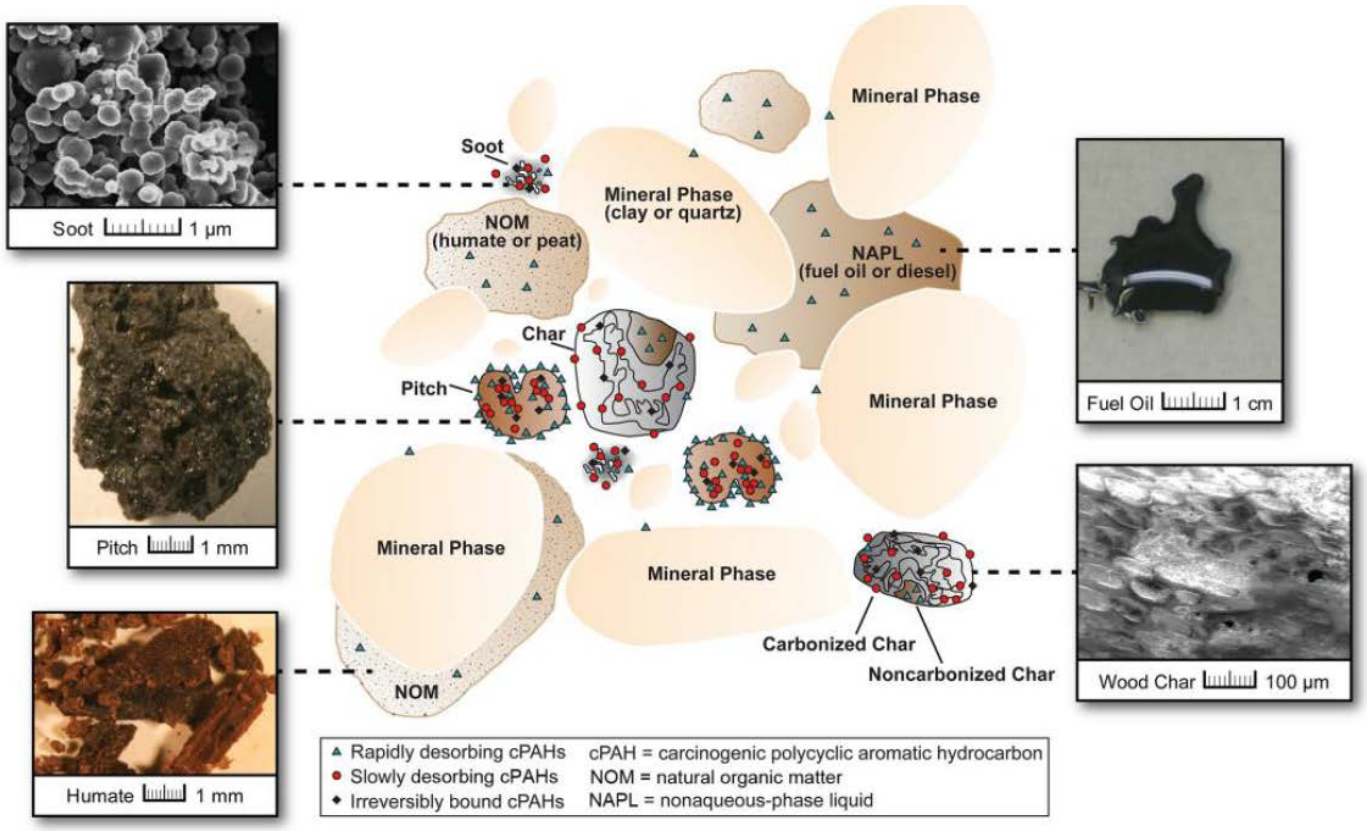


Figure 1. PAH Bioavailability from Soils – Schematic of factors controlling oral or dermal absorption as a function of PAH source materials and soil chemistry (from Ruby et al. 2016, with permission).

2 Identification of Relevant PAH Sources, Mixtures, and Exposure Pathways

For full details of this research, see Appendix A.

2.1 Problem Addressed/Background

The objective of this initial task is to provide important background information for focusing the direction of the project research in a manner to ensure applicability to PAH contamination at DoD sites. The work performed under this task provides perspectives on current activities being conducted by EPA with regard to characterizing toxicity of PAHs and provides a retrospective review of RODs to assess which specific PAHs have previously driven remedial decisions at DoD sites. These two components are then used together to provide an understanding of which PAHs are likely to drive remedial decisions in the future and thereby to identify the PAHs of primary interest for this research project. The RODs were also evaluated for information on sources of PAH contamination to soils at DoD sites. Together with information gained from conversations with risk assessors from various military branches, this provided a basis for selecting source materials of PAHs for inclusion in the study.

2.2 Review of Regulatory Toxicology

2.2.1 Technical Approach

When present, PAHs invariably exist in the environment as mixtures. Although the total number of PAHs is unknown, there are hundreds of PAHs present as components of mixtures. To the extent possible, the regulatory approach to health assessment of PAHs considers the interaction between the individual PAHs in the mixture. For this task, the regulatory history and various approaches used to evaluate individual PAHs and PAH mixtures were evaluated.

2.2.2 Results

For PAH mixtures that have not been evaluated for toxicity, EPA and other regulatory agencies have utilized relative potency factors (RPFs) to assess the toxicity of individual PAHs. In the RPF approach, the doses of individual components acting through a similar mechanism of action are summed after scaling to the relative potency of an index chemical in the group for which the most complete dose-response characterization is available. For PAHs, BaP has been selected as the index chemical because: 1) it is typically present in environmental settings where PAHs are detected; 2) it has the most robust toxicological dataset among the PAHs and a formal dose-response assessment has been conducted based on chronic rodent bioassays; 3) there is a large database of *in vivo* and *in vitro* studies directly comparing the toxic potency of various PAHs with BaP; and 4) it is one of the most potent carcinogens in PAHs tested. EPA has proposed updated RPFs for an expanded list of PAHs.

2.2.3 Conclusions and Implications for Human Health Risk Assessment/Implementation

Proposed modifications to PAH health assessment are fundamentally consistent with the long-standing regulatory approach of using RPFs to evaluate the carcinogenic risk of PAHs in mixtures and to evaluate the noncancer effects on a chemical-specific basis. Nevertheless, the proposed changes could have significant effects on environmental assessment of PAHs depending to some degree on how the new guidance is implemented. At a minimum, the developing EPA guidance on the RPF approach will likely result in increased analysis, lower cleanup levels, and a lag time before background data are available to assess the larger list of chemicals.

2.3 Identification of Primary Exposure Pathways

2.3.1 Technical Approach

To ensure the research was focused on generating information relevant to assessing potential human risks from exposure to PAHs, an initial component of the project evaluated exposure pathways to determine which ones were primary risk drivers in the assessment of PAH-contaminated sites. This effort was completed based on guidance regarding default risk assessment approaches and on review of the primary literature.

2.3.2 Results

Applying standard EPA default exposure values, ingestion accounts for 73% and dermal exposure accounts for 23% of risks from direct contact with PAHs in soil. Risks from inhalation exposures are assumed by EPA to be negligible and therefore are not included as a topic of study in this project. Dermal exposures are specifically addressed in this research project, because they account for approximately one-fourth of exposures when applying default exposure assumptions and also because they become relatively more important if estimates of oral exposure are reduced. For example, if PAHs present in soil at a site were determined to have a relative bioavailability of 0.2 (i.e., the absorption of PAHs from soil was only one-fifth the absorption of PAHs from rodent chow), then oral exposure estimates would be reduced 5-fold and would be calculated to account for only 39% of risk from exposure to PAHs in soil at the site, and dermal exposures would account for the remaining 61% of risk. The weight of evidence indicates uptake of PAHs into the edible portion of plants is low and therefore dietary intake is not a significant exposure pathway.

2.3.3 Conclusions and Implications for Human Health Risk Assessment/Implementation

For PAHs in soil, direct-contact risks are dominated by the ingestion route, followed by the dermal route and, several orders of magnitude lower, the inhalation route. Because of the evidence indicating the dermal pathway contributes significantly to PAH exposure, dermal absorption of PAHs from soil is included in this research project.

2.4 Review of DoD Records of Decision

2.4.1 Technical Approach

The RODs database is maintained by EPA, and contains full-text RODs. An ROD provides the justification for the remedial action (treatment) chosen at a Superfund site. The RODs database was searched for information on reported PAH concentrations at DoD sites. Search criteria included all states for fiscal years 2009 and 2010. Each ROD was reviewed to find information on individual (as opposed to total) PAH concentrations in either surface or subsurface soil media. If the ROD contained PAH data, the data were extracted into tables. Sometimes both surface and subsurface soil concentrations were reported, and in those cases both types of data were extracted. Average and minimum concentrations were not always reported in the RODs; therefore, only maximum soil concentrations were used. The data were then screened using EPA Regional Screening Levels for residential exposure and screening levels modified to incorporate revised RPFs proposed by EPA.

2.4.2 Results

Data from 11 different RODs were identified for inclusion in this analysis, including DoD installations located in California, New York, New Jersey, Maryland, Florida, Virginia, Pennsylvania, Massachusetts, and Wyoming. When screened against current EPA residential soil screening criteria, BaP was the overwhelming driver for risks at the DoD sites that were included in this analysis. However, when proposed EPA residential soil screening criteria were used, the results indicated that dibenz[a,h]anthracene became the primary driver of human health risk because of the very conservative RPF value assigned to that PAH in the proposed RPF approach. More than 70% of the sites identified in this RODs search exceeded residential soil screening criteria for BaP, benz[a]anthracene, and benzo[b]fluoranthene, independent of whether current or proposed EPA RPF values were used.

2.4.3 Conclusions and Implications for Human Health Risk Assessment/Implementation

This analysis indicates that the current primary human health risk drivers are the larger PAHs (i.e., four- to six-ring) associated with cancer endpoints. Thus, BaP, benz[a]anthracene, benzo[b]fluoranthene, indeno[1,2,3-cd]pyrene, and dibenz[a,h]anthracene will likely be risk drivers for human health risk assessment at DoD sites.

2.5 Sources of PAHs at DoD Sites

2.5.1 Technical Approach

Two primary sources of information were used in this task. First, we reviewed DoD RODs to identify areas of PAH contamination, activities conducted in those locations, and other information relating to

sources of PAHs. Second, we interviewed DoD site personnel, including site historians, to identify activities conducted at DoD sites that could result in PAH contamination.

2.5.2 Results

During the review of DoD RODs, soil PAH data were collected for 18 exposure units at 11 sites. These data indicate that PAHs at DoD sites are found primarily in soils at landfills and general waste disposal sites; areas where petroleum products were stored, used, spilled, or disposed of; vehicle staging and cleaning areas; fire training facilities; and areas where ordnance, pesticides, and ores were stored. Total PAH concentrations ranged from 0.53 to 37,900 mg/kg, with most exposure units (all but five) exhibiting maximum total PAH concentrations below 40 mg/kg. Primary historical sources of PAHs at DoD sites are combustion by-products and petroleum products. Skeet shooting ranges are an emerging concern for DoD as a type of site where PAHs are present in soil.

2.5.3 Conclusions and Implications for Human Health Risk Assessment/Implementation

Based on this evaluation of potential sources of PAHs to DoD sites, it was concluded that source materials for the research should include skeet, a fuel, and a combustion source. In doing so, the range of likely sources to DoD sites would be captured in the study substrates, and the potential influence of different sources on the bioavailability of PAHs from soil could be characterized.

2.5.4 Overall Conclusions and Future Research Needs

This task confirmed that incidental ingestion and dermal contact are the exposure pathways of relevance for assessing potential risks from PAH-contaminated soils and that therefore the appropriate focus of our study was on factors controlling bioavailability of PAHs from soil. The task results were also important in focusing the work on the larger, four- to six-ring PAHs and for identifying the types of PAH source that are most prevalent at DoD sites.

3 Soil/PAH Interactions

For full details of this research, see Appendix B.

3.1 Problem Being Addressed/Background

PAH source materials and the way PAHs interact with soil components influence how tightly PAHs are bound to the soil matrix. The goals of this portion of the project were to characterize 1) the interactions between PAHs and soil components across different PAH sources and soil compositions and 2) to define the effect these interactions have on the solubility of PAHs from soil.

3.2 Technical Approach

To enable a rigorous and controlled evaluation of the effect of PAH source materials, soil compositions, and PAH concentration on the soil-chemical interactions of PAH, a series of artificial soils were constructed with a range of PAH concentrations, soil compositions, and different PAH source materials of specific relevance to DoD sites (skeet fragments, soot and fuel oil). As described in the project Work Plan, rather than attempt to select a representative soil from among the large array of soils available in the environment, and then adjust characteristics by amending the soil, this project used a standardized ASTM soil to serves as the "baseline." Then, to achieve a range of PAH concentrations and to provide insights into the impacts of soil composition on PAH bioavailability, soils were prepared with different proportions and types of its respective components (peat, clay, and sand), and spiked with different concentrations of PAH source materials (skeet, soot, fuel oil). Charcoal fines were also introduced into some of the soil to allow the project to investigate the effect of increasing the sorptive capacity of the soil. This ability to control these factors that might affect the soil-chemical interactions was identified as more important to the project goals than a less-controlled study design using contaminated field soils. Table 1 summarizes the matrix of soils generated for the study, including the soil compositions evaluated and the target PAH concentrations spiked to the soils.

The soils were weathered using wet-dry cycles in the laboratory to simulate the aging of soils in the environment. The weathering and aging procedure was developed by the Environmental Toxicology Branch lab at the U.S. Army Edgewood Chemical Biological Center, and has been used on previous SERDP projects for artificial weathering of soils (Kuperman et al. 2005, 2006, and 2009). The process involved exposing the spiked soils to alternating hydrating and air-drying cycles for two months at ambient environmental conditions in a greenhouse. Each of the spiked soils was spread to a thickness of 2.5–4 cm thick in an open glass container and hydrated with ASTM type I water to 60 percent of the soil's water holding capacity, then placed in the greenhouse to dry. After each week, each soil container was reweighed to determine moisture loss rehydrated to the original weight, and then thoroughly mixed by hand. This process continued on a weekly basis for eight weeks, after which the soils were air dried, disaggregated, sieved to <150 µm, and returned to UMC for testing. These weathered soils were used for several aspects of the project, including chemical evaluation and modeling of soil-PAH interactions,

providing data to inform selection of samples for *in vivo* testing, and as study substrates for the development of an initial PBET method.

Table 1. Test soil matrix for project research, including description of soil compositions evaluated, PAH source materials, and target PAH concentrations.

Test Soil Matrix (ASTM synthetic soil; 70% sand, 20% clay, 10% peat)						
PAH Sources	Synthetic soil	Synthetic soil with 2 percent charcoal fines	Synthetic soil with peat content reduced to 1%	Synthetic soil with kaolinite content reduced to 2%	Synthetic soil with kaolinite replaced with montmorillonite	Synthetic soil with peat replaced with humus
Solvent spike	0.1, 1.0, 10, and 100	0.1, 1.0, and 10	1.0	1.0	1.0	1.0
Soot	0.1, 1.0, 10, and 100	1.0	—	—	—	—
Skeet Particles	0.1, 1.0*, 10, and 100	1.0	—	—	—	—
Fuel Oil	0.1, 1.0*, 10	0.1, 1.0, and 10	1.0	1.0	—	—

Aqueous equilibrium partitioning tests were conducted on the weathered soils, the source materials, and the individual soil components. This enabled the calculation of partitioning constants (K_D) of PAHs to each of the soils and soil components, which served a number of purposes:

1. To quantify the importance of each component on overall PAH partitioning within the soil matrix.
2. To provide a basis for development of a predictive model that describes partitioning among the different sorption domains within an aqueous system.
3. To assess whether there were any sorptive interactions between soil components.
4. To evaluate the relationship between aqueous equilibrium partitioning of PAHs and PAH solubility under simulated physiological conditions of the human gastro-intestinal tract (PBET).
5. To provide data to inform the evaluation of the relationship between aqueous equilibrium partitioning and the RBA and dermal uptake of PAHs investigated under other tasks of this SERDP research effort.

3.3 Results

A summary of the log K_D and comparison against concentration in water for the different PAH sources and PAHs evaluated is provided in **Figure 2**. The results indicate that the source material (solvent, fuel oil, soot, or skeet) had a greater impact on the aqueous equilibrium partitioning behavior of PAHs than did soil characteristics or PAH concentrations. Soils containing skeet generally exhibited the highest K_D values, followed by soot-, fuel oil- and solvent-spiked soils. The difference in K_D values due to source material spanned over an order of magnitude, confirming the importance of PAH source material on PAH partitioning. Among all soil compositions, the addition of 2% charcoal to the soil had the largest enhancement of K_D. The mineral components of soil, on the other hand, generally had a very small impact on overall PAH partitioning.

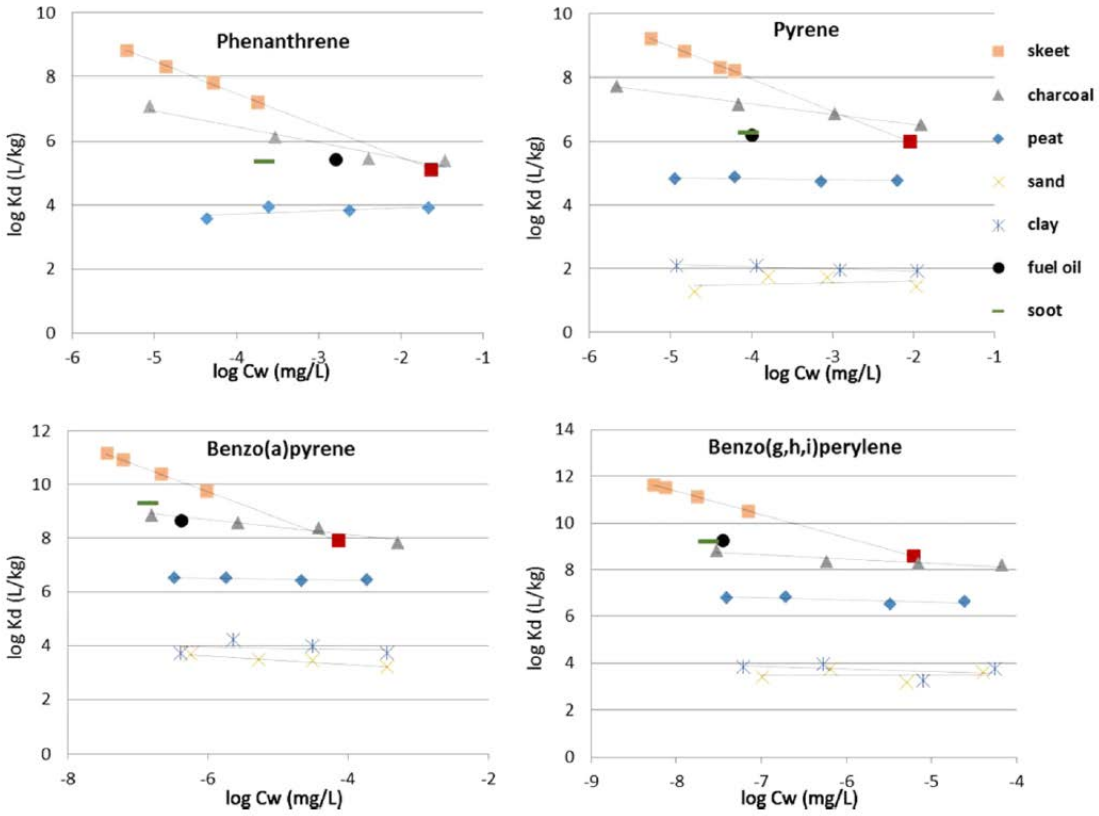


Figure 2. Comparison of measured partition coefficients (log K_D) for soil components and source materials. Dark red squares represent skeet measured with minimal depletion. Orange squares represent K_D measured with increasing depletion of PAHs from skeet. Soot and fuel oil were not evaluated for depletion (Xia et al. 2016).

PAH partitioning behavior in the weathered soils could not be predicted by traditional one carbon model (organic carbon) or two carbon partitioning models (organic carbon and BC) (**Figure 3**). Including independently measured partitioning behavior of the soil components and PAH sources allowed better prediction but still suffered from issues of interaction (fuel oil absorption into peat) and highly nonlinear

partitioning with depletion (for skeet). This highly nonlinear partitioning behavior with increasing depletion in skeet was investigated further, and a 2–3 orders of magnitude increase in partitioning was observed with only a small fraction loss of PAH from the skeets particles. This suggests that residual PAHs left in skeet following a small amount of PAH depletion are increasingly strongly bound.

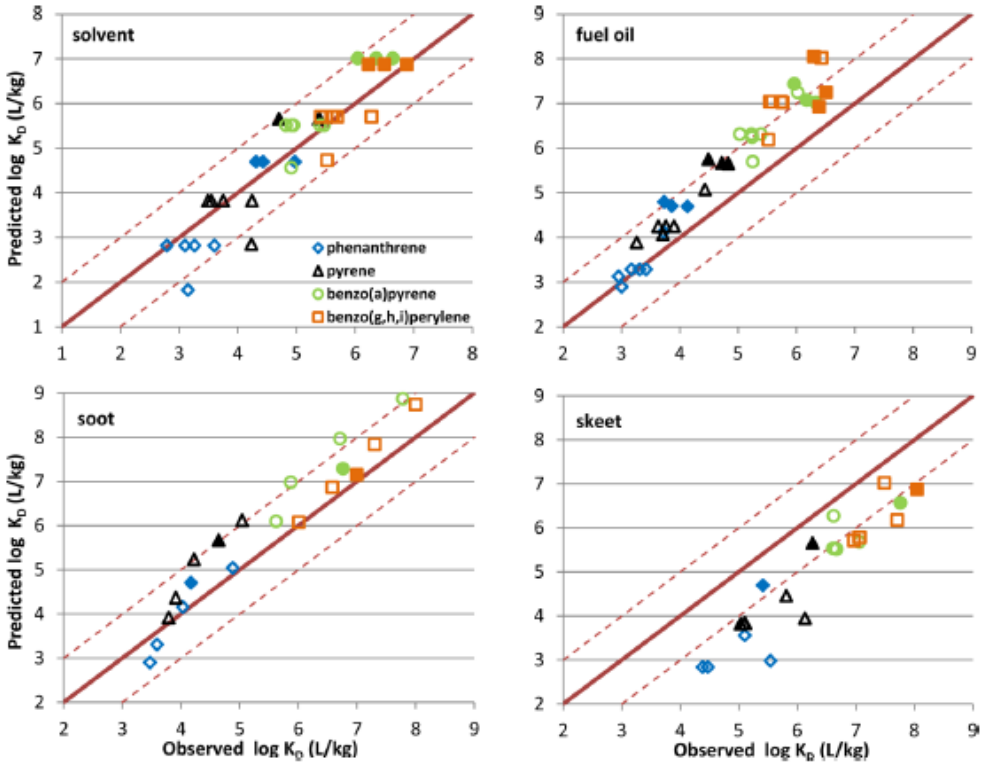


Figure 3. Comparison between predicted K_D from multidomain sorption model and observed K_D for soil with different source materials. Solid Fill for each shape represents soils with addition of 2% charcoal (Xia et al. 2016).

3.4 Implications for Human Health Risk Assessment/Implementation

The results of this research indicate that the sources of PAH contamination in soil play the dominant role in controlling PAH partitioning in PAH-impacted soils. Soil composition is also important, especially the presence of BC. These results have a number of implications:

1. Identifying the probable PAH sources to a contaminated soil can provide some indication of the strength of binding of PAHs in contaminated soils and, therefore, may serve as an indication of whether bioavailability assessment may be warranted at a Site.

2. The highly nonlinear partitioning with depletion for skeet observed suggests that initial weathering of coal-tar derived PAH sources in the field, such as may occur for skeet, may have an important influence on subsequent partitioning behavior for these sources in soil. Specifically, partitioning of PAHs out of the source material may decrease substantially after initial weathering effects in the natural environment.

3.5 Future Research Needs/Suggested Follow-On Research

Further investigation of the partitioning behavior of PAHs in soils weathered in the natural environment may provide data to confirm that partitioning behavior changes rapidly over time with the binding within the soil matrix becoming stronger, particularly for coal-tar derived PAH sources.To allow rigorous study of soil properties that influence PAH sorption to soil (and associated effects on bioavailability), this research utilized “constructed” soils that were subjected to accelerated “weathering” via 8 weeks of weekly wet-dry cycles. Research by others has demonstrated that such aging can affect the sequestration of PAH in soils, and there is some evidence (e.g., Northcott and Jones 2001) the sequestration process can be fastest in early phases of weathering. Further weathering would be required to fully account for the extensive weathering PAHs may endure in the field, and these effects may warrant future study, and particularly whether the strong influence of PAH source on partitioning behavior remains constant over time in soils from contaminated sites. However, the findings of this research indicate that PAH source is likely to be important at all sites, outweighing other site-specific considerations, thus informing site investigations.

4 Initial *In Vitro* Testing

For full details of this research, see Appendix C.

4.1 Background

The implementation of bioavailability adjustments at contaminated PAH sites would benefit from the development of a robust *in vitro* method that has a demonstrated ability to predict PAH bioavailability to humans across the whole range of possible PAH-impacted soil types. The research conducted in this project included two elements related to the development of *in vitro* methods to predict RBA. The initial *in vitro* testing used the study soils constructed at UMBC that formed the basis for the aqueous equilibrium partitioning tests. These initial tests are described here. A later task, described further below, involved the comparison of *in vitro* extraction testing of soils against the RBA values for those same soils that were derived from animal testing.

The goal of this initial *in vitro* extraction testing was to develop a PBET that yields reproducible and repeatable results in the laboratory. This test was used to evaluate PAH bioaccessibility from different PAH source materials and from the full suite of weathered test soils developed in earlier components of the research project. This research expands on the 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 PBET of the same soils.

4.2 Technical Approach

To select the most appropriate initial PBET to work with, an extensive literature review was conducted to assess the most important parameters controlling PAH desorption in the mammalian gastrointestinal (GI) tract. The initial *in vitro* extraction method developed for this research combines the end-over-end mixing method of Drexler and Brattin (2007) with a simplified PBET modified from a method developed for organic contaminants (Ruby et al. 2002) that included two “phases” (simulated gastric and simulated intestinal phases), a lipid sink, and ingredients that favored micelle formation. These methods (Drexler and Brattin 2007 and Ruby et al. 2002) are based on mammalian physiology, but rather than attempting to completely mimic the conditions of the gastrointestinal tract, they incorporate what are believed to be the most important factors for controlling the dissolution of PAHs from soil. The presence of micelles and a lipid sink have been previously shown to enhance PAH dissolution into GI fluid. However, our research identified that inclusion of these components in the PBET fluid also favors the formation of emulsions when attempting to extract the PAHs from the PBET fluid with solvents, especially under the vigorous agitation specified in the method. The emulsions prevented complete recovery of PAHs from the PBET solution. Therefore, a comprehensive method development study was conducted to optimize the extraction of PAHs from the PBET fluid.

To enable a rigorous and controlled evaluation of the effect of PAH source materials, soil characteristics, and soil compositions on PAH bioaccessibility, all the artificial soils described previously were extracted with the PBET method. This also provided the data to allow an evaluation of the relationship between PAH partitioning (as measured in earlier efforts of the project) and PAH bioaccessibility.

4.3 Results

As was found for PAH aqueous equilibrium partitioning (described above, in section 3), the initial PBET indicated that PAH source materials had a large impact on PAH bioaccessibility (**Figure 4,** expressed as individual PAHs and the same data expressed as BaP-equivalent concentrations). PAHs introduced to the soils in soot or skeet showed significantly lower solubility under the physiologic condition of these extractions. PAHs introduced to the soils in fuel oil demonstrated bioaccessibility in this system that was equal to or higher than that observed for PAHs spiked into soils with a solvent (**Figure 4**).

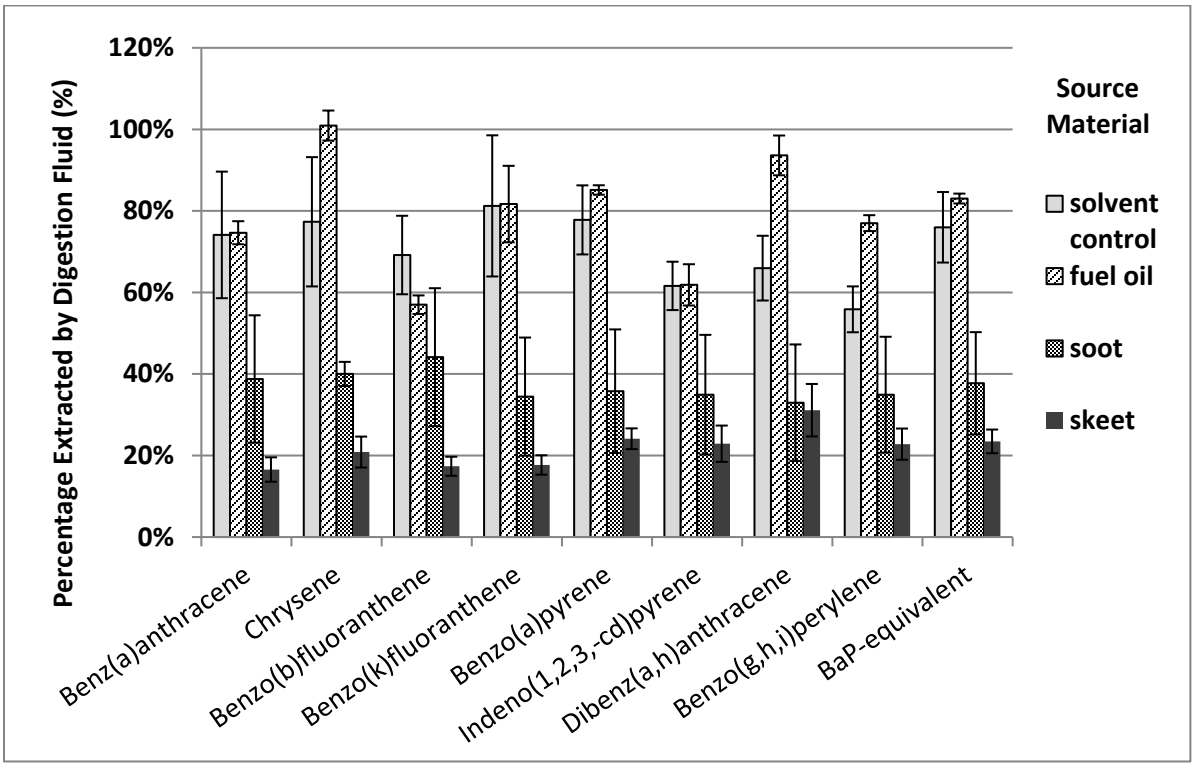


Figure 4. Effect of PAH source materials (control, fuel oil, soot, and skeet particles) on percentage of individual PAHs or BaP-equivalent concentration extracted by simulated gastrointestinal fluid from soils with 1mg/kg target BaP (n = 3, error bars represent standard error).[1]

[1] BaP-equivalent concentration was calculated using the RPF approach specified in Table 8 of U.S. EPA 1993. Data shown represent the BaP-equivalent concentration of PAHs in the simulated gastrointestinal fluid, relative to the BaP-equivalent concentration of PAHs in the soil before extraction.

The addition of charcoal to the soil also had a large effect on PAH bioaccessibility as it did with PAH partitioning (**Figure 5,** expressed as individual PAHs and the same data expressed as BaP-equivalent concentrations). This similarity between PAH partitioning and bioaccessibility is reflected in a strong inverse relationship between K_D and bioaccessibility in the weathered soils (**Figure 6**), confirming PAH partitioning has a strong effect on the observed PAH bioaccessibility.

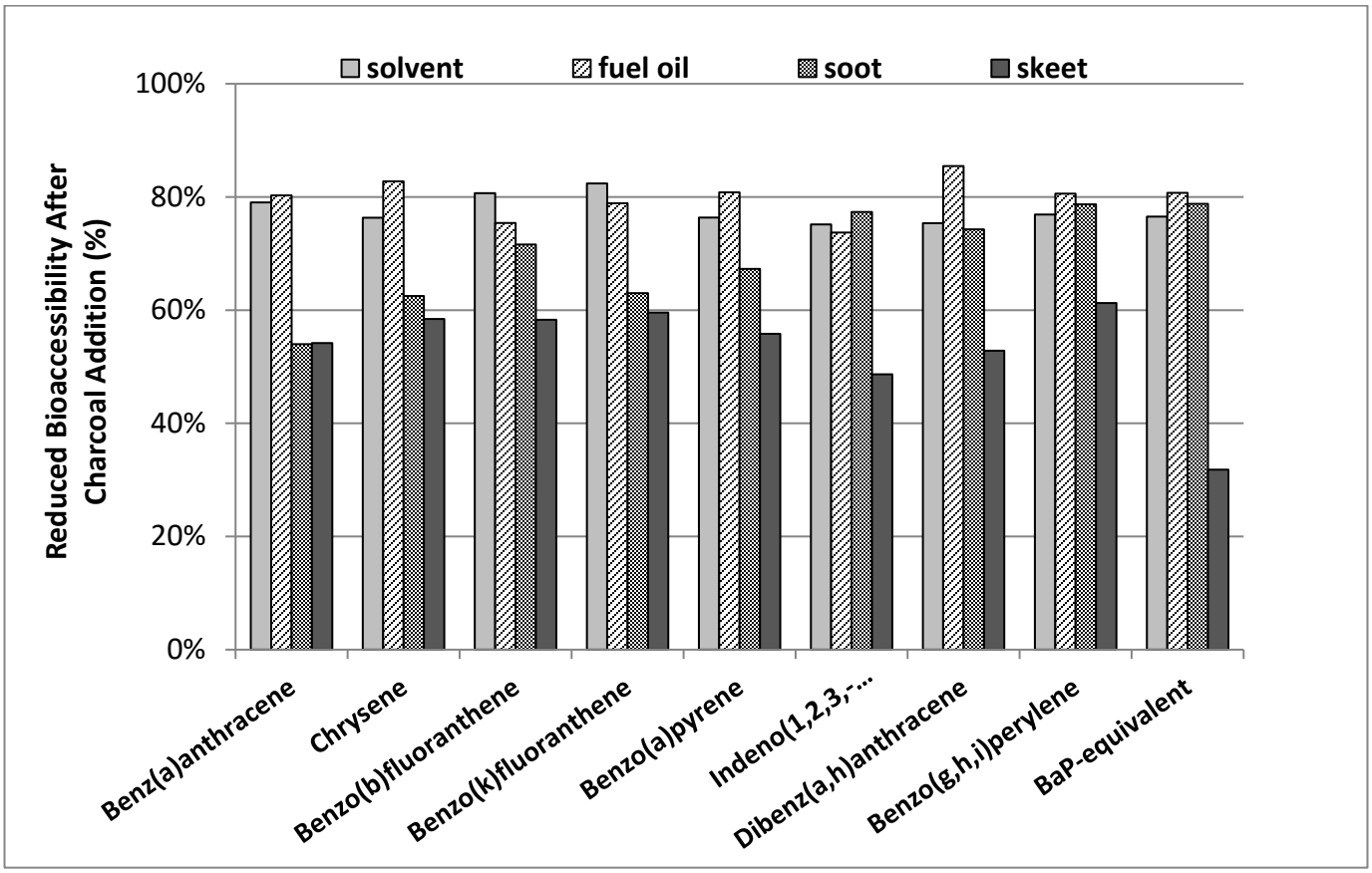


Figure 5. Reduction of PAH bioaccessibility after charcoal addition for soils spiked with different source materials (data shown represent soils spiked to achieve 1mg/kg target BaP). Data reported for individual PAHs and the BaP-equivalent concentration.

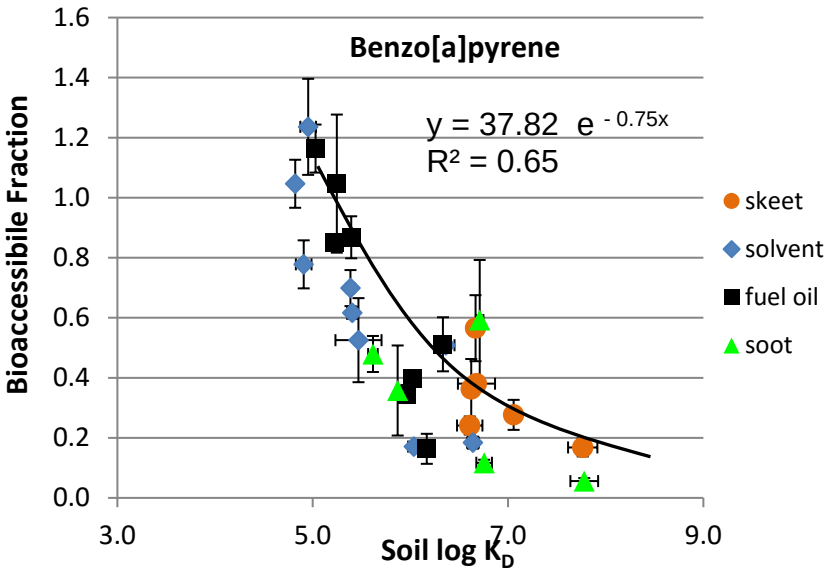


Figure 6. Correlation between measured soil K_D and PBET for benzo[a]pyrene. Error bars represent standard error.

4.4 Implications for Human Health Risk Assessment/Implementation

The results of this research indicate that PAH sources to soil play the dominant role in PAH bioaccessibility as measured with this PBET system. It also demonstrates that the PAH partitioning behavior is correlated with PAH bioaccessibility. These results have a number of implications:

1. Identifying the probable PAH sources for a contaminated soil can provide some indication of how tightly the PAHs are bound to the soil and the extent to which the PAHs can be solubilized in a PBET system. This information could be used to inform decisions of whether a bioavailability investigation is warranted at a Site.

2. Aqueous equilibrium partitioning of PAHs may also provide an indication of whether a bioavailability adjustment is likely to be significant at a Site.

3. The presence of charcoal substantially reduces PAH bioaccessibility, suggesting that further investigation may be warranted to better understand the possibility of BC amendments to reduce bioaccessibility (and ultimately bioavailability) for PAH-impacted soils.

4.5 Future Research Needs/Suggested Follow-On Research

The results indicate that aqueous equilibrium partitioning is predictive of PAH solubility under simulated physiological conditions. The relationships between PAH partitioning and oral bioavailability or dermal absorption need to be further evaluated. Importantly these relationships need to be tested against reliable *in vivo* methods. The results of these studies also indicate it may be useful to conduct *in vivo* investigations to confirm whether the use of BC amendments for soil remediation could reduce PAH absorption following incidental ingestion.

5 *In Vivo* Assessment of the RBA of PAHs from Soil

For full details of this research, see Appendix D.

5.1 Problem Addressed/Background

The objective of the *in vivo* studies is to develop an animal model for *in vivo* measurement of the RBA of PAHs from soil. Although there exist several reports in the literature regarding the measurement of PAH bioavailability, our review indicates that nearly all suffer from one or both of two primary deficiencies: 1) the method used to measure absorbed dose has not been demonstrated as being valid or 2) the doses of PAHs used in the studies are substantially larger than those associated with exposure from environmental media. These, and other limitations of the available literature, were published by our project research team (Ruby et al. 2015). Our research sought to identify a reliable measurement endpoint for PAH-bioavailability determination at environmentally-relevant PAH doses in a suitable animal model.

5.1.1 Technical Approach

The project Interim Report describes the various approaches evaluated for use in this component of the research project. Ultimately, our investigations identified evaluation of RBA based on concentrations of tritium-labeled BaP in blood as the most reliable measurement endpoint. Although other measurement endpoints, such as urinary metabolites or lipid concentrations, might in theory yield useful metrics of absorbed dose, those approaches did not result in reliable results in our investigation.

Using a rat research model, the RBA of PAHs from a library of soils was investigated. The soils were constructed and weathered in a manner that paralleled the processes used at UMBC for the development of constructed soils used in the assessment of soil-chemical interactions and which served as the basis for the initial PBET extractions described above. The differences between the soils constructed at UMBC and those developed at the University of Florida in Gainesville (UFl) were primarily the presence of tritium-labeled BaP and the smaller amount of each soil created at UFl. Contaminated soil samples were constructed using PAHs from three source materials—solvent, soot, and fuel oil—to which ^{3}H-BaP (total BaP concentrations of 1, 10, and 100 ppm) was added in a mixture of PAHs. The soils were weathered for eight weeks using weekly wet-dry cycles. To minimize enzyme induction that would affect BaP metabolism, each soil was administered as a single dose to rats, and blood samples were taken over six days. A schematic of this is provided in **Figure 7**. RBA of the BaP from soil was estimated by comparing the area under the curve (AUC) for ^{3}H concentration versus time in blood with the AUC observed from the same PAH mixture dosed in a food matrix (food was identified as the appropriate reference matrix for determining RBA because the studies upon which the toxicity values are based were conducted using doses of BaP in food).

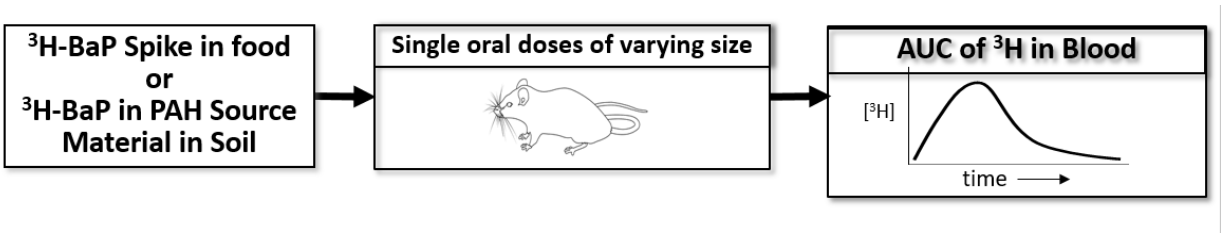


Figure 7. Simplified schematic of study design for in vivo testing

5.1.2 Results

Eighteen constructed soils were evaluated that varied in terms of total BaP concentration (1, 10, and 100 mg/kg), PAH source material (solvent, soot, or fuel oil), and soil characteristics ("baseline" soil; soil with reduced proportions of clay or peat, or the addition of charcoal fines) (Table 2). RBA values were estimated for each of the 15 soils that did not show evidence of ^{3}H-BaP decomposition. Results indicated that RBA values were generally independent of BaP dose when the source was solvent. When the source was soot, BaP RBA was decreased at the highest BaP dose, while RBA values generally increased with BaP dose when fuel oil was the source. The elimination of three soils from the study because of BaP degradation during weathering greatly reduced the comparisons that could be made in BaP RBA from soils with different compositions. The addition of charcoal to soil decreased BaP RBA by two-thirds or more regardless the PAH source. Reducing the clay or peat content of the soil had little or no effect on BaP RBA.

Table 2. Relative Oral Bioavailability of BaP from Soils

matrix	BaP, ppm (μg dose)	source material					
		solvent		soot		fuel oil	
		AUC[a]	RBA	AUC[a]	RBA	AUC[a]	RBA
baseline synthetic soil	1 (0.5)	455 ± 30	0.558 ± 0.082	442 ± 27	0.542 ± 0.074	529 ± 3	0.648 ± 0.008
	10 (5)	413 ± 8	0.505 ± 0.021	550 ± 26	0.673 ± 0.072	785 ± 97	0.961 ± 0.267
	100 (50)	426 ± 65	0.552 ± 0.078	192 ± 13	0.235 ± 0.035	869 ± 119	1.064 ± 0.325
baseline synthetic soil + charcoal	10 (5)	103 ± 16	0.126 ± 0.020	171 ± 4	0.210 ± 0.013	249 ± 24	0.305 ± 0.066
baseline synthetic soil–reduced clay	10 (5)	335 ± 49	0.410 ± 0.060	NA[b]	NA[b]	698 ± 40	0.854 ± 0.111
baseline synthetic soil–reduced peat	10 (5)	—[c]	—[c]	NA[b]	NA[b]	621 ± 70	0.761 ± 0.191
food	[d]	817 ± 28					

[a] nCi-h/ml; mean ± SD, N=5
[b] NA = not determined per experimental design; see Table 1
[c] Not evaluated due to evidence of BaP degradation during weathering
[d] Average of AUCs obtained from animals receiving BaP in food in concentrations of 1, 10, and 100 ppm, corresponding to doses of 0.5, 5, and 50 μg total BaP

5.1.3 Conclusions and Implications for Human Health Risk Assessment/Implementation

This research indicates that the source of PAH contamination in soils can have a significant effect on the bioavailability of PAHs from soil measured in this animal model. The addition of charcoal to the soil reduced RBA, but altering the amount of clay or peat in the soil had little impact on RBA. Soils for which soot was the source of PAHs demonstrated lower RBA, especially at higher PAH concentration. (Note, because the PAHs were introduced to the soil in soot, higher PAH concentration in the soot soils means that more soot was also in the soils, hence possibly providing greater sorption sites within the soil.) PAHs introduced to soils in fuel oil have the highest measured RBA, particularly at high concentrations where soil sorption sites may have been saturated.

The RBA values derived in this study are based on the total BaP radioactivity present in the soil doses given to the rats. At the conclusion of the weathering of soil, it was found that some radioactivity present in the soil was intractable to extraction using an acetone-dichloromethane with a modified Method 3550C. This method is among the most common for preparation of environmental soil samples for PAH analysis. Among the soils for which RBA was measured, there was no evidence from radiochromatography of biodegradation of the BaP or loss of the label; essentially all of the radioactivity in extracted material was confined to a single peak corresponding to BaP. This led to the conclusion that the radioactivity remaining in weathered soil after solvent extraction was sequestered, non-extractable BaP. The formation of non-extractable bound residues is a well-documented process during the weathering and ageing of PAHs and other organic compounds in soils.

Although the use of radiolabeled BaP in this study allows for a unique perspective on the behavior of BaP in the soil, the sequestration of BaP and other PAHs that occurs during weathering raises the question of the most relevant way to quantify administered dose—the total amount present in soil that is ingested or only the amount liberated by standard EPA methods? The RBA values above were derived based upon total BaP present in soil, which could be directly measured in this study because radiolabeled BaP was used. Other methods that fail to capture a sequestered fraction would result in different findings.

5.2 Overall Conclusions and Future Research Needs

The study here demonstrates that the RBA of BaP in soil can be significantly lower than the default assumption of 100% and that the specific RBA will depend significantly on the source of the BaP in the soil and to some extent on soil characteristics, especially the content of BC. Another significant finding pertains to the importance of how “total concentrations” of PAHs in soil are characterized and to the fact that the gastrointestinal tract of mammals may be more efficient at extracting BaP than some solvent extraction methods, at least for some soils.

Opportunities to expand the understanding of the RBA of PAHs from soil could include:

1. Investigations of site soils: Despite the many advantages of using laboratory weathered, constructed soil samples with radiolabeled BaP in exploring critical factors' influences bioavailability, it is possible that some differences from contaminated site soils may exist. Thus, it will be important to confirm the findings reported herein, to the extent possible, with PAH-contaminated site soils.

2. Sequestration processes: This research using radiolabeled BaP provided elucidation of sequestration processes that may not be achieved using unlabeled soils. Further characterization of the sequestration processes should be undertaken, and the use of labeled material may provide a unique tool for better understanding the complex nature of these processes.

3. Incorporating alternate comparison doses: All of the RBA values reported in this research are based on food as the relevant comparison. Food is the appropriate basis for comparison based on the existing cancer slope factor for BaP. EPA is considering changing the slope factor to be derived from toxicity studies based on gavage exposures. It may be appropriate to update the database and calculated RBA values for soil to reflect gavage doses as the basis of comparison.

6 *In Vitro* Method Development and Correlation to RBA Measured *In Vivo*

For full details of this research, see Appendix E.

6.1 Background

The goal of one of the final phases of the project was to assess the ability of bench-top extraction methods to predict the RBA as measured in animals. Although measurements conducted following animal exposures serve as a "gold standard" for assessing bioavailability, the significant costs and time requirements for animal testing can preclude the development of bioavailability information for incorporation into human health risk assessments on a site-specific basis. Additionally, as discovered in the development of the animal research model in this project, the detection limits imposed in animal research models preclude the use of animal research to develop RBA estimates for PAHs in soil at concentrations of relevance to decision making for contaminated sites; we found that a radiolabel was required to provide the necessary detection limits, a condition that cannot be met with field-contaminated soils. These are some of the many reasons that suggest the need for a robust, inexpensive laboratory method for estimating the RBA of PAHs at environmentally-relevant PAH concentrations (i.e., in the range of 0.1–100 mg/kg).

6.2 Technical Approach

This component of the research draws on information generated in earlier efforts of the project, most directly using information from the initial PBET extraction and the results from the *in vivo* RBA testing to inform the development of an *in vitro* extraction method that predicts RBA. Although physiologically-based methods have generally been the focus in the past for predicting the RBA of organic compounds in soil to humans, this effort investigated both the use of PBET systems and simpler extraction methods, under the concept that the effort was more focused on predicting the RBA results from animal testing than on ensuring that the extractions specifically mimic the mammalian gastrointestinal tract.

Five different types of extraction conditions were evaluated in this effort:

- Two mild-solvent extraction systems. One was "ScintiVerse" scintillation cocktail, which is largely comprised of C10-13-alkyl derivatives of benzene (83%) and the emulsifier/dispersant, dioctyl sulfosuccinate (13%). The other solvent system was n-butanol (99+% purity), selected based on peer-reviewed publications conducted with this method.

- A simplified physiologically-based extraction system. The approach was simplified from the initial PBET used in the earlier phases of this SERDP research project because the initial PBET identified problems with emulsions forming that confounded the analyses of the extraction fluid.

- EPA Method 3550C. This method is a standard EPA method for extracting nonvolatile and semivolatile organic compounds from solids, including soils. Although the EPA documentation for the method states that it "may not be as rigorous as other extraction methods," it is frequently used in characterizing concentrations of PAHs in soils from contaminated sites. As described above, our animal research revealed that the gastrointestinal tract of the rat can be as or more efficient at extracting BaP from soils, so we included this method to investigate whether a stable relationship existed between the chemical extraction method and the animal RBA results, i.e., whether the BaP concentration determined through Method 3550C can be used to predict, without any further analysis, the relevant, orally bioavailable concentration in soil.

- Solid phase absorption sink. Recent studies have indicated that *in vitro* extraction methods that include a solid matrix as an "infinite sink" can increase the bioaccessibility of PAHs. Therefore, to assess whether a solid phase sink affects the *in vitro* extraction results for our soils, and also whether it might simplify chemical analyses, our efforts include a trial with a silicone rod as an absorption sink.

All extractions were performed in replicate on the library of soils evaluated for RBA in rats. Average results from the extraction testing were compared to the RBA results from animal testing and evaluated for correlations in results.

6.3 Results

Initial trials with the extraction method that included the solid phase adsorption sink indicated poor recovery of PAHs and poor reproducibility, so no further investigation with this method was pursued.

Reproducibility in the results from the extraction testing with fluids indicated good reproducibility across replicates and good recovery of spikes. Bioaccessibility results were therefore averaged across replicates prior to comparison to RBA values.

Results of extraction of soils with ScintiVerse scintillation fluid showed little correlation with RBA (R^2 values less than 0.1), indicating that this fluid does not provide a good prediction of RBA.

Results of extraction of soils with the simplified PBET or extraction with n-butanol each showed good correlation with RBA as measured in rats, R^2 of 0.57 and 0.74, respectively. With the

simplified PBET method, one soil (the soil with fuel oil as the PAH source at a BaP concentration of 100 mg/kg) appears to be an outlier. With that soil removed from the evaluation, the R^2 value increases to 0.69.

Results of extraction of soils with EPA Method 3550C demonstrated lower correlation with the animal RBA results ($R^2 = 0.43$). In some instances, the 3550C results over predict RBA by over 3-fold, while in other instances the extraction method under predicts RBA by nearly 2-fold. The only patterns that appear to emerge in the relationship between 3550C results and RBA are that 3550C results are most likely to over-predict RBA at the highest concentrations of BaP (100 mg/kg) and that the percentage recovery of total BaP by 3550C increases with concentration. The over prediction of RBA by 3550C was greatest for the soils with charcoal added, where the ratio of 3550C/RBA ranged from 2.1 to 3.0.

Figure 8 presents the "in vitro to in vivo correlation" (IVIVC) for these extraction methods.

RBA results were also compared to the K_D values for similarly prepared soils evaluated in the earlier phases of this research project. Because the K_D values were calculated based on concentrations in soils as measured by EPA Method 3550C, the comparison of K_D against RBA was performed both with the K_d values as reported in the investigation and with K_D calculated based on the target concentration of BaP spiked into the soils. Little relation could be found between K_d and RBA for K_D values calculated either on measured or target spike concentrations of BaP.

6.4 Conclusions and Implications for Human Health Risk

6.4.1 Assessment/Implementation

The research conducted under this SERDP-funded project provides data on the most extensive library of PAH-contaminated soils conducted to data, reflecting diverse PAH sources, several soil characteristics, and a range of PAH concentrations. The results of this assessment of in vitro and in vivo correlation (IVIVC) between bioavailability measurements demonstrate that extraction methods exist that reasonably predict RBA as measured in rats. The two extraction methods that show the best predictive relationship are a physiologically-based method and a simple solvent extraction using n-butanol. The ability of these methods to predict the *in vivo* results holds across most of the diverse soils tested, indicating that the extraction methods likely capture the PAHs that are liberated from the soils during transit through the gastrointestinal tract of the animals. These results form a strong basis for suggesting *in vitro* methods to estimate the RBA of BaP from soils at contaminated sites.

6.4.2 Overall Conclusions and Future Research Needs

Radiolabeling was used in the analysis of extraction tests because it was available for the soils investigated and it allowed for more efficient analyses. It also allowed for quantitation of BaP that was *not* extracted so that total mass of BaP could be followed. However analytical detection limits should be adequate to characterize the concentrations of BaP in these extracts without the label. It should be confirmed that conventional chemical methods yield the same results in these *in vitro* extraction methods as was achieved using the radiolabel.

In both instances, for PBET and n-butanol, the *in vitro* extraction slightly under predicts RBA. Although the correlation between *in vitro* and *in vivo* results is an important component in assessing a predictive relation, our experience with regulatory agencies is that they may be more comfortable with methods that over predict. Therefore, it may be prudent to make slight adjustments to the methods used in this research to identify a method that provides slightly more aggressive dissolution without detrimental effects to the correlation.

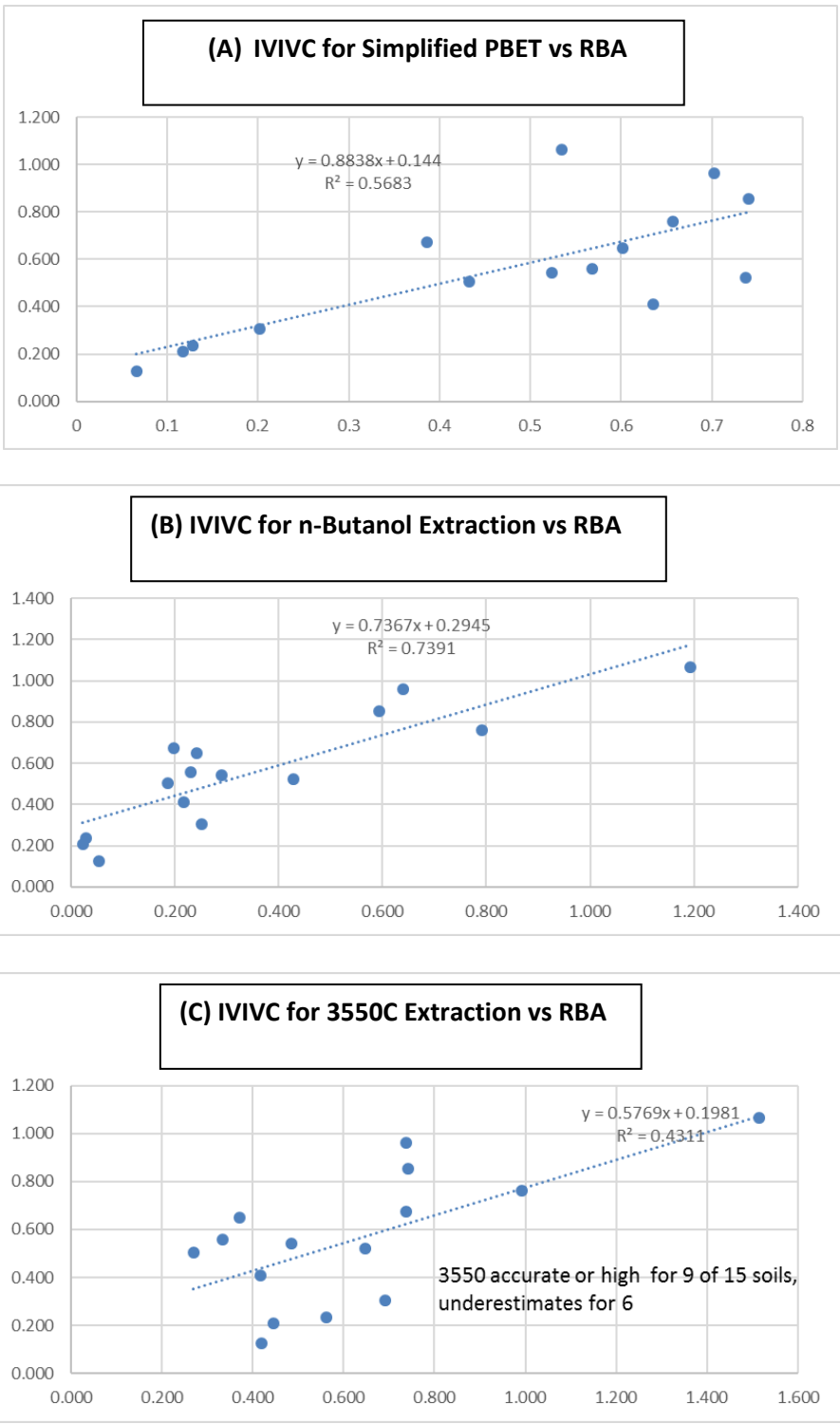


Figure 8. Figures A, B, and C present the "in vitro to in vivo correlation" (IVIVC) for three extraction conditions tested. In all cases, the results of the extraction tests are presented on the x-axis, and the RBA values, as measured in rats, presented on the y-axis. Both x and y axes are reported as the fraction of the total BaP from the soil (e.g., a value of 1 means 100%).

7 Evaluation of Dermal Absorption of BaP from Soil

For full details of this research, see Appendix F.

7.1 Problem being addressed/Background

Soil cleanup standards and assessment of human health risks at contaminated sites are based in part on predicted human exposure to soil contaminants, including from direct skin contact. Available investigations of dermal absorption from soil are relatively sparse and have been conducted with a variety of different methods, many of which fail to account for important physical and chemical drivers of skin permeation. To improve understanding of the soil-dermal exposure pathway and the influence of soil characteristics on skin permeation, *in vitro* assessments of BaP absorption through human epidermis were conducted.

7.2 Technical Approach

In vitro assessments of ^{14}C-benzo[a]pyrene (BaP) absorption through human epidermis were conducted with the sub-63µm fraction of four test soils containing different amounts of organic and black carbon.

Table 3. Soils, source, and chemical characterization for soils used in assessment of dermal absorption of BaP. Chemical characterization provided by UMBC.

Soil	Soil Source	TOC (%) §	Black Carbon (%) §
CSU	Colorado agricultural soil	0.99	0.14
ISU	Iowa agricultural soil	3.13	0.23
MTSS	Montana soil near smelter	3.91	1.23
Yolo	Yolo County, California soil	0.97	0.09

The research design included:

- Small particles that adhere to skin (<63 µm sieve fraction)
- Varying total organic carbon (TOC) content (1–4%) and varying BC content (0.1–1.2%) (Table 3)
- Yolo soil chosen because EPA default absorption for BaP (13%) was measured using this soil

Soils were artificially weathered for eight weeks and applied to heat-separated epidermis at nominal BaP concentrations of 3 and 10 mg/kg for 8 or 24 h. Experiments were also conducted for 24 h with unweathered soils and with BaP deposited onto skin from acetone at a comparable chemical load.

7.3 Any Deviations from the Work Outlined in the Interim Report

The original plan called for experiments to be conducted with both BaP and fluoranthene. The ^{14}C-fluoranthene received from the vendor failed testing for required purity, which significantly delayed and limited the corresponding experiments. Ultimately the limited fluoranthene results were inadequate in number and scope to logically compliment the BaP experiments and were excluded from the publication. The original plan was also for use of a target high-end concentration of 30 ppm BaP in soil. Concern over potential soil supersaturation led to reduction of that value to 10 ppm.

7.4 Results

A total of 126 trials were attempted, with four excluded due to probable membrane failures (n = 2; both from weathered soil trials) or having total radioactive recoveries below 50% (n = 2; both from acetone vehicle trials). The average total radioactivity recovered was 101% (83–117% range) for weathered soil, 89% (85–93%) for unweathered soil, and 80% (61–98%) for acetone.

For weathered soils, no significant differences were seen among the four soil types for any of the endpoints measured (**Figure 9**). Absorption was independent of soil type, the mass in the receptor fluid was proportional to exposure duration but independent of concentration, and the mass recovered in the skin after washing was proportional to concentration and independent of exposure time.

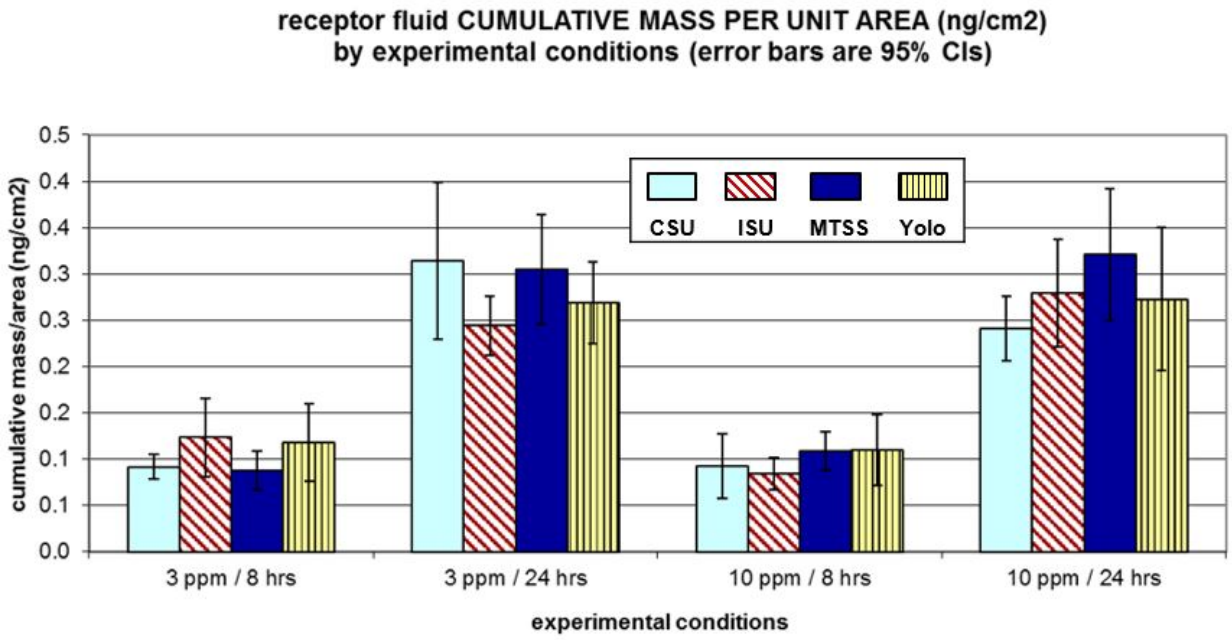


Figure 9. Dermal absorption of benzo[a]pyrene from weathered soils.

Results from the weathered and unweathered soils were essentially similar, although in 10 mg/kg trials, mass of BaP recovered in washed skin per area of skin (M_{sk}/A) was larger in unweathered vs. weathered soils by a statistically significant difference.

Although the cumulative absorption varied with time, the measured flux remained the same at the different time points measured. It is plausible that this was caused by soil particles left on the skin after washing.

Compared with the experiments on weathered and unweathered soil at similar BaP load (3-mg/kg concentration), M_{sk}/A for BaP delivered in acetone was between one and two orders of magnitude greater (**Figure 10**). After 24 h, mass of BaP recovered in receptor fluid per area of skin (M_{rf}/A) was greater from the acetone-delivery experiments by approximately one order of magnitude compared to soil experiments at both concentrations.

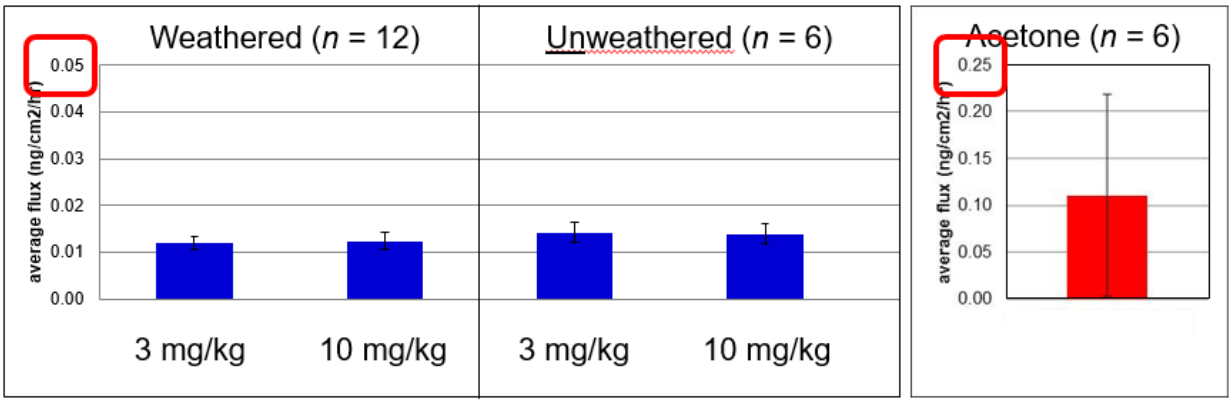


Figure 10. Absorption of benzo[a]pyrene from soils was much lower than from solvent

7.5 Conclusions/Benefits

Taken together the results of this study suggest that BaP concentrations tested, although low, may have exceeded the sorbent capacity of the soils used in this study and that the quantity of BaP measured in the skin was primarily attributable to residual soil on the skin surface that was not removed by the washing step. While alternative hypotheses can explain individual observations (e.g., mass of BaP in the skin is proportional to the soil concentrations below saturation), we were unable to identify other explanations that were consistent with all primary observations. For example, BaP soil concentrations less than saturation can explain the observed concentration dependence of the mass of BaP in the skin but not the absence of a concentration effect in the mass of BaP in the receptor fluid.

Among the factors this research was designed to investigate was the influence of BC content on the binding of BaP to the soil and the associated effect on dermal absorption. Other components of the research conducted under this project (i.e., investigation of equilibrium partitioning of PAHs from soil, or RBA) showed the addition of 2% charcoal to the soil matrix resulted in increased binding of the PAHs to soil and lower bioavailability. Our findings in the study of dermal absorption are in contrast to this observation of the impact of added charcoal but not necessarily contradictory.

The four soils used in the evaluation of dermal BaP absorption were all field soils spiked with BaP in the lab. Soils were characterized for BC content, which ranged from 0.9 to 1.2%. It is possible the BC content of these soils was too low to influence the binding of BaP (the soils constructed at UMBC and UFl contained 2% added charcoal). More likely, the lack of observed effect of BC on dermal absorption in this limited study was due to the use of field soils where the full nature of the BC in the soils was not characterized. The high carbon soil in the dermal absorption study was from a smelter site, where the carbon could have been influenced by many factors, whereas the BC added to soils for the equilibrium partitioning, bioavailability, and bioaccessibility studies was a laboratory-grade, cleaned activated charcoal (S79959, Fisher

Scientific), a form of BC that is likely more highly porous than BC generally found in soils. Future controlled studies of the influence of the source and concentration of BC on dermal absorption could elucidate these effects.

7.6 Implications for Human Health Risk Assessment/Implementation

BaP is the index chemical for PAH risk assessment. In 2013, EPA proposed, for the first time, a dermal carcinogenic slope factor for BaP. That value is undergoing further review but will likely substantially increase the importance of dermal exposures in evaluation of risks from PAH contaminated sites. Review of the cancer bioassay studies upon which the proposed dermal slope factor was derived suggests that estimates of cancer risk should be based on absorbed rather than exposed dose, making the results of the experiments reported here directly relevant. The presumed primacy of the Wester et al. (1990) *in vivo* soil experiment results is called into question by their similarity to *in vitro* acetone-deposition results reported here. Evidence is also presented for rapid adherence of a portion of soil-borne contaminant. Some of that rapidly adhering mass may be on fine particles that are not easily removed. A health-protective assumption would be that non-removable particle-bound material is functionally equivalent to the same mass of neat compound in the outer layers of skin.

Results of this study also highlight the importance of soil concentration relative to sorption capacity. Soils may be weak sorbents, with low mg/kg levels of BaP representing soil saturation. BaP is routinely found in soils at or above concentrations used in this study, suggesting soils in the environment might exist at super-saturated conditions with obvious implications for transfer to skin. However, the apparent saturation limit of soils might be influenced by both the duration of weathering and the source of the BaP, which were not evaluated in this preliminary investigation. Sorption capacity might increase if soil is amended with partitioning phases in the form of soot or other carbonaceous material. Soils in experiments reported here were spiked with pure chemicals using a volatile solvent, a procedure that might have contributed to saturation exceedance. Nevertheless, results reported here do suggest that uptake from saturated soil is slower than uptake from a similar amount of BaP deposited from acetone.

7.7 Future Research Needs/Suggested Follow-On Research

This research highlights the potential for soil saturation at relatively low soil concentrations of spiked PAHs. The solubility of chemicals in soil can be assessed experimentally. If vapor pressure of a soil contaminant is adequate, then thermodynamic activity can be assessed by measuring contaminant concentration in the head space in equilibrium with the contaminated soil compared with the pure contaminant. For less volatile compounds such as BaP, contaminant uptake into a sorbent material from soil compared with the neat contaminant or differential scanning calorimetry could be used. Because soil saturation was not anticipated, the scope of the present study did not include such measurements. It would be useful to include soil saturation

measurements in future studies of contaminated soils. Based on the results of other components of the project research, it is important to assess the influence of source materials of PAHs in soil on the dermal absorption. It is likely that the factors that affect aqueous partitioning, physiologically-based extraction testing, and oral bioavailability of PAHs will also affect partitioning from soil to skin. Since source material has been identified as the primary controlling factor for these other measurements evaluated in other tasks, it is likely that source material will be important to controlling dermal absorption of PAHs from soil. Finally, for reasons described above, in section 7.5, controlled studies of the influence of the type and concentration of BC on dermal absorption could elucidate factors that are important to understanding risk following human contact with soils.

Appendix A

Identification of Relevant PAH Sources, Mixtures, and Exposure Pathways

This task was completed prior to submission of the Interim Report. Therefore, this appendix presents the information as presented in the Interim Report. It is reproduced here in the final report to ensure that all major pieces of work product are reflected in this Final Project Report.

3 TASK: IDENTIFICATION OF RELEVANT PAH SOURCES, MIXTURES, AND EXPOSURE PATHWAYS

3.1.1 Objective

The objective of this initial task is to provide background information that is important for focusing the direction of the project research in a manner to ensure applicability to PAH contamination at DoD sites. Specifically, the effort is intended to ensure that subsequent research tasks are focused on the specific PAHs that drive remedial decisions, that the soil ingestion pathway is, in fact, an important exposure pathways for sites contaminated with PAHs, and finally, to provide insights into the types of contaminant sources responsible for introducing PAHs into contaminated DoD facilities.

This task has been completed, and findings were reported in the project Work Plan that was finalized in March 2012. A summary is provided herein, and Appendix A to this report includes the full complement of technical information that was communicated in the Work Plan.

The work performed under this task provides perspectives on current activities being conducted by EPA with regard to characterizing toxicity of PAHs, and provides a retrospective review of Records of Decision (RODs) to assess which specific PAHs have previously driven remedial decisions at DoD sites. These two components are then used together to provide an understanding of which PAHs are likely to drive remedial decisions in the future, and thereby to identify the PAHs of primary interest for this research project. The RODs were also evaluated for information on sources of PAH contamination to soils at DoD sites. Together with information gained from conversations with risk assessors from various military branches, this provided a basis for selecting source materials of PAHs for inclusion in the study.

3.1.2 Review of Regulatory Toxicology of PAHs

When present in environmental media, PAHs invariably exist as mixtures. There are hundreds of PAHs potentially present as components of these mixtures (USEPA 2010), and to the extent possible, the regulatory approach to health assessment of PAHs considers the interactions among the individual PAHs in the mixture. Although there is limited dose-response information for some sources of PAHs (e.g., coke oven emissions, creosote, diesel engine exhaust, and coal tar preparations), PAHs in environmental media are highly variable and, in most cases, are too dissimilar to the specific mixtures for which dose-response data are available to be directly applicable. In this case, EPA guidance recommends evaluation of the individual components of the mixture and adding together the risks from each component (USEPA 2000).

For PAH mixtures, EPA and other regulatory agencies (e.g., California Environmental Protection Agency [CalEPA]) have used relative potency factors (RPFs) to assess the toxicity of individual PAHs. Using RPFs, the doses of individual components acting through similar mechanisms of action are summed after scaling to the relative potency of an index chemical in the group for which the most complete dose-response characterization is available. For PAHs, benzo[a]pyrene

Based on this evaluation of potential sources of PAHs to DoD sites, it was concluded that source materials for the research should include skeet, a fuel, and a combustion source. In doing so, the range of likely sources to DoD sites would be captured in the study substrates, and the potential influence of different sources on the bioavailability of PAHs from soil could be characterized.

3.2 CONCLUSIONS DERIVED FROM THIS TASK

This task confirmed that incidental ingestion and dermal contact are the exposure pathways of relevance for assessing potential risks from PAH-contaminated soils, and should therefore remain the focus of our study on factors controlling bioavailability of PAHs from soil. The task results were also important in focusing the work on the larger, four- to six-ring PAHs, and for identifying the types of PAH source that are most prevalent at DoD sites and should be the focus of this study.

(BaP) has been selected as the index chemical, because: 1) it is typically present in environmental settings where PAHs are detected; 2) it has the most robust toxicological data set among the PAHs, and a formal dose-response assessment has been conducted; 3) there is a large database of *in vivo* and *in vitro* studies directly comparing the potency of various PAHs with BaP; and 4) it is one of the most potent carcinogens of the PAHs tested.

Several modifications have been made to the approaches used by regulatory agencies for addressing the potential toxicity following human exposure to PAHs. Table 3-1 provides a summary of activities regarding PAH regulation by EPA and CalEPA from 1992 to the present. Among the changes that are most relevant to assessing remedial decisions at DoD sites are the implications of a RPF approach proposed by EPA in 2010. Prior to 2010, EPA provided RPF values for cancer risks for seven individual PAHs. These were order-of-magnitude estimates of potency relative to BaP, with no compounds ranked higher in toxicity than BaP. Under the proposed approach, EPA provides RPF values for 25 individual PAHs, spanning values of potency relative to BaP from 0.009 to 60, with six individual PAHs having potency values higher than BaP. These are benzo[c]fluorene, benz[j]aceanthrylene, benz[l]aceanthrylene, dibenz[a,c]anthracene, dibenz[a,h]anthracene, and dibenzo[a,l]pyrene, with RPF values of 20, 60, 5, 4, 10, and 30, respectively. Table 3-1 provides a comparison of the RPFs for PAHs under the current paradigm and under the revised approach that was proposed by EPA in 2010. The implications of the different RPF approaches are illustrated in Table 3-3, which provides a summary of the health-based residential soil screening levels for the individual PAHs using the different RPFs. For comparison, Table 3-3 also provides information on background concentrations of PAHs in soil for rural and urban locations. In several instances, the background concentrations are higher than the risk-based screening levels, which have important implications for conducting risk assessment at “contaminated” sites.

Table 3-1. Regulatory history of PAHs in the United States

Year	Event	Reference
1992	EPA derives current benzo[a]pyrene oral cancer slope factor of 7.3 per mg/kg-day	USEPA 1992
1993	EPA releases *Provisional Guidance for Quantitative Risk Assessment of PAHs*	USEPA 1993
1993	CalEPA derives inhalation unit risk for benzo[a]pyrene of 1.1×10^{-3} per µg/m^3	OEHHA 1993
1993	CalEPA derives relative potency factors for PAHs	OEHHA 1993
2004	EPA releases external peer review dravt of the *Toxicological Review of Naphthalene* with revised inhalation unit risk of 1×10^{-4} per µg/m^3	USEPA 2004
2004	CalEPA derives inhalation unit risk for naphthalene of 3.4×10^{-5} per µg/m^3	OEHHA 2004
2010	CalEPA derives revised oral cancer slope factor for benzo[a]pyrene of 2.9 per mg/kg-day	OEHHA 2010
2010	EPA releases external review draft of *Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures*	USEPA 2010
2012	Target date for finalization of EPA's *Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures*	IRIS Track[a]
2012	Target date for external peer review and finalization of EPA's updated *Toxicological Review of Benzo[a]pyrene*	IRIS Track[a]

[a] EPA IRIS Track website: http://cfpub.epa.gov/ncea/iristrac/

Notes:

CalEPA = California Environmental Protection Agency

EPA = U.S. Environmental Protection Agency

Table 3-2. Comparison of relative potency factors for polycyclic aromatic hydrocarbons

	USEPA 1993[a]	CalEPA (2009)[b]	USEPA 2010 Proposed[c]
Anthanthrene	--	--	0.4
Benzo(a)pyrene	1	1	1
Benzo[a]anthracene	0.1	0.1	0.2
Benz[b,c]aceanthrylene, 11H-	--	--	0.05
Benzo[b]fluoranthene	0.1	0.1	0.8
Benzo[c]fluorene	--	--	20
Benz[e]aceanthrylene	--	--	0.8
Benzo[g,h,i]perylene	--	--	0.009
Benz[j]aceanthrylene	--	--	60
Benzo[j]fluoranthene	--	0.1	0.3
Benzo[k]fluoranthene	0.01	0.1	0.03
Benz[l]aceanthrylene	--	--	5
Chrysene	0.001	0.01	0.1
Cyclopenta[c,d]pyrene	--	--	0.4
Cyclopenta[d,e,f]chrysene, 4H-	--	--	0.3
Dibenzo[a,e]fluoranthene	--	--	0.9
Dibenz[a,j]acridine	--	0.1	--
Dibenz[a,h]acridine	--	0.1	--
7H-dibenzo(c,g)carbazole	--	1	--
Dibenzo[a,e]pyrene	--	1	0.4
Dibenz[a,c]anthracene	--	--	4
Dibenz[a,h]anthracene	1	0.34	10
Dibenzo[a,h]pyrene	--	10	0.9
Dibenzo[a,i]pyrene	--	10	0.6
Dibenzo[a,l]pyrene	--	10	30
Fluoranthene	--	--	0.08
Indeno[1,2,3-c,d]pyrene	0.1	0.1	0.07
Naphtho[2,3-e]pyrene	--	--	0.3
5-methylchrysene	--	1	--
1-nitropyrene	--	0.1	--
4-nitropyrene	--	0.1	--
1,6-dinotropyrene	--	10	--
1,8-dinotropyrene	--	1	--
6-nitrochrysene	--	10	--
2-nitrofluorene	--	0.01	--
7,12-dimethylbenzanthracene	--	21	--
3-methylcholanthrene	--	1.8	--
5-nitroacenaphthene	--	0.01	--

Notes:

[c] USEPA. 2010. Development of a relative potency factor (RPF) approach for polycyclic aromatic hydrocarbon (PAH) mixtures. Draft. February. EPA/635/R-08/012A. U.S. Environmental Protection Agency, Washington D.C.

[a] USEPA. 1993. Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons. July. EPA/600/R-93/089. U.S. Environmental Protection Agency, Washinton D.C.

[b] CalEPA. 2009. Technical Support Document for Describing Available Cancer Potency Factors: Methodologies for derivation, listing of available values, and adjustments to allow for early life stage exposures. Appendix A: Hot Spots Unit Risk and Cancer Potency Values. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. May.
CalEPA RPFs calculated by dividing the oral slope factor listed for each chemical by the oral slope factor for benzo[a]pyrene.

Table 3-3. Polycyclic aromatic hydrocarbon soil background concentrations and health-based screening levels derived using relative potency factors (µg/kg)[a]

	Soil Background Concentrations[b]		Residential Soil Screening Level[c]	
PAH	Rural	Urban	Based on USEPA 1993	Based on USEPA 2010
Anthanthrene	NA	NA		38
Benzo(a)pyrene	2–1,300	165–220	15	15
Benz[a]anthracene	5–20	169–59,000	150	75
Benz[b,c]aceanthrylene, 11H-	NA	NA	--	300
Benzo[b]fluoranthene	20–30	15,000–62,000	150	19
Benzo[c]fluorene	NA	NA	--	0.75
Benz[e]aceanthrylene	NA	NA	--	19
Benzo[g,h,i]perylene	10–70	900–47,000	--	1,667
Benz[j]aceanthrylene	NA	NA	--	0.25
Benzo[j]fluoranthene	NA	NA	--	50
Benzo[k]fluoranthene	10–110	300–26,000	1,500	500
Benz[l]aceanthrylene	NA	NA	--	3.0
Chrysene	38.3	251–640	15,000	150
Cyclopenta[c,d]pyrene	NA	NA	--	38
Cyclopenta[d,e,f]chrysene, 4H-	NA	NA	--	50
Dibenzo[a,e]fluoranthene	NA	NA	--	17
Dibenzo[a,e]pyrene	NA	NA	--	38
Dibenz[a,c]anthracene	NA	NA	--	3.8
Dibenz[a,h]anthracene	NA	NA	15	1.5
Dibenzo[a,h]pyrene	NA	NA	--	17
Dibenzo[a,i]pyrene	NA	NA	--	25
Dibenzo[a,l]pyrene	NA	NA	--	0.5
Fluoranthene	0.3–40	200–166,000	--	188
Indeno[1,2,3-c,d]pyrene	10–15	8,000–61,000	150	214
Naphtho[2,3-e]pyrene	NA	NA	--	50

[a]EPA Regional Screening Levels available at: http://www.epa.gov/reg3hwmd/risk/human/rb-concentration_table/Generic_Tables/index.htm

[b]Background soil concentrations of polycyclic aromatic hydrocarbons measured in the United States (ATSDR 1995).

[c]Residential soil screening levels for PAHs that are based on the a carcinogenic relative potency factor. Screening levels based on USEPA 1993 are taken directly from EPA Regional Screening Levels table. Screening levels based on USEPA 2010a were calculated by dividing the benzo[a]pyrene screening level by the proposed relative potency factor.

Notes:

NA = background data not available

Evolving approaches to health assessment of PAHs will affect how environmental assessment is conducted and cleanup levels are derived in the future. The EPA's draft RPF approach, when finalized and incorporated into environmental policy, will potentially raise significant issues for environmental assessment of PAHs, including:

- The need for new EPA-approved analytical methods for analysis of the 18 PAHs with new RPF values, and detection limits sufficiently low to detect concentrations below soil screening levels.

- Requirements for tracking of additional analytes that have, to date, not generally been tracked in environmental characterizations.

- Treatment of undetected PAHs could provide challenges and conceptually result in site risks being driven by chemicals that are not detected in environmental samples (e.g., if concentrations are assumed to be equal to one-half of the analytical detection limit).

- Changes to the RPFs could result in significant reduction to soil screening levels for PAHs that currently have screening levels (for all PAHs with the exception of indeno[1,2,3-cd]pyrene) (Table 3-2). Current residential screening levels range from 15 to 15,000 µg/kg, and application of the new RPFs would result in screening levels from 1.5 to 500 µg/kg for these PAHs. When the 18 new PAHs with screening levels are included, residential soils screening levels would range from 0.25 to 1,667 µg/kg.

- In the absence of background data for the 18 new PAHs with RPFs, it will be impossible to assess whether the presence of these PAHs on a site is attributable to site-related activities or to normal background sources (Table 3-3).

The proposed modifications to PAH health assessment are fundamentally consistent with the long-standing regulatory approach for assessing mixtures: using RPFs to evaluate the carcinogenic risk of PAHs in mixtures and evaluating noncancer effects on a chemical-specific basis. Nevertheless, the proposed changes could have significant effects on environmental assessment of PAHs, depending on how the new guidance is implemented. At a minimum, the draft guidance from EPA on the RPF approach for PAHs will likely result in a requirement for more analyses, lower cleanup levels, and a lag time before background data are available to assess the larger list of individual PAHs included.

Additional details regarding the regulatory toxicology of PAHs are provided in the information compiled in Appendix A.

3.1.3 Primary Exposure Pathways for PAHs

To ensure that the research is focused on generating information relevant to assessing potential human risks from exposure to PAHs, an initial component of the project evaluated exposure pathways to determine which ones were primary risk drivers in the assessment of PAH-

contaminated sites. This effort was completed based on guidance regarding default risk assessment approaches, and on review of the primary literature.

For PAHs in soil, direct-contact risks are dominated by the ingestion route, followed by the dermal route and, several orders of magnitude lower, the inhalation route. Applying standard EPA default exposure values, ingestion accounts for 73 percent and dermal exposure accounts for 23 percent of risks from direct contact with PAHs in soil. Risks from inhalation exposures are assumed by EPA to be negligible and therefore are not included as a topic of study in this project. Dermal exposures are specifically addressed in this research project, because they account for approximately one-fourth of exposures when applying default exposure assumptions and also because they become relatively more important if estimates of oral exposure are reduced. For example, if a site were determined to have an RBA of 0.2 (i.e., the absorption of PAHs from soil was only one-fifth the absorption of PAHs from rodent chow), then oral exposure estimates would be reduced 5-fold and would be calculated to account for only 39 percent of risk from exposure to PAHs in soil at the site, and dermal exposures would account for the remaining 61 percent of risk. Therefore, dermal absorption of PAHs from soil is included in this research project to address all of the relevant exposure pathways.

In literature from the 1980s and 1990s, some published studies suggested that dietary intake of PAHs can be a significant route of exposure to the general population where vegetables and grains are a major source of dietary PAHs. However, opposite suggestions were also published. If PAHs are taken up into plant tissues, this could present a pathway for human exposures to PAHs, and understanding whether uptake into plants is a predominant pathway of exposure to PAHs in soil is therefore essential to determining the value of bioavailability research that assesses absorption from exposures to soil (i.e., if PAHs in plants provide the predominant pathway of exposure to PAHs in soils, then understanding the RBA of PAHs from soil ingestion becomes less relevant).

The weight of evidence from available studies indicates that, although crops and vegetation may be contaminated with PAHs, this contamination results from direct soil contact and from atmospheric deposition of PAHs onto plants, rather than from uptake of PAHs from soil into plant tissues. Overwhelmingly, research indicates that the roots of plants create microbial conditions that are conducive to dissipating PAHs from soil, as opposed to taking PAHs up into the roots and shoots. Therefore, uptake of PAHs directly from soil into the edible portion of crops is not a significant exposure pathway for humans and is not a subject of further investigation in this project.

3.1.4 Review of DoD Records of Decision

The overall goal of this research project is to understand the soil/chemical interactions and the extent of human exposure to PAHs in soil. Defining those objectives, however, is complicated by the fact that the term “PAHs” refers to a class of compounds composed of more than 100 individual chemicals of varying chemical characteristics and toxicity. EPA has historically regulated 16 PAHs as Priority Pollutants, of which only seven have RPFs and are therefore considered carcinogens. At this writing, EPA is considering expanding the list of PAHs with

RPFs to include a total of 25 PAHs (the original 7 plus an additional 18) (this topic is summarized above and discussed in detail in Appendix A). To best focus this research effort on creating a useful tool for decision making at DoD sites, an initial effort has been undertaken to ensure that we understand which specific PAHs are driving risks at DoD sites, and if possible, to anticipate which PAHs may be risk drivers in the future. This was accomplished by compiling available data from the most recently evaluated DoD sites where PAHs were identified as requiring remediation, and combining this site-specific information regarding the concentration of individual PAHs in contaminated site soils with toxicity information, to assess which chemicals are the risk drivers at DoD sites. In all cases, the specific PAHs analyzed are included in the list of 16 Priority Pollutant PAHs, sometimes with the addition of 2-methylhaphthalene.

The methods used in this evaluation and results are provided below, followed by a brief discussion of the implications of the analysis. Additional details of this evaluation are provided in Appendix A.

3.1.4.1 Methods

The RODS database is maintained by EPA, and contains full-text RODs. A ROD provides the justification for the remedial action (treatment) chosen at a Superfund site. The RODs database[1] was searched for information on reported PAH concentrations at DoD sites. Search criteria included all states for fiscal years 2009 and 2010. Each ROD was reviewed to find information on individual (as opposed to total) PAH concentrations in either surface or subsurface soil media. If the ROD contained PAH data, the data were extracted into tables. Sometimes both surface and subsurface soil concentrations were reported, and in those cases, both types of data were extracted. Average and minimum concentrations were not always reported in the RODs; therefore, only maximum soil concentrations were used. These data were then subjected to the screening process discussed in Appendix A.

3.1.4.2 Results

Data from 11 different RODs were identified for inclusion in this analysis, including DoD installations located in California, New York, New Jersey, Maryland, Florida, Virginia, Pennsylvania, Massachusetts, and Wyoming.

When screened against current EPA residential soil screening criteria, BaP was the overwhelming driver for risks at the DoD sites that were included in this analysis. However, when proposed EPA residential soil screening criteria were used, the results indicated that dibenz[a,h]anthracene became the primary driver of human health risk because of the very conservative RPF value assigned to that PAH in the proposed RPF approach (Table 4). The other major change was that chrysene replaced indeno[1,2,3-cd]pyrene as one of the top five risk drivers when the proposed RPF values were used. More than 70 percent of the sites identified in this RODs search exceeded residential soil screening criteria for BaP, benz[a]anthracene, and benzo[b]fluoranthene, independent of whether current or proposed EPA RPF values were used.

[1] See http://www.epa.gov/superfund/sites/rods/

Figures 3-1 and 3-2 present a synthesis of the findings for the magnitude of current risk-based screening criteria exceedances and the percentage of sites that exceed those criteria for individual PAHs, respectively. In summary, this analysis indicates that the current primary human health risk drivers are the larger PAHs (i.e., four- to 6-ring) associated with cancer endpoints of toxicity:

- BaP
- Benz(a)anthracene
- Benzo(b)fluoranthene
- Indeno[1,2,3-cd]pyrene
- Dibenz(a,h)anthracene.

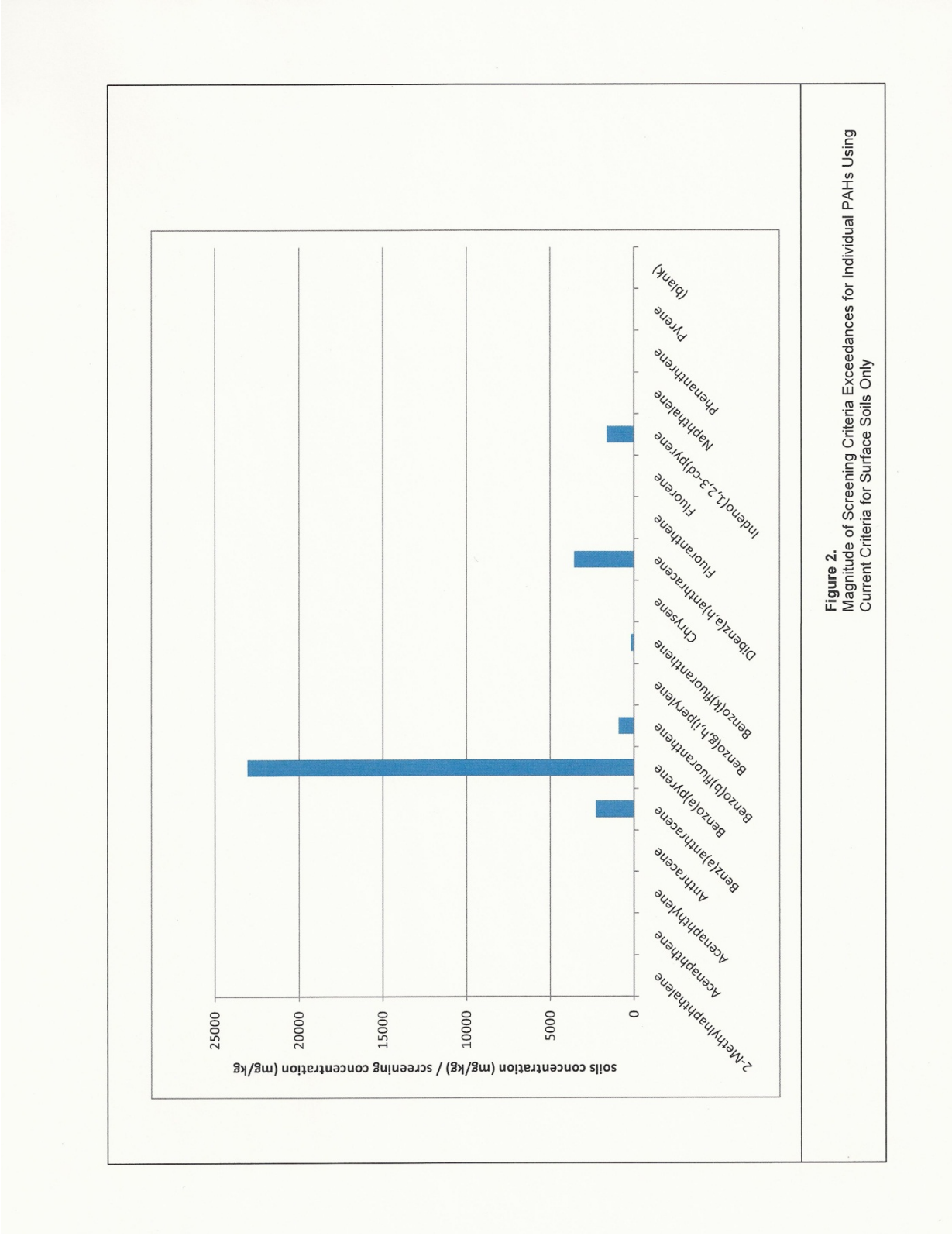


Figure 3-1. Reproduced from Project Work Plan

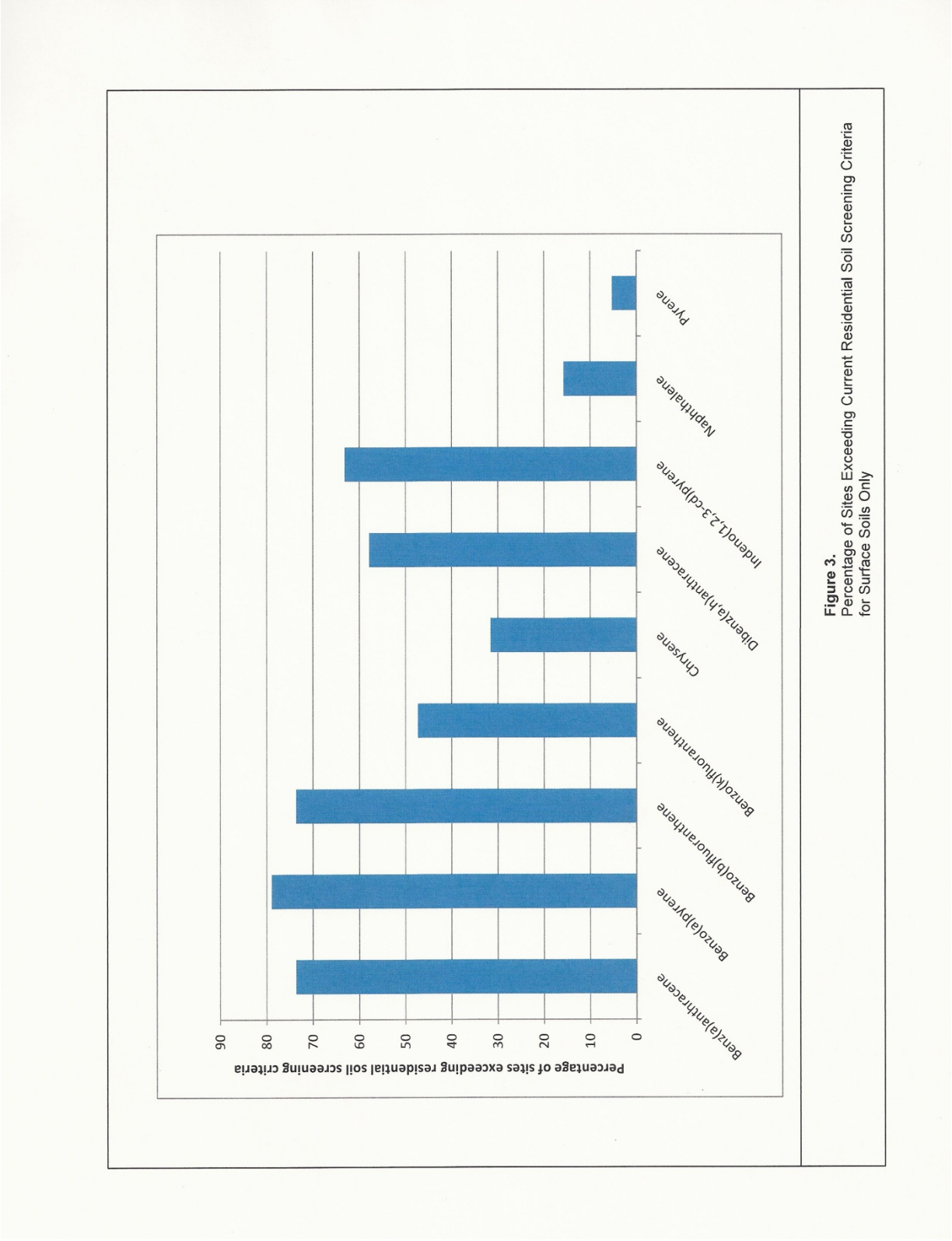


Figure 3-2. Reproduced from Project Work Plan

When available, the actual soil clean-up goals used at each site were extracted from the RODs and are presented in Table 3-4. Residential (unrestricted use) soil clean-up goals for BaP range from 0.062 to 1.1 mg/kg.

Table 3-4. Summary of PAH clean-up goals and DoD sites

ROD#	Site Name	PAHs	Soil Cleanup Goal (mg/kg)
FL6170024412	**Naval Air Station Jacksonville, FL**	Benzo(a)pyrene equivalent	0.7
NJ3210020704	**Picatinny Arsenal, NJ**		
	Residential Soil Clean-up Criteria	Benz(a)anthracene	4
		Benzo(a)pyrene	0.66
		Benzo(b)fluoranthene	4
		Benzo(k)fluoranthene	4
		Dibenz(a,h)anthracene	0.66
		Indeno(1,2,3-c,d)pyrene	4
NY0213820830	**Seneca Army Depot, NY**		
	Residential Use Soil Cleanup Objectives	Acenaphthene	20
		Acenaphthylene	100
		Anthracene	100
		Benzo(a)anthracene	1
		Benzo(a)pyrene	1
		Benzo(b)fluoranthene	1
		Benzo(ghi)perylene	100
		Benzo(k)fluoranthene	0.8
		Chrysene	1
		Dibenz(a,h)anthracene	0.33
		Fluoranthene	100
		Fluorene	30
		Indeno(1,2,3-cd)pyrene	0.5
		Phenanthrene	100
		Pyrene	100
	Commercial Use Soil Cleanup Objectives	Acenaphthene	500
		Acenaphthylene	500
		Anthracene	500
		Benzo(a)anthracene	5.6
		Benzo(a)pyrene	1
		Benzo(b)fluoranthene	5.6
		Benzo(ghi)perylene	500
		Benzo(k)fluoranthene	56
		Chrysene	56
		Dibenz(a,h)anthracene	0.56
		Fluoranthene	500
		Fluorene	500
		Indeno(1,2,3-cd)pyrene	5.6
		Phenanthrene	500
		Pyrene	500

ROD#	Site Name	PAHs	Soil Cleanup Goal (mg/kg)
	Industrial Use Soil Cleanup Objectives	Acenaphthene	1000
		Acenaphthylene	1000
		Anthracene	1000
		Benzo(a)anthracene	11
		Benzo(a)pyrene	1.1
		Benzo(b)fluoranthene	11
		Benzo(ghi)perylene	1000
		Benzo(k)fluoranthene	110
		Chrysene	110
		Dibenz(a,h)anthracene	1.1
		Fluoranthene	1000
		Fluorene	1000
		Indeno(1,2,3-cd)pyrene	11
		Phenanthrene	1000
		Pyrene	1000
WY5571924179	**F.E. Warren Air Force Base, WY**		
	Residential Preliminary Remediation Goal	Benzo(a)pyrene	0.062

Notes:

= PAHs of greatest importance at DOD sites based on current RPF values (see Table 4).

3.1.5 Sources of PAHs at DoD Sites

PAHs are generally released into the environment either as by-products of combustion and pyrolysis processes (pyrogenic PAHs), or as spills of petroleum products, like crude oil, fuel oil, or diesel (petrogenic PAHs). Pyrogenic PAHs are found in the environment in the form of coal tar, creosote, coke, soot, and char, while petrogenic PAHs are released within a non-aqueous-phase liquid (NAPL) matrix. Table 3-5 lists the dominant sources of PAHs to soils, broken down as natural, industrial, and non-industrial types of sources. PAHs in soils at DoD sites originate from non-industrial sources, because DoD has not conducted the types of industrial operations that produce extensive PAH contamination at industrial sites (e.g., wood treating, coking, or refining of petroleum products). Discussions with various DoD personnel, including site historians, have not produced any indications that DoD operated manufactured gas plants at their facilities in the early part of the 20th century, which is the only industrial activity that DoD might reasonably have conducted that would have produced extensive PAH contamination of soils.

During the review of DoD RODs, soil PAH data were collected for 18 exposure units at 11 sites. These data indicate that PAHs at DoD sites are found primarily in soils at landfills and general waste disposal sites; areas where petroleum products were stored, used, spilled, or disposed of; vehicle staging and cleaning areas; fire training facilities; and areas where ordnance, pesticides, and ores were stored. Total PAH concentrations ranged from 0.53 to 37,900 mg/kg, with most exposure units (all but five) exhibiting maximum total PAH concentrations below 40 mg/kg.

Table 3-5. Sources of carcinogenic PAHs to soils[a]

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 oil
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
	Refineries	Soot, various NAPLs (crude oil, fuel oil, diesel, etc.)
	Carbon black manufacture	Soot, oil tar
	Fuel spills and/or disposal	Various NAPLs (crude oil, fuel oil, waste oil, diesel, jet fuel, etc.)
Non-industrial sources	Skeet	Coal tar pitch or bitumen (used as binder in targets)
	Asphalt sealants	Coal tar
	Landfills	Creosote (treated wood), soot, char
	Incinerators (municipal, hospital)	Soot
	Open burning	Soot, char
	Fire training	Soot
	Fires	Soot, char
	Auto/truck emissions	Soot

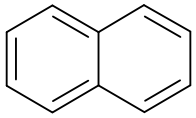
[a] Includes both current and historical sources of PAHs to soils

A review of DoD site work and conversations with DoD personnel have indicated that skeet shooting ranges are an emerging concern for DoD as a type of site where PAHs are present in soil. Historically, skeet[2] were made of a mixture of crushed limestone (70 percent) and coal tar (30 percent; used as a binder). Some ranges are still using limestone and coal tar skeet, while others are using "environmentally friendly" skeet. Maximum total PAH concentrations across six U.S. Navy skeet sites sites (202 samples) was 2,823 mg/kg, with average and geometric mean concentrations of 109 mg/kg and 2.1 mg/kg, respectively. Adjusting the geometric mean concentrations of each PAH by its respective RPF (if it has one) yields a value of 0.32 mg/kg BaP equivalents for these skeet sites. The BaP equivalents were dominated by BaP, dibenz[a,h]anthracene, and benzo[b]fluoranthene. These data indicate that soils at skeet sites contain significant concentrations of PAHs. Based on comparison to other types of DoD sites, soils at skeet sites appear to contain total PAH concentrations on the high end of DoD site types (an average of 109 mg/kg total PAHs for all six skeet sites, versus a maximum of <40 mg/kg for most other DoD sites).

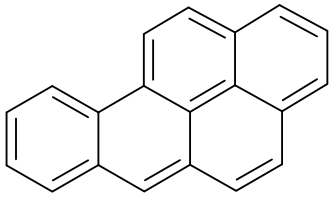
[2] The clay targets ("clay pigeons") used in skeet shooting are referred to herein as "skeet."

APPENDIX A
REGULATORY TOXICOLOGY OF POLYCYCLIC AROMATIC HYDROCARBONS

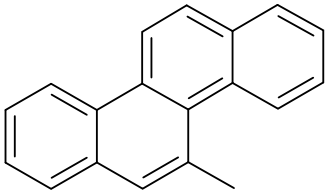
Polycyclic aromatic hydrocarbons (PAHs) are a class of organic chemicals consisting of two or more fused, unsubstituted aromatic hydrocarbon rings and their alkyl-substituted derivatives. The chemical class does not include heterocyclic compounds containing oxygen, nitrogen, or sulfur as part of the ring structure, or PAHs substituted with oxygen, nitrogen, or sulfur. Some example PAHs include:



Naphthalene
(unsubstituted)



Benzo[a]pyrene
(unsubstituted)



5-Methylchrysene
(alkyl-substituted)

This technical memorandum summarizes the current regulatory approach to health assessment of PAHs, proposed modifications to the regulatory approach, and the implications of those modifications to future health-based assessment of PAHs. Table A-1 provides a summary of key milestones in the regulatory history of PAHs.

REGULATORY APPROACHES

When present, PAHs invariably exist in the environment as mixtures. Although the total number of PAHs is unknown, there are hundreds of PAHs present as components of mixtures (USEPA 2010a). To the extent possible, the regulatory approach to health assessment of PAHs considers the interaction between the individual PAHs in the mixture. U.S. Environmental Protection Agency (EPA) (1986; 2000) guidance for risk assessment of chemical mixtures recommends three approaches for evaluation of chemical mixtures, depending on the available data. In order of preference, the three approaches are:

1. Use of dose-response data for the mixture of interest
2. Use of dose-response data for a "sufficiently similar" mixture

3. Use of dose-response data for the individual components.

EPA has evaluated and developed dose-response assessments for three chemical mixtures that include PAHs formed through pyrolysis: coke oven emissions, creosote, and diesel engine exhaust.[1] Cancer bioassay data also exists for manufactured gas plant residue (Weyand et al., 1995) and coal tar preparations (Culp et al., 1998); two PAH-containing mixtures. Complex mixtures of PAHs in the environment, however, are highly variable; in most cases too dissimilar to the specific mixtures for which dose-response data are available to be directly applicable. In this case, EPA guidance recommends evaluation of the individual components of the mixture and, when those components are believed to act through a similar mechanism of action, adding together the risks from each component (USEPA 2000). For PAH mixtures, EPA (1993; 2010a), California Environmental Protection Agency (CalEPA) (OEHHA 2009), and others have utilized relative potency factors (RPFs) to assess the toxicity of individual PAHs. In the RPF approach, the doses of individual components acting through a similar mechanism of action are summed after scaling to the relative potency of an index chemical in the group for which the most complete dose-response characterization is available. For PAHs, benzo[a]pyrene (BaP) has been selected as the index chemical because: 1) it is typically present in environmental settings where PAHs are detected; 2) it has the most robust toxicological dataset among the PAHs and a formal dose-response assessment has been conducted based on chronic rodent bioassays; 3) there is a large database of *in vivo* and *in vitro* studies directly comparing the potency of response of various PAHs with BaP; and 4) it is one of the most potent carcinogens of PAHs tested.

Cancer Assessment

EPA initially derived RPFs in 1993 for six PAHs, in addition to BaP, calling the approach the estimated order of potential potency (EOPP) (USEPA 1993). The EOPPs were based solely on skin tumor formation data from four mouse skin painting studies. Therefore, unlike the toxicity equivalence factors (TEFs) developed by EPA (2010b) for dioxins and furans, EOPPS are applicable only to carcinogenic potency and not noncancer endpoints. EPA defines TEFs as RPFs supported by enough data to conclude a specific mode of action is relevant for all health effects and relevant to all exposure routes and durations. EPA (1993; 2010a) notes that existing PAH toxicology studies have largely focused on metabolism, genotoxicity, and cancer, and there are inadequate data available to identify a mechanism(s) of action relevant to noncancer endpoints. EPA (1993) also indicated the EOPPs should only be applied to oral exposures using the oral slope factor for the index chemical, BaP, because at the time there was no inhalation unit risk for BaP, no data to

[1] Coke oven emissions – http://www.epa.gov/iris/subst/0395.htm; creosote – http://www.epa.gov/iris/subst/0360.htm; diesel engine exhaust – http://www.epa.gov/iris/subst/0642.htm.

support a conclusion of dose equivalency between oral and inhalation exposures, and inadequate data to judge the accuracy of the EOPPs for inhalation exposures.

CalEPA developed RPFs (termed potency equivalency factors, PEFs) for 20 PAHs, in addition to BaP, for regulating PAHs under its toxic air contaminants program (OEHHA 1993; 1994). Unlike EPA (1993), CalEPA assumed that relative cancer potency was equivalent across exposure routes and did not limit data selection to studies using only one protocol (i.e., the skin painting studies). CalEPA also derived unit risks for four additional PAHs or PAH derivatives (dibenz[a,h]anthracene, 7,12-dimethylbenzanthracene, 3-methylcholanthrene, and 5-nitroacenaphthene) and calculated RPFs for those PAHs based on the ratio of their unit risk to that of BaP. CalEPA continues to evaluate new data and modify the RPFs when they conclude the data support a revision. The most recent CalEPA RPFs are applied in the agency's latest cancer potency factor guidance (OEHHA 2009).

EPA (2010a) recently released a draft RPF approach guidance that proposes RPFs for 24 PAHs (in addition to BaP) based on carcinogenic potency. Unlike the EOPPs described in USEPA (1993), the new RPFs are intended to apply to all exposure routes (oral, inhalation, dermal). A EPA Scientific Advisory Board reviewed the draft RPF approach guidance and provided final comments in March of 2011 (USEPA 2011) and the final assessment is scheduled to be completed in the first quarter of fiscal year 2012 (October–December 2011).[2]

EPA (2010a) evaluated unsubstituted PAHs with three or more fused aromatic rings containing only carbon and hydrogen; the PAHs likely to act through a mode of action similar to BaP. For each compound they considered oral, inhalation, and dermal exposure studies that tested one or more PAH simultaneously with BaP in rodent carcinogenicity bioassays and *in vivo* or *in vitro* assays for cancer-related endpoints (e.g., DNA adduct formation, mutagenicity, and other genotoxicity or tumor endpoints). Beginning with a target list of 74 PAHs that have been identified in environmental media, EPA conducted a comprehensive literature search encompassing studies from the 1950s through 2009, and identified studies with relevant dose-response data for 51 PAHs. RPFs were calculated for each study as the ratio of the slope of the dose-response curves for the subject PAH and BaP. Sixteen of the 51 PAHs evaluated were excluded because only one *in vitro* study was available. A weight of evidence evaluation for potential carcinogenicity was conducted for the remaining 35 PAHs, giving the greatest weight to *in vivo* tumor bioassays. Of these 35 PAHs, EPA concluded there was adequate evidence for lack of carcinogenicity for three PAHs, inadequate data to evaluate carcinogenicity in eight PAHs, and adequate evidence to conclude the remaining 24 PAHs (in addition to BaP) are carcinogenic and to derive final RPFs. The final RPF for each PAH was calculated as the arithmetic mean of RPFs from the available cancer bioassays or, in the absence of a cancer bioassay, from the available cancer-

[2] http://cfpub.epa.gov/ncea/iristrac/index.cfm?fuseaction=viewChemical.showChemical&sw_id=1062

related endpoint studies. Only studies with positive outcomes were included, which would tend to bias the final RPF estimate high. For most PAHs, three or fewer studies formed the basis of the final RPF. In addition, EPA assigned a relative confidence rating to the estimate based on the number and quality of cancer bioassays, the number and quality of supporting cancer-related endpoint data, and the availability of data for multiple routes of exposure, both sex, and multiple species.

The RPFs currently in use by EPA (1993) and by CalEPA (OEHHA 2009), along with the new proposed RPFs by EPA (2010a), are summarized in Table A-2.

Benzo[a]pyrene

BaP is the index chemical for all PAH RPF approaches. EPA identifies BaP as a *probable human carcinogen*, based on adequate evidence from animal studies and inadequate evidence in humans. The current EPA oral cancer slope factor was derived in 1992, a correction to the estimate from the previous year's Dose-Response Analysis of Ingested Benzo[a]pyrene.[3] The slope factor of 7.3 per mg/kg-day is the geometric mean of the range of slope factors (4.5 to 11.7 per mg/kg-day) derived based on forestomach tumors in two strains of mice, and forestomach, laryngeal, and pharyngeal tumors in rats. BaP is currently being reassessed under EPA's IRIS program. An updated BaP toxicological review document is scheduled to be completed and released for external peer review in the second quarter of fiscal year 2012 (January–March 2012).[4]

In the updated *Public Health Goal for Benzo(a)pyrene in Drinking Water*, CalEPA derived a new oral slope factor of 2.9 per mg/kg-day for BaP based on forestomach and oral cavity tumors in female mice (from Culp et al., 1998), after adjustment for potential *in utero* early life exposures (OEHHA 2010). This new value attributes less than one-quarter the carcinogenic potency to BaP as the previous CalEPA slope factor of 12 per mg/kg-day (OEHHA 2009). CalEPA decided not to derive slope factors for the older studies on which both U.S. EPA's slope factor and CalEPA's earlier slope factor were based because of deficiencies in study design. CalEPA (OEHHA 1993) also derived an inhalation unit risk of 1.1×10^{-3} per $\mu g/m^3$ for BaP based on respiratory tract tumor incidence in male hamsters (from Thyssen et al., 1981).

Naphthalene

Naphthalene is a PAH composed of two unsubstituted aromatic rings. Because it has only two aromatic rings, the type of metabolic activation in the so-called "bay region" that can

[3] http://www.epa.gov/iris/subst/0136.htm

[4] http://cfpub.epa.gov/ncea/iristrac/index.cfm?fuseaction=viewChemical.showChemical&sw_id=1007

occur in certain PAHs with ≥3 rings, a molecular step that is critical for the mechanism of action assumed in the RPF approach cannot occur. The National Toxicology Program first listed naphthalene as *reasonably anticipated to be a human carcinogen* in the 11th Report on Carcinogens (NTP 2004) based on an increased incidence of invasive nasal tumors in rats and lung tumors in female mice exposed to naphthalene by inhalation in chronic bioassays (NTP 1992; 2000). The IRIS file for naphthalene indicates that EPA considers naphthalene a *possible human carcinogen*, based on inadequate data in humans and limited data in animals for carcinogenicity by the inhalation route.[5] However, EPA concluded there are inadequate data to quantify carcinogenicity (i.e., to develop a cancer potency value) because of the lack of a chronic oral study and the weakness of the evidence from the inhalation studies. In 2004, EPA released an external review draft of the updated toxicological review and IRIS summary, which includes an inhalation unit risk for the carcinogenicity of naphthalene by the inhalation route (1×10^{-4} per $\mu g/m^3$) (USEPA 2004) based on nasal tumors in rats and mice following 2-year bioassays (Abdo et al. 2001; NTP 1992, 2000). They continued to conclude there are inadequate data to determine carcinogenicity by the oral route. Although the final report from the external reviewers is available, the 2004 toxicological review was never finalized. IRIS Track indicates that the next milestone is "Draft Development" and the next milestone due date is to be determined.

CalEPA has derived an inhalation unit risk for naphthalene of 3.4×10^{-5} per $\mu g/m^3$ (OEHHA 2004) based on increased nasal tumor incidence in male rats (NTP 2000).

Age-Dependent Adjustment Factors (ADAFs)

BaP is considered to be a "complete" carcinogen, meaning it likely both *initiates* tumor formation by directly damaging DNA and *promotes* tumor growth (USEPA 2010a). Therefore, because it is likely carcinogenic by a mutagenic mode of action, according to EPA (2005) guidance for assessing early life exposures to carcinogens, any assessment that includes BaP exposures prior to the age of 16 years should incorporate the appropriate ADAFs. By extension, assessment of PAH mixtures using the RPF approach should apply the appropriate ADAFs to the entire mixture of PAHs because use of an RPF assumes that all the individual PAHs act through the same mechanism as BaP. Application of ADAFs will depend on the specific exposure scenario and age group being assessed: a 10-fold adjustment factor is used when children less than 10 years of age may be exposed, and a 3-fold adjustment factor is used for children 2–15 years of age. The use of ADAFs for PAHs not included in the RPF approach should be evaluated on a case-by-case basis. For example, naphthalene is not considered to be mutagenic and so an ADAF is not applied.

[5] http://www.epa.gov/iris/subst/0436.htm

Noncancer Health Effects

As noted above, the RPFs derived for PAHs are applicable only to carcinogenic potency. Data are not available to support a single mechanism of action relevant to the various noncancer health effects associated with different PAHs. The approach to evaluating the noncancer health effects of PAHs is the same as for other individual chemicals: use of a chemical-specific RfD or RfC derived based on animal or human studies for the specific PAH of interest.

EPA has derived oral RfDs for seven PAHs (acenaphthene, anthracene, fluoranthene, fluorene, 2-methylnaphthalene, naphthalene, and pyrene) and an inhalation RfC for napthalene. EPA does not, however, have either an oral reference dose (RfD) or an inhalation (RfC) for BaP. CalEPA evaluated noncancer toxicity of BaP and derived an oral RfD of 0.0017 mg/kg-day based on kidney toxicity in rats (from Knuckles et al., 2001).

IMPLICATIONS FOR ENVIRONMENTAL ASSESSMENT OF PAHS

Evolving approaches to PAH health assessment will affect how environmental assessment is conducted and cleanup levels derived as part of that assessment. The practical implications of PAH potential changes in regulatory toxicology are discussed in this section.

Pending Revisions to Dose-Response Assessments

EPA currently lists only noncancer toxicity values for naphthalene in IRIS, an RfD of 0.02 mg/kg-day and an RfC of 0.003 mg/m^3.[6] But the EPA Regional Screening Level tables use the inhalation unit risk developed by CalEPA (3.4×10^{-5} per µg/m^3) to derive a residential soil screening level based on cancer risk following dust inhalation. The cancer-based soil screening level (3.6 mg/kg) is less than the screening level for combined ingestion, dermal, and inhalation exposure based on the noncancer toxicity (140 mg/kg). Therefore, the cancer risk-based, dust inhalation-only screening level is also the overall residential soil screening level. If the proposed EPA inhalation unit risk of 1×10^{-4} per µg/m^3 is finalized without modification, the resulting residential soil screening level would be decreased to 1.2 mg/kg, reflecting cancer risks from dust inhalation exposures.

The revised EPA toxicological assessment for BaP has been in internal review draft since 2004. Evaluation of the practical implications of the revised assessment will not be possible until the external peer review draft is released (scheduled for 2012).

[6] http://www.epa.gov/iris/subst/0436.htm

Draft RPF Approach

EPA's draft RPF approach, when finalized and incorporated into environmental policy, will raise significant issues for environmental assessment of PAHs, including:

- **Additional analytes**–the draft guidance increases the number of carcinogenic PAHs with RPFs from seven to 25, including BaP. Taken at face value, this would mean an additional 18 chemicals to analyze and evaluate, many of which have not commonly been analyzed in the past.
- **Treatment of undetected PAHs**–depending on how PAHs that are not detected are treated in the risk assessment process, undetected PAHs could contribute significantly to total PAH risk estimates. If half the detection limit is used for undetected chemicals, as is common practice when applying a TEF approach to calculate TCDD toxic equivalence, PAHs that may not be present could potentially drive risk estimates. In particular, detection limits for PAHs with high RPFs but which have not typically been analyzed in the past and may not be present at all, could drive risk estimates. For example, benz[j]aceanthrylene and dibenzo[a,l]pyrene have RPFs of 60 and 30, respectively.
- **Changes to existing RPFs** – of the six carcinogenic PAHs with existing RPFs (excluding BaP), the proposed RPF has increased for five and decreased for one. An increased RPF corresponds to an increased risk estimate, a decreased screening level, and potentially, a decreased cleanup level. Whereas the residential screening levels currently range from 15 to 15,000 µg/kg, application of the new RPFs would result in screening levels from 1.5 to 500 µg/kg for these PAHs. Screening levels would range from 0.25 to 1,667 µg/kg for all 25 PAHs with RPFs in the EPA (2010) proposed guidance (Table A-3).
- **Background concentrations** – Assessing site-related PAHs is challenging because of the presence of both anthropogenic, non-point source impacts and "natural" background levels (e.g., fires). It's a particularly important issue in urban and/or industrialized settings. Background data exist for a few cPAHs, primarily for those that have traditionally been evaluated, but generally lacking for most of the cPAHs for which EPA has developed new RPFs (USEPA 2010a). There will be a lag time before implementation of the new guidance, with an increased number of chemicals to analyze, and the availability of background data for those chemicals. In urban areas, background levels of cPAHs in soil typically exceed health-based screening levels. Table A-3 compares typical rural and urban soil background levels for cPAHs (ATSDR 1995) to current EPA regional screening levels for residential soil and screening levels that incorporate the proposed RPFs from EPA (2010a).

CONCLUSIONS

Proposed modifications to PAH health assessment are fundamentally consistent with the long-standing regulatory approach: use of RPFs to evaluate the carcinogenic risk of PAHs in mixtures and evaluation of noncancer effects on a chemical-specific basis. Nevertheless, the proposed changes could have significant effects on environmental assessment of PAHs, depending, to some degree on how the new guidance is implemented. At a minimum, the draft EPA (2010a) guidance on the RPF approach will likely result in increased analysis, lower cleanup levels, and a lag time before background data are available to assess the larger list of chemicals.

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Table A-1. Regulatory History of PAHs in the United States

Year	Event	Reference
1992	EPA derives current benzo[a]pyrene oral cancer slope factor of 7.3 per mg/kg-day	USEPA 1992
1993	EPA releases *Provisional Guidance for Quantitative Risk Assessment of PAHs*	USEPA 1993
1993	CalEPA derives inhalation unit risk for benzo[a]pyrene of 1.1×10^{-3} per $\mu g/m^3$	OEHHA 1993
1993	CalEPA derives relative potency factors for PAHs	OEHHA 1993
2004	EPA releases external peer review dravt of the *Toxicological Review of Naphthalene* with revised inhalation unit risk of 1×10^{-4} per $\mu g/m^3$	USEPA 2004
2004	CalEPA derives inhalation unit risk for naphthalene of 3.4×10^{-5} per $\mu g/m^3$	OEHHA 2004
2010	CalEPA derives revised oral cancer slope factor for benzo[a]pyrene of 2.9 per mg/kg-day	OEHHA 2010
2010	EPA releases external review draft of *Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures*	USEPA 2010
2011	Target date for finalization of EPA's *Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures*	IRIS Track[a]
2011	Target date for external peer review and finalization of EPA's updated *Toxicological Review of Benzo[a]pyrene*	IRIS Track[a]

[a] EPA IRIS Track website: http://cfpub.epa.gov/ncea/iristrac/

Notes:

CalEPA = California Environmental Protection Agency

EPA = U.S. Environmental Protection Agency

Table A-2. Comparison of Relative Potency Factors for Polycyclic Aromatic Hydrocarbons

	USEPA 1993[a]	CalEPA (2009)[b]	USEPA 2010 Proposed[c]
Anthanthrene	--	--	0.4
Benzo(a)pyrene	1	1	1
Benzo[a]anthracene	0.1	0.1	0.2
Benz[b,c]aceanthrylene, 11H-	--	--	0.05
Benzo[b]fluoranthene	0.1	0.1	0.8
Benzo[c]fluorene	--	--	20
Benz[e]aceanthrylene	--	--	0.8
Benzo[g,h,i]perylene	--	--	0.009
Benz[j]aceanthrylene	--	--	60
Benzo[j]fluoranthene	--	0.1	0.3
Benzo[k]fluoranthene	0.01	0.1	0.03
Benz[l]aceanthrylene	--	--	5
Chrysene	0.001	0.01	0.1
Cyclopenta[c,d]pyrene	--	--	0.4
Cyclopenta[d,e,f]chrysene, 4H-	--	--	0.3
Dibenzo[a,e]fluoranthene	--	--	0.9
Dibenz[a,j]acridine	--	0.1	--
Dibenz[a,h]acridine	--	0.1	--
7H-dibenzo(c,g)carbazole	--	1	--
Dibenzo[a,e]pyrene	--	1	0.4
Dibenz[a,c]anthracene	--	--	4
Dibenz[a,h]anthracene	1	0.34	10
Dibenzo[a,h]pyrene	--	10	0.9
Dibenzo[a,i]pyrene	--	10	0.6
Dibenzo[a,l]pyrene	--	10	30
Fluoranthene	--	--	0.08
Indeno[1,2,3-c,d]pyrene	0.1	0.1	0.07
Naphtho[2,3-e]pyrene	--	--	0.3
5-methylchrysene	--	1	--
1-nitropyrene	--	0.1	--
4-nitropyrene	--	0.1	--
1,6-dinotropyrene	--	10	--
1,8-dinotropyrene	--	1	--
6-nitrochrysene	--	10	--
2-nitrofluorene	--	0.01	--
7,12-dimethylbenzanthracene	--	21	--
3-methylcholanthrene	--	1.8	--
5-nitroacenaphthene	--	0.01	--

Notes:

[a] USEPA. 1993. Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons. July. EPA/600/R-93/089. U.S. Environmental Protection Agency, Washinton D.C.

[b] CalEPA. 2009. Technical Support Document for Describing Available Cancer Potency Factors: Methodologies for derivation, listing of available values, and adjustments to allow for early life stage exposures. Appendix A: Hot Spots Unit Risk and Cancer Potency Values. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. May.
CalEPA RPFs calculated by dividing the oral slope factor listed for each chemical by the oral slope factor for benzo[a]pyrene.

[c] USEPA. 2010. Development of a relative potency factor (RPF) approach for polycyclic aromatic hydrocarbon (PAH) mixtures. Draft. February. EPA/635/R-08/012A. U.S. Environmental Protection Agency, Washington D.C.

Table A-3. Polycyclic Aromatic Hydrocarbon Soil Background Concentrations and Health-Based Screening Levels Derived Using Relative Potency Factors (µg/kg)[a]

	Soil Background Concentrations[b]		Residential Soil Screening Level[c]	
PAH	Rural	Urban	based on USEPA 1993	based on USEPA 2010
Anthanthrene	NA	NA		38
Benzo(a)pyrene	2–1,300	165–220	15	15
Benz[a]anthracene	5–20	169–59,000	150	75
Benz[b,c]aceanthrylene, 11H-	NA	NA	--	300
Benzo[b]fluoranthene	20–30	15,000–62,000	150	19
Benzo[c]fluorene	NA	NA	--	0.75
Benz[e]aceanthrylene	NA	NA	--	19
Benzo[g,h,i]perylene	10–70	900–47,000	--	1,667
Benz[j]aceanthrylene	NA	NA	--	0.25
Benzo[j]fluoranthene	NA	NA	--	50
Benzo[k]fluoranthene	10–110	300–26,000	1,500	500
Benz[l]aceanthrylene	NA	NA	--	3.0
Chrysene	38.3	251–640	15,000	150
Cyclopenta[c,d]pyrene	NA	NA	--	38
Cyclopenta[d,e,f]chrysene, 4H-	NA	NA	--	50
Dibenzo[a,e]fluoranthene	NA	NA	--	17
Dibenzo[a,e]pyrene	NA	NA	--	38
Dibenz[a,c]anthracene	NA	NA	--	3.8
Dibenz[a,h]anthracene	NA	NA	15	1.5
Dibenzo[a,h]pyrene	NA	NA	--	17
Dibenzo[a,i]pyrene	NA	NA	--	25
Dibenzo[a,l]pyrene	NA	NA	--	0.5
Fluoranthene	0.3–40	200–166,000	--	188
Indeno[1,2,3-c,d]pyrene	10–15	8,000–61,000	150	214
Naphtho[2,3-e]pyrene	NA	NA	--	50

[a]EPA Regional Screening Levels available at: http://www.epa.gov/reg3hwmd/risk/human/rb-concentration_table/Generic_Tables/index.htm

[b]Background soil concentrations of polycyclic aromatic hydrocarbons measured in the United States (ATSDR 1995).

[c]Residential soil screening levels for PAHs that are based on the a carcinogenic relative potency factor. Screening levels based on USEPA 1993 are taken directly from EPA Regional Screening Levels table. Screening levels based on USEPA 2010a were calculated by dividing the benzo[a]pyrene screening level by the proposed relative potency factor.

Notes:

NA = background data not available

PAH UPTAKE BY PLANTS

INTRODUCTION

More than 90 percent of PAHs in the environment reside in surface soil (Zhan et al. 2010). As a result, concerns have arisen that plants grown in PAH-contaminated soils can become contaminated with PAHs due to absorption processes (Zhan et al. 2010). If PAHs are taken up into plant tissues, this could present a pathway for human or animal exposures to PAHs. Understanding the predominant pathways for plant uptake is therefore essential to protect human and ecological health when exposure to contaminated soil occurs (Gao and Collins 2009). This document provides a review of available information regarding the relation between soil concentrations of PAHs plants grown in those soils, to understand whether this is potentially a meaningful exposure pathway that should be considered in the risk assessment process.

In literature from the 1980s and 1990s, some published studies suggested that dietary intake of PAHs can be a significant route of exposure to the general population where vegetables and grains are a major source of dietary PAHs (Menzie et al. 1992; Phillips 1999). However, opposite suggestions were also published. For example, as stated in Wild and Jones (1992): "It is known that PAHs are not readily taken up by plants. The chemical properties of PAHs dictate that they are strongly adsorbed onto soil organic matter, and uptake via foliar and or root mechanisms is inefficient. Several laboratory experiments have shown that some PAHs may have the propensity to move, albeit inefficiently, from the soil environment into plant tissues. However, several uncertainties remain."

In the 2000s, the literature was dominated by studies evaluating the efficiency and usefulness of some plants as phytoremediation tools for PAHs. For some chemicals, it has been established that phytoremediation is a promising alternative approach to soil remediation, due to its cost effectiveness, convenience, and environmental acceptability (Cheema et al. 2010).

Although plants have been shown to take up some contaminants from soils, as in the case of metals, the research on PAH-contaminated soils suggests that plant species considered for phytoremediation may actually not "take up" PAHs, but instead, may "transform" them. Plants may contribute to the dissipation of PAHs through various mechanisms, such as plant uptake and accumulation, increase of microbial activities, improvement of physical and chemical conditions of soils, and adsorption of pollutants in the rhizosphere (Cheema et al. 2010).

The objective of this summary is to review recent papers (published since 2000) that describe research conducted to determine whether plants accumulate PAHs from soil into

vegetation (or crops). This summary document is divided into two sections. The first focuses on papers that examine plant uptake of PAHs with regard to human or wildlife health concerns, and the second focuses on papers that examined plant uptake of PAHs for phytoremediation potential.

PAH Uptake in Plants: Crop Uptake Studies

Plant uptake of organic pollutants is important when considering the transfer of pollutants from soils into the food chain. PAHs are detected frequently in agricultural and urban soils and can therefore raise concern (Lu and Zhu 2009). In general, contaminants may enter plants via several pathways: root uptake from the contaminated soil, vapor uptake from the atmosphere, and particle-phase deposition onto the waxy cuticle of the leaves (Lu and Zhu 2009). Lu and Zhu (2009) suggested that toxic organic contaminants could enter vegetables cultivated on the contaminated soils and may threaten the product quality, as well as human health (Fismes et al. 2002; Gao and Zhu 2004; Khan et al. 2008; Samsoe-Petersen et al. 2002; Wennrich et al. 2002; Wild and Jones 1992). Understanding the occurrence and predominant pathways for plant uptake is therefore essential to protect human and ecological health when exposure to contaminated soil occurs (Gao and Collins 2009).

- Samsoe-Petersen et al. (2002) examined vegetables grown in two contaminated soils and in a reference soil. They also collected fruits from both uncontaminated and contaminated private gardens. For PAHs, linear regression did not show good correlation between soil and crop concentrations, but results did show elevated levels of several trace elements and PAHs in the vegetables grown in contaminated soil. Samsoe-Petersen et al. (2002) demonstrated that the main route of exposure to PAHs for leaf crops was via direct contact the leaves being in direct contact with the soil, not from translocation from the soil to the edible portion of the plant. No correlations were found between concentrations in the soil and concentrations in the fruits (Samsoe-Petersen et al. 2002). However, the authors did suggest that berries growing close to the soil surface may be contaminated via direct uptake from soil deposited on their surface.
- Fismes et al. (2002) collected PAH-contaminated soils from a gasworks and conducted greenhouse studies with lettuce, potatoes, and carrots. These authors reported that concentrations of PAHs in peeled potatoes were very low and were not correlated with the PAH concentrations in soils. Lettuce roots had higher PAH concentrations than the leaves. The PAH levels in whole tubers were higher than those in peeled tubers. They suggested that this was likely because the peels have higher lipid contents than the pulp. The authors also reported that the bioconcentration factors from soil to plants were very low, and probably overestimated, because pot experiments can exaggerate the availability of pollutants.

- Residues of PAHs in soils from organic farms, and their uptake by four varieties of organically produced potatoes and three varieties of organic carrots from England, were investigated by Zohair et al. (2006). Samples of soils and crops (with and without peels) were analyzed. PAH concentrations were more than two orders of magnitude lower in shoots than in soils. PAHs were more abundant in the peels of potatoes and carrots than in the cores. Peeling carrots and potatoes was found to remove 56%–100% of the PAH residues, depending on the crop variety and the properties of the contaminants.
- Khan et al. (2008) reported that leafy vegetables, particularly lettuce grown on wastewater-contaminated soils, contain PAHs at elevated concentrations. The low-molecular-weight PAHs (LMW-PAHs) dominated in shoots and roots due to their higher water solubility than high-molecular-weight PAHs (HMW-PAHs), resulting in greater uptake and translocation of PAHs into plants. The concentrations of LMW-PAHs and HMW-PAHs in the roots were two to three times lower than the soil concentrations. LMW-PAH concentrations in shoots were four to five times lower than the respective soil concentrations. The authors suggested that soil-to-plant transfer is one of the major pathways of PAH transport into shoots and roots of plants grown in wastewater-contaminated soils.
- Su and Zhu (2008) investigated the distribution and transport mechanisms of three PAHs (naphthalene, phenanthrene, and pyrene) in rice-seedling/water/ soil systems. They also investigated the observed effect of rice rhizosphere on PAH removal from soils. The PAH concentrations in soils exhibited little toxicity to rice seedlings, as both root and shoot biomasses did not show significant changes when seedlings were exposed to increasing concentrations of the three PAHs, in comparison to the control. Concentrations of PAHs in rice roots were higher than those in shoots. The ratios of PAH concentrations in roots to those in soils or external solutions increased with increasing log K_{ow} values of the compounds. The authors suggested that the transport of contaminants from roots to shoots through xylem contributed little to the accumulation of PAHs in shoots. PAH uptake by rice shoots appeared to occur mainly via direct uptake from the atmosphere. Results from the study indicated that contributions of plant uptake and rhizosphere effects are relatively insignificant in removing naphthalene, phenanthrene, and pyrene from soils. The authors concluded that rice roots have a lesser rhizosphere effect on PAH removal than other plant species such as ryegrass or white clover.

PAH Uptake in Plants: Phytoremediation Studies

The successful application of plants to clean up soils and sediments contaminated with petroleum compounds has been well documented in the published literature (Lu et al. 2010). A variety of plant species, including sorghum (Nedunuri et al. 2000), ryegrass (Nedunuri et al. 2000; White et al. 2006), fescue (Hutchinson et al. 2001; White et al. 2006), Bermuda grass (Hutchinson et al. 2001), pine (Palmroth et al. 2006), and poplar (Palmroth

et al. 2006), increase the degradation of hydrocarbons (Lu et al. 2010). Harvey et al. (2002) described the ready adsorption of all PAH congeners on the root surface, but absorption into the root is extremely limited and highly variable, depending on the species and environmental conditions. HMW-PAHs are extremely water-insoluble and partition preferentially into the humic fraction of soils rather than the aqueous phases. The authors noted that degradation of certain PAHs occurs in the rhizospheres of the various plants. They concluded that plants both stimulate microbial degradation and have the ability to mobilize and accumulate hydrophobic pollutants from the rhizosphere soils through the transpiration stream. Additional studies are summarized below.

- Early research on phytoremediation was conducted by Aprill and Sims (1990). They examined the beneficial effects of eight prairie grasses on biodegradation of PAHs (benzo(a)pyrene, benz(a)anthracene, chrysene, and dibenz(a,h)anthracene) in a greenhouse experiment. All eight plant species were present in each pot, except in unvegetated controls. The removal of PAHs was significantly greater from vegetated soils than from unvegetated soils after 150 days of incubation, and the rate of disappearance was related directly to water solubility.
- Reilley et al. (1996) investigated the effect of vegetation on anthracene and pyrene in the soil environment. They reported significantly enhanced dissipation (probably through biodegradation) of anthracene and pyrene in the presence of plants. They suggested that this is most likely the result of carbon exudation from plant roots into the rhizosphere, which supports an increased microbial population. Enhanced degradation of the four-ring PAH, pyrene, in vegetated soils suggested that degradation of other PAHs with four or more rings, such as the carcinogens benz(a)anthracene and benzo(a)pyrene, may also be enhanced in rhizosphere soils.
- Binet et al. (2000) investigated the fate of PAHs in the rhizosphere and mycorrhizosphere of plants, via mechanisms that included biodegradation, uptake, and adsorption. Experiments were conducted with ryegrass (inoculated with or without mychorrizae) cultivated in pots filled with soil spiked with anthracene or a mixture of eight PAHs. In both experiments, 36%–66% of the initial extractable PAH concentrations were dissipated, 0.006%–0.11% were adsorbed to roots, 0.003%–0.16% were found in root tissue, and 0.001% were found in shoot tissue. The authors stated that the major portion of the PAH dissipation in rhizosphere soil was due to biodegradation or biotransformation. For non-mycorrhizal plants, anthracene and PAH phytoextraction was accomplished mainly through adsorption. Accumulation in root tissue was limited, and only traces were found in shoot tissue. In mycorrhizal plants, anthracene and PAH were less adsorbed to roots, and shoot tissue concentrations were lower than in non-myccorhizal plants.
- Gao and Ling (2006) studied the uptake of phenanthrene and pyrene by ryegrass from either soil or water. Root concentrations of phenanthrene and pyrene for ryegrass uptake were larger than shoot concentrations, regardless of the system (soil/plant or water/plant). However, root and shoot concentrations for ryegrass

uptake from culture solution were always much higher than those for ryegrass uptake from soils, indicating that PAHs in culture solution are more available for uptake by plants than those in interstitial water in soil (Gao and Ling 2006).

- Uptake of three PAHs (naphthalene, phenanthrene, and pyrene) from soils by ryegrass, white clover, and soybean were invested in an 8-week pot experiment by Yang et al. (2007). They observed that vegetation had no significant effect on the naphthalene and phenanthrene concentrations in soil, and suggested that the major dissipative pathway for those two PAHs was likely evaporation into air, because it was demonstrated that both mineralization of PAHs and transport from roots to shoots in plants were minimal and slow. In contrast to naphthalene and phenanthrene, vegetation significantly enhanced the dissipation of pyrene. Pyrene accumulation by ryegrass, soybean, and white clover was small compared to the pyrene decreases in soils (<0.01% of total dissipation). The authors suggested that pyrene dissipation occurs mainly via microbial degradation or mineralization. Plants could stimulate microbial activity by releasing root exudates, which may lead, in turn, to the enhanced degradation of persistent organic chemicals such as PAHs.
- Xu et al. (2009) suggested that translocation of pyrene from roots to shoots is still ambiguous and that the impact of these processes has not been clearly established. These authors conducted a pot experiment to investigate the potential for phytoremediation of pyrene from spiked soils using white clover. Results indicated that white clover showed no sign of stress, and treatments and controls produced similar biomass. The authors reported that the residual pyrene concentrations in the contaminated soils were much lower than the initial values. Also, pyrene remaining in the vegetated soils was significantly lower than that in the non-vegetated soils. They suggested that plant-enhanced dissipation of soil pyrene may be predominantly the result of plant-promoted microbial degradation, whereas direct uptake and accumulation of pyrene by white clover was very small compared to the microbial degradation pathway.
- Cheema et al. (2010) investigated the capability of four plant species (tall fescue, ryegrass, alfalfa, and rape seed) to degrade phenanthrene and pyrene in spiked soil. After 65 days of plant growth, results showed that the presence of vegetation significantly enhanced the dissipation of phenanthrene and pyrene from contaminated soils. Higher PAH degradation rates were observed in the combined-plant cultivation compared to single-plant cultivation. According to Cheema et al. (2010), the contribution of direct plant uptake and accumulation of phenanthrene and pyrene was very low compared to the plant-enhanced dissipation. These authors concluded that plant-promoted biodegradation was the predominant contribution to the removal of PAHs from soil.
- Lu et al. (2010) performed a 5-month greenhouse study to evaluate the effectiveness of goosegrass in phytoremediation of petroleum-contaminated soils and to investigate the fate of hydrocarbons in soil and plant tissue. In the planted

treatments, 32% of PAHs were removed. In contrast, only 5% of the PAHs were dissipated in the unvegetated treatment. In this study, there was no significant uptake of PAHs by goosegrass; the accumulation of total measured PAHs in the plant tissue was 1.4% of that present in the soil, suggesting that the major part of PAH dissipation in the rhizosphere soil was due to biodegradation or biotransformation.

- Gao and Zhu (2004) reported that shoot concentrations in plants grown in soils treated with phenanthrene or pyrene were much lower than root concentrations. Although shoot accumulation of phenanthrene and pyrene consistently increased with increasing soil concentrations, the concentrations of the compounds were statistically far lower than in the roots. Plant off-take of phenanthrene and pyrene accounted for less than 0.01% of dissipation enhancement for phenanthrene, and for 0.24% for pyrene, in planted versus unplanted control soils. Gao and Zhu (2004) suggested that plant-promoted biodegradation was the predominant contribution to enhanced remediation of soil.

CONCLUSIONS

The objective of this literature review was to determine whether PAHs are taken up from soils into vegetation. Published studies that examined the usefulness of plants for phytoremediation of PAH-contaminated soils were reviewed, as well as studies that examined the potential contamination of crops such as vegetables and fruits via uptake of PAHs from soils.

The majority of studies related to the transfer of PAHs from soils to crops suggested that root crops, such as potatoes and carrots, adsorb PAHs from the soil onto the root. Results indicate that crop roots are more likely to contain PAHs than the shoot portions; generally, the peels of root crops contain higher concentrations than the cores. This suggests that direct contact with soil is responsible for PAH concentrations in crops, and not translocation from soil to the edible portions. In addition, rice crops appear to be take up PAHs in their shoots via direct uptake from the atmosphere, not from the soil.

With regard to uptake of PAHs into leafy crops such as lettuce research has indicated that PAH concentrations in the leafy tissues are related to direct contact of crops with PAH-contaminated soil and not from direct uptake from soil into the plants. As Collins et al. (2006) described, uptake of PAHs in leafy portions is more likely due to atmospheric deposition than to root uptake from soil. Hydrophobic organic compounds (HOCs) are strongly bound to soil, and in particular with soil organic matter, and only a very limited fraction would be expected to be available for plant uptake (Tao et al. 2009). After uptake by roots, these compounds partition strongly onto the root epidermis and are therefore poorly translocated to shoots (Tao et al. 2009). As a consequence, HOCs in above-ground plant tissues are considered to be derived mainly from the atmosphere (Tao et al. 2009).

This conclusion corresponds with the findings of Wild et al. (2005). These authors used excitation microscopy to visually track the uptake and movement of anthracene and phenanthrene from a growth medium into living roots of maize and wheat over a 56-day period. They suggested that the longitudinal movement of both compounds was not observed to extend beyond the root base into the stem or vegetative parts of the plant. Wild et al. (2005) observed that the degradation of anthracene to partial breakdown products occurred directly in the zones of root elongation.

The second part of this review focused on published papers regarding bioremediation potential. A variety of greenhouse experiments were conducted using soils contaminated with PAHs, wherein some were planted with vegetation and others were not. The results of this type of research suggest that the amount of PAHs taken up by roots and shoots is minimal, and instead of removing PAHs from soils through uptake into plant tissues, concentrations of PAHs in the soils decrease or dissipate through biodegradation or biotransformation processes in the rhizosphere.

In conclusion, crops and vegetation are more likely contaminated with PAHs due to direct soil contact or atmospheric deposition of PAHs onto plants. A large body of research indicates that the roots of plants create microbial conditions that are conducive to dissipating PAHs from soil through biotransformation or biodegradation, as opposed to taking PAHs up into the roots and shoots.

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REVIEW OF U.S. DEPARTMENT OF DEFENSE RECORDS OF DECISION

The goal of this research project is to understand the soil–chemical interactions and the potential for human exposure to polycyclic aromatic hydrocarbons (PAH) in soil. Defining those objectives, however, is complicated by the fact that the term "PAHs" refers to a class of compounds comprised of over 100 individual chemicals of varying chemical characteristics and toxicity. The U.S. Environmental Protection Agency (EPA) has historically regulated 16 PAHs as priority pollutants, of which only 7 have relative potency factors (RPFs) and are considered carcinogens. EPA is currently considering expanding the list of PAHs with RPFs to include 18 additional PAHs (refer to Appendix A for details). To best target this research effort for decision-making at U.S. Department of Defense (DOD) sites, an initial effort was undertaken to identify the specific PAHs that are driving human health risks at DOD sites, and (if possible) to anticipate which PAHs may be future risk drivers. This was accomplished by compiling data on PAH concentrations in soil at DOD sites from recent records of decision (RODs) and screening these data against residential soil screening criteria, which then enabled the extent to which individual PAHs are risk drivers at DOD sites to be assessed.

The methods used in this evaluation and the results are provided below, followed by a discussion of the implications of the analysis. As an additional analysis, the data compiled for this effort were evaluated to identify the types of site activities that have led to PAH contamination in soils.

METHODS

The record of decision system (RODS) database is maintained by EPA and contains full-text RODs. A ROD provides the justification for the remedial action (treatment) chosen at a Superfund site. It also contains site history, site description, site characteristics, community participation, enforcement activities, past and present activities, contaminated media, the contaminants present, scope and role of response action, and the remedy selected for cleanup[1].

[1] See http://www.epa.gov/superfund/sites/rods/

On January 11, 2011, the RODS database[2] was searched for information on reported PAH concentrations at DOD sites and included search criteria for all states for fiscal years 2009 and 2010.

Eleven RODs were identified that contained data on PAH concentrations in surface soils, subsurface soils, or both. The 11 corresponding DOD installations are located in California, New York, New Jersey, Maryland, Florida, Virginia, Pennsylvania, Massachusetts, and Wyoming. Some RODS contained more than one exposure area (typically termed exposure points in the RODs). For the purposes of this analysis, each exposure area was treated as a separate location, and therefore 22 separate locations were included. However, when only surface soil data were considered, the data set included only 18 separate locations.

Each ROD was reviewed to find information on individual (as opposed to total) PAH concentrations in either surface or subsurface soil media. If the ROD contained PAH data, then the data was extracted into tables. Sometimes both surface and subsurface soil concentrations were reported, and in those cases both types of data were extracted. Average and minimum concentrations were not always reported in the RODs, and therefore only maximum soil concentrations were used in this analysis. A flow chart of the screening process is depicted in Figure C-1.

Screening Conducted Using Current Criteria

The soil concentrations were compared to health-based soil screening criteria specific to PAHs, available from EPA's regional screening levels (RSL) for chemical contaminants at superfund sites[3] and are presented in Table C-1. The residential soil screening levels from the regional screening levels summary table were used. Three of the PAHs that were reported in the DOD RODs did not have available screening criteria (acenapthylene, benzo[g,h,i]perylene, and phenanthrene), therefore screening was not conducted on those PAHs.

The maximum soil concentrations for individual PAHs reported for each DOD site were divided by the current residential screening criteria presented in Table C-1 to assess the magnitude of exceedance. Both surface and subsurface soil concentrations were evaluated (e.g., one screening was performed using only surface soil data and another was performed using both surface and subsurface soil data). Values less than 1 were excluded because only those results that are greater than 1 indicate a PAH concentration that is of potential

[2] See http://www.epa.gov/superfund/sites/rods/

[3] Available at http://www.epa.gov/region9/superfund/prg/index.html

concern for human health risk. Based on maximum soil concentrations, the results were averaged across all sites and are presented in Figures C-2 and C-3. The results are also presented as the percent of sites where the maximum soil concentration exceeded the soil screening criteria for individual PAHs (Figure C-4 for surface and subsurface soil and Figure C-5 for surface soil only).

Screening Conducted Using Proposed Criteria

Additional screenings, similar to those described above, were conducted using EPA's proposed residential soil screening values, which are discussed in Section 2.1 of the Work Plan and reviewed in detail in Appendix A. These proposed residential screening criteria are more conservative than the current screening criteria and are listed in Table C-2.

As with the screening against current criteria, one screening was performed using only surface soil data, while another screening was performed using both surface and subsurface soil data. Figures C-6 and C-7 illustrate the magnitude of proposed screening criteria exceedances for the individual PAHs averaged across all sites (surface and subsurface versus surface soils only, respectively), while Figures C-8 and C-9 illustrate the percent of sites exceeding the proposed residential soil screening criteria.

RESULTS

The location of the 11 sites for which PAH data were found, and the general types of activities conducted at those locations are as follows:

- F.E. Warren Air Force Base, ROD #WY5571924179.—The spill Site 8-wash rack area was used for cleaning vehicles. Site EAOF04 was established as a demilitarization and disposal area for ordnance, equipment, and chemical warfare agents, white phosphorus, chlorinated solvents, and metals.
- Naval Weapons Industrial Reserve Plant, ROD #MA6170023570.—Site 4 BTEX Plume contamination is due to a combination of the former transportation building operations and a 7600 gallon leaking underground storage tank. The building was used for equipment storage and vehicle maintenance. Some waste petroleum may have been released to the ground from garage operations.
- Site 8, Ore Storage Area used for Naval Support Activity, ROD #PA3170022104.—This area was used for storage of various ore piles including chromium, manganese, kyanite, and aluminum oxide.
- South Base, Edwards Air Force Base, ROD #CA1570024504.—Site 14 was used as a fire-fighting training facility, Site 29 was an abandoned sanitary landfill, and Site 5 was a former waste storage area for petroleum, oil, and lubricants.

- Seneca Army Depot, ROD #NY021320830.—SEAD-59 was used for disposal of construction debris and oil sludge. The SEAD-59 Stockpile was where vehicles and materials were staged, including roads and grounds debris. SEAD-71 was an alleged paint disposal area. SEAD-71 Fenced Area Excluded was an area that contained construction debris, including sheet metal, asphalt, chain-link fencing, stone, piping, railroad ties, wood, and cinders.
- Naval Air Station, Patuxent River, ROD #MD7170024536.—Operable Unit 2 (Area 4b) was a former fire-fighting training area. Operable Unit 3 (Area 4c) encompassed former disposal trenches. Site 4 was a waste and debris disposal area between 1943 and 1960. Throughout the site, waste and debris were placed either on the ground surface or in long narrow trenches. Waste included miscellaneous station waste, construction debris, sewage sludge, petroleum, oil, and lubricant products, paints, thinner, solvents, pesticides, and laboratory wastes.
- Langley Air Force Base, ROD #VA2800005033.—Site LF-01 was a former waste disposal area; Site LF-05 is an abandoned landfill; Site LF-18 is a former disposal area located adjacent to NASA property, near the Munitions Storage Area; Site LF-22 is a former waste disposal area; and Site FT-41 is a former fire-training area.
- Naval Air Station Jacksonville, ROD #FL6170024412.—The site consists of a former pesticide mixing, usage, and storage area, and a former pesticide underground storage tank.
- Andrews Air Force Base, ROD #MD0570024000.—Site FT-03 was used for fire-training activities from 1959 until 1972. Hazardous flammable materials such as waste oil, jet fuel, paint thinner, and other liquid wastes were stored in drums.
- Picatinny Arsenal, ROD #NJ3210020704.—Site 61 was originally used for photographic laboratory, laboratory equipment storage, and ammunition sampling. Site 104 was used for propellant and ammunition analyses.
- U.S. Army Garrison, Aberdeen Proving Ground, ROD #MD2210020036.—New O-Field was an active site from the 1950s through the late 1970s as a destruction, demilitarization, disposal, and training area. Burning operations were conducted in trenches in the northern portion of the open field.

Screening Results Based on Current Screening Values

The average screening results for all sites using current residential soil screening criteria indicate that benzo[a]pyrene (BaP) had the greatest magnitude of criteria exceedances. This was true when subsurface soils were either included or excluded (Figures C-2 and C-3, respectively). Dibenz[a,h]anthracene exceeded current screening criteria to the second greatest extent, followed by benz[a]anthracene, indeno[1,2,3-c,d]pyrene, and benzo(b)fluoranthene (Figures C-2 and C-3).

Based on the DOD sites for which PAH data were available in the RODS database for fiscal years 2009 and 2010, the average percentage of sites where current residential screening criteria were exceeded was greatest for BaP, followed by benz[a]anthracene, benzo(b)fluoranthene, indeno[2,3-c,d]pyrene, and dibenz[a,h]anthracene (Figure C-4). For each of those PAHs, the screening criteria were exceeded at more than 50 percent of the sites. The rank ordering of PAHs were identical when subsurface soils were included in the analyses and when they were not (Figures C-4 and C-5, respectively).

Screening Results Based on Proposed Screening Values

The average screening results for all sites using proposed screening criteria indicate that dibenz[a,h]anthracene had the greatest extent of criteria exceedances. This was true when subsurface soils were included or excluded in the screening (Figures C-6 and C-7, respectively). BaP exceeded current screening criteria to the second greatest extent, followed by benzo[b]fluoranthene, benz[a]anthracene, chrysene, and indeno[1,2,3-c,d]pyrene (Figures C-6 and C-7, respectively). Again, the results were similar when subsurface soils were included and when they were excluded from the analyses.

Based on the DOD sites for which PAH data were available for fiscal years 2009 and 2010, the average percentage of sites where proposed residential screening criteria were exceeded was greatest for BaP and benzo[b]fluoranthene, followed by benz[a]anthracene and indeno[1,2,3-c,d]pyrene (Figure C-8). For each of those PAHs, the screening criteria were exceeded at more than 50 percent of the sites. The rank ordering of PAHs were identical when subsurface soils were included or excluded from the analyses (Figures C-8 and C-9).

DISCUSSION

The rank ordering of PAHs identified using the surface soil screening results were identical to the trends when both surface and subsurface soil data were used. Therefore, for simplicity, from this point forward, this analysis will not distinguish between surface and subsurface soils.

When screened against current EPA residential soil screening criteria, BaP was the overwhelming driver for risks at the DOD sites that were included in this study. However, when proposed EPA residential soil screening criteria were used, the results indicated that dibenz[a,h]anthracene became the primary driver of human health risk, due to the very conservative RPF value assigned to that PAH in EPA's proposed set of criteria. Benzo[b]fluoranthene becomes a larger risk driver under the proposed criteria, whereas it was less of a driver when the current screening criteria were used. Chrysene and

benzo[b]fluoranthene become risk drivers when the proposed criteria are used, but were virtually absent as a driver when the current criteria were used.

Over 70 percent of the sites identified in this ROD search exceeded residential soil screening criteria for BaP, benz[a]anthracene, and benzo[b]fluoranthene, independent of whether current or proposed EPA RPF values were used.

Figures C-3 and C-5 present a synthesis of the findings for the magnitude of current risk-based screening criteria exceedances and the percentage of sites that exceed those criteria for individual PAHs. In summary, this analysis indicates that the current primary human health risk drivers are:

- BaP
- Benz[a]anthracene
- Benzo[b]fluoranthene
- Indeno[1,2,3-c,d]pyrene
- Dibenz[a,h]anthracene

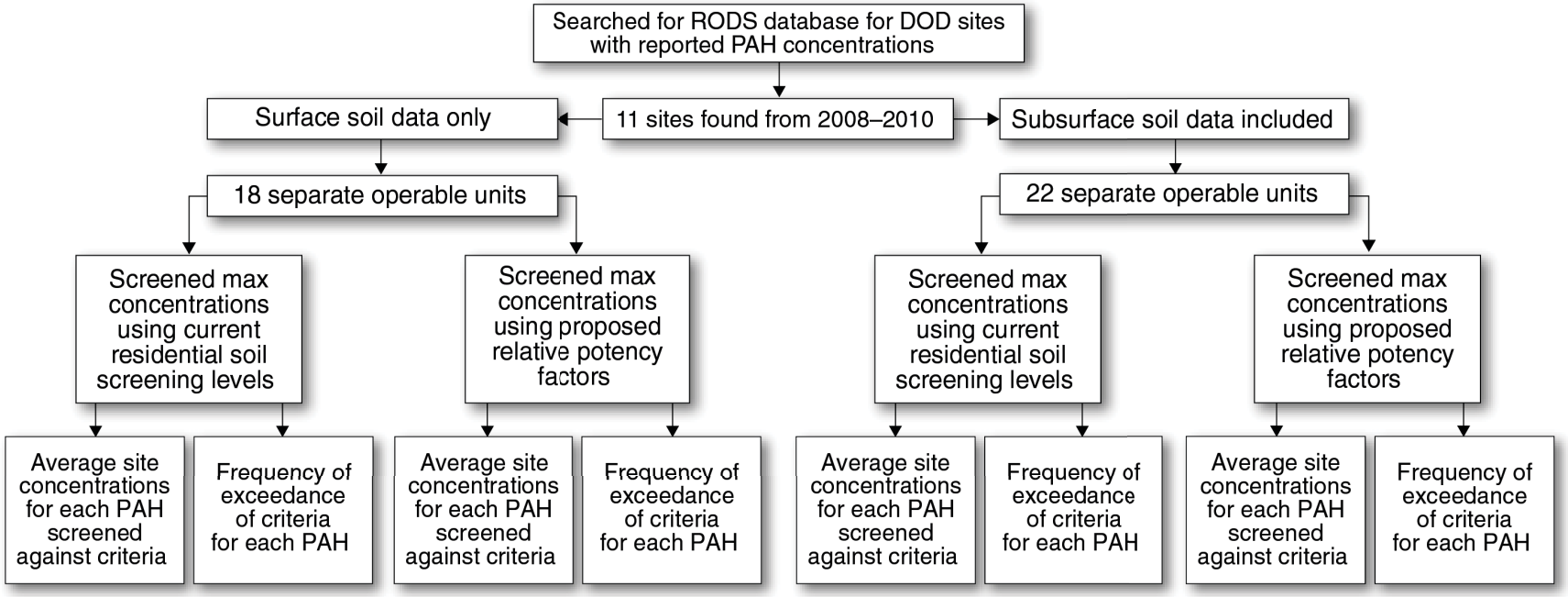
Table C-1. Current Regional Screening Levels for PAHs at Superfund Sites

PAH	Screening Concentration (mg/kg)	Endpoint
Acenaphthene	3400	Noncancer endpoint
Anthracene	17000	Noncancer endpoint
Benz(a)anthracene	0.15	Cancer endpoint
Benzo(a)pyrene	0.015	Cancer endpoint
Benzo(b)fluoranthene	0.15	Cancer endpoint
Benzo(k)fluoranthene	1.5	Cancer endpoint
Chrysene	15	Cancer endpoint
Dibenz(a,h)anthracene	0.015	Cancer endpoint
Fluoranthene	2300	Noncancer endpoint
Fluorene	2300	Noncancer endpoint
Indeno(1,2,3-cd)pyrene	0.15	Cancer endpoint
1-Methylnaphthalene	22	Cancer endpoint
2-Methylnaphthalene	310	Noncancer endpoint
Naphthalene	3.6	Cancer endpoint
Pyrene	1700	Noncancer endpoint

Table C-2. Proposed Residential Screening Criteria

PAH	Screening Concentration (mg/kg)	Endpoint
Acenaphthene	3400	Noncancer endpoint
Anthracene	17000	Noncancer endpoint
Benz[a]anthracene	0.075	Cancer endpoint
Benzo[g,h,i]perylene	1.7	Cancer endpoint
Benzo[a]pyrene	0.015	Cancer endpoint
Benzo[b]fluoranthene	0.019	Cancer endpoint
Benzo[k]fluoranthene	0.5	Cancer endpoint
Chrysene	0.15	Cancer endpoint
Dibenz[a,h]anthracene	0.0015	Cancer endpoint
Fluoranthene	0.19	Noncancer endpoint
Fluorene	2300	Noncancer endpoint
Indeno[1,2,3-c,d]pyrene	0.21	Cancer endpoint
1-Methylnaphthalene	22	Cancer endpoint
2-Methylnaphthalene	310	Noncancer endpoint
Naphthalene	3.6	Cancer endpoint
Pyrene	1700	Noncancer endpoint

FIGURES



DOD = U.S. Department of Defense
PAH = polycyclic aromatic hydrocarbon
RODS = record of decision system

Figure C-1.
Methods Used in Screening Data to Identify Which Individual PAHs are Risk Drivers at PAH-contaminated DOD Sites

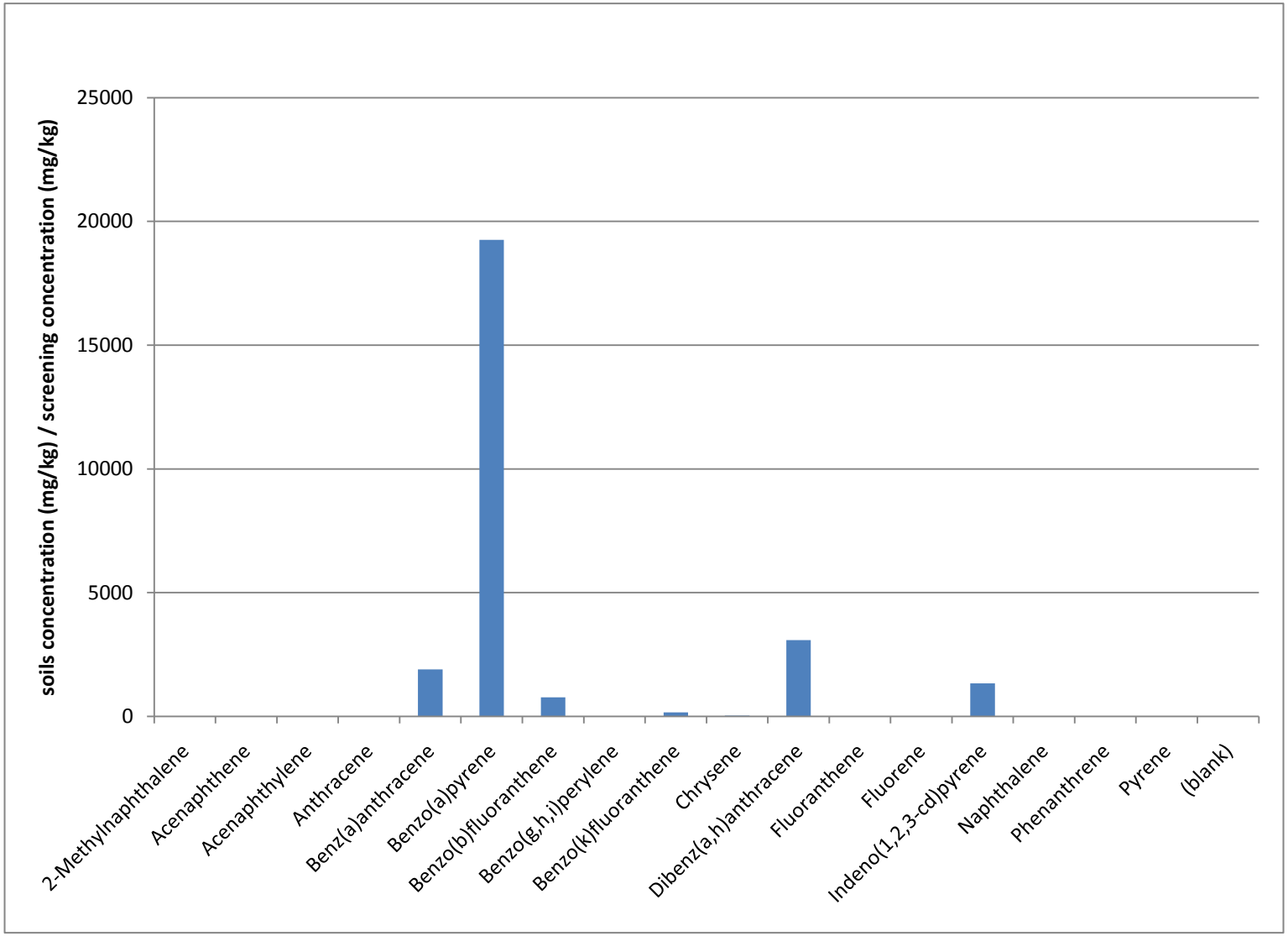


Figure C-2.
Magnitude of Screening Criteria Exceedances for Individual PAHs Using Current Criteria for Surface and Subsurface Soils

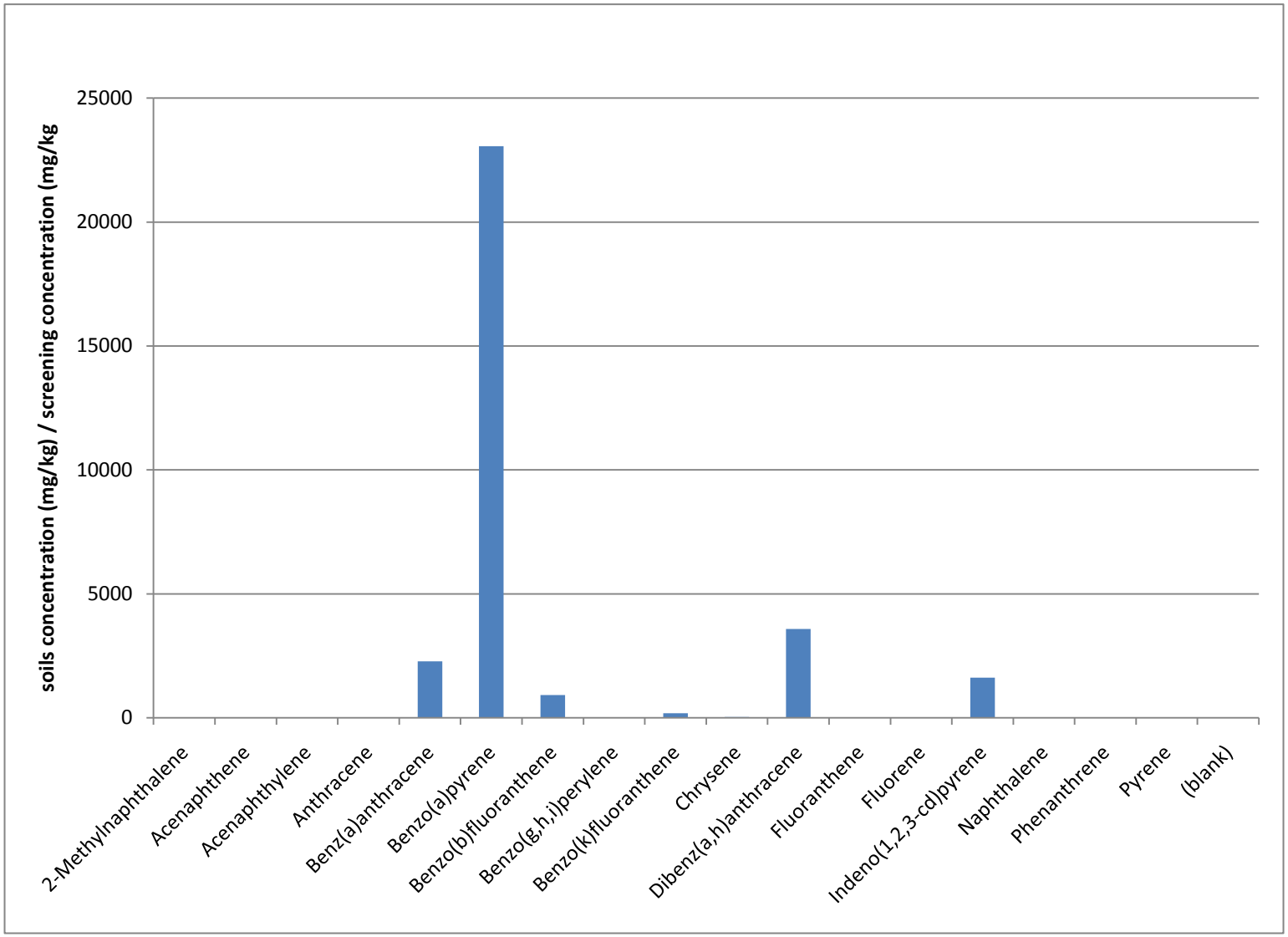


Figure C-3.
Magnitude of Screening Criteria Exceedances for Individual PAHs Using Current Criteria for Surface Soils Only

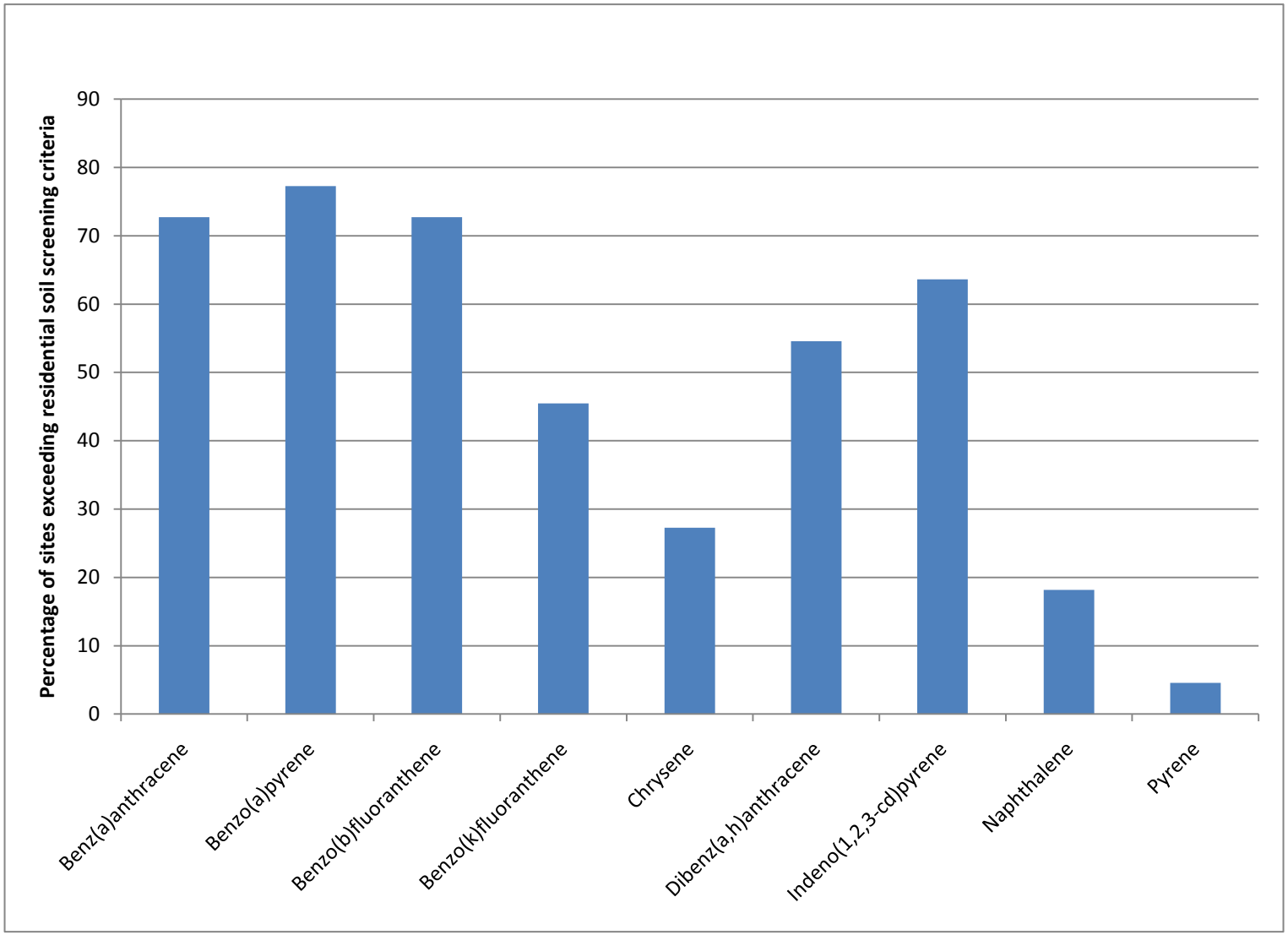


Figure C-4.
Percentage of Sites Exceeding Current Residential Screening Criteria for Surface and Subsurface Soils

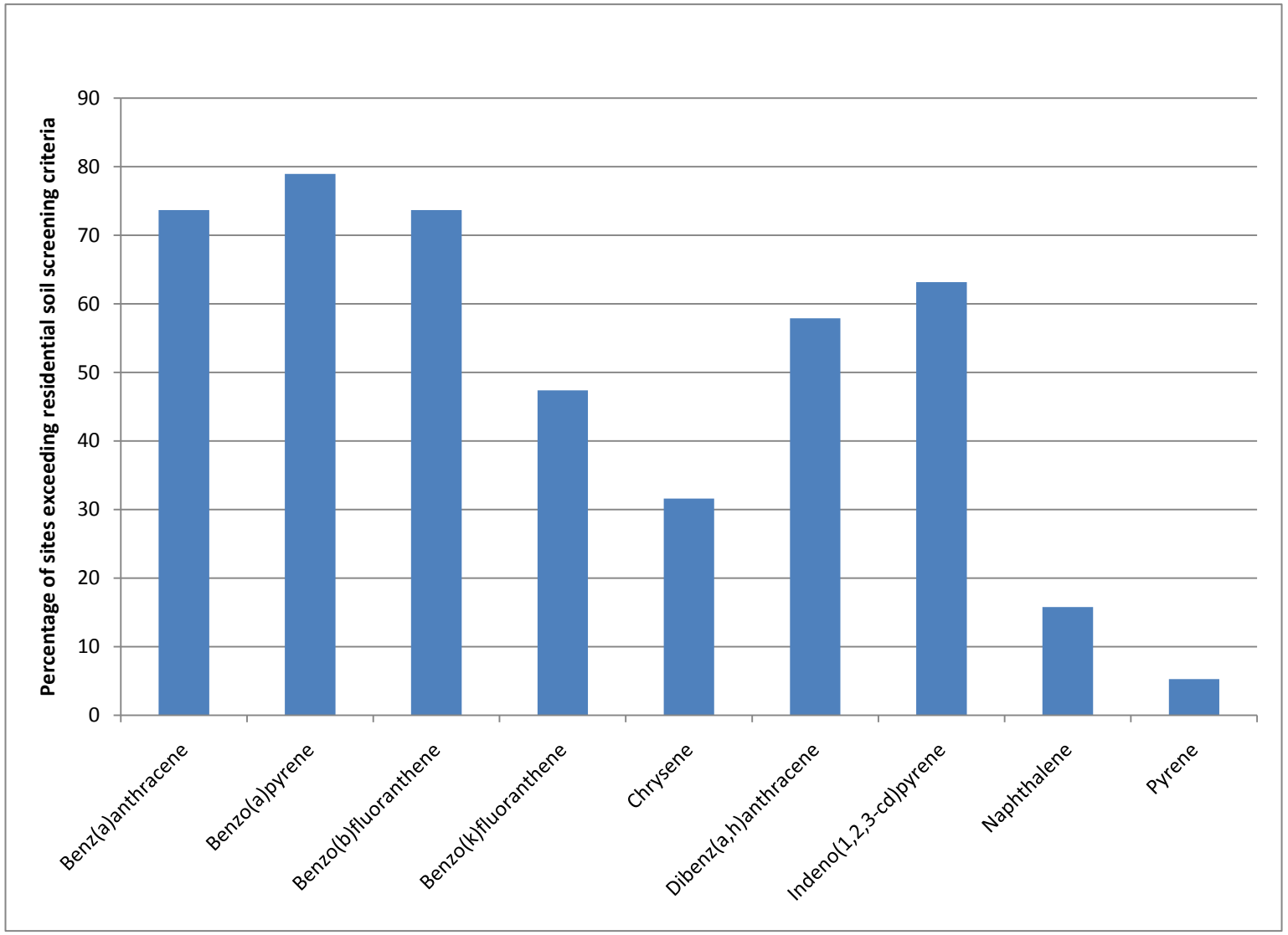


Figure C-5.
Percentage of Sites Exceeding Current Residential Soil Screening Criteria for Surface Soils Only

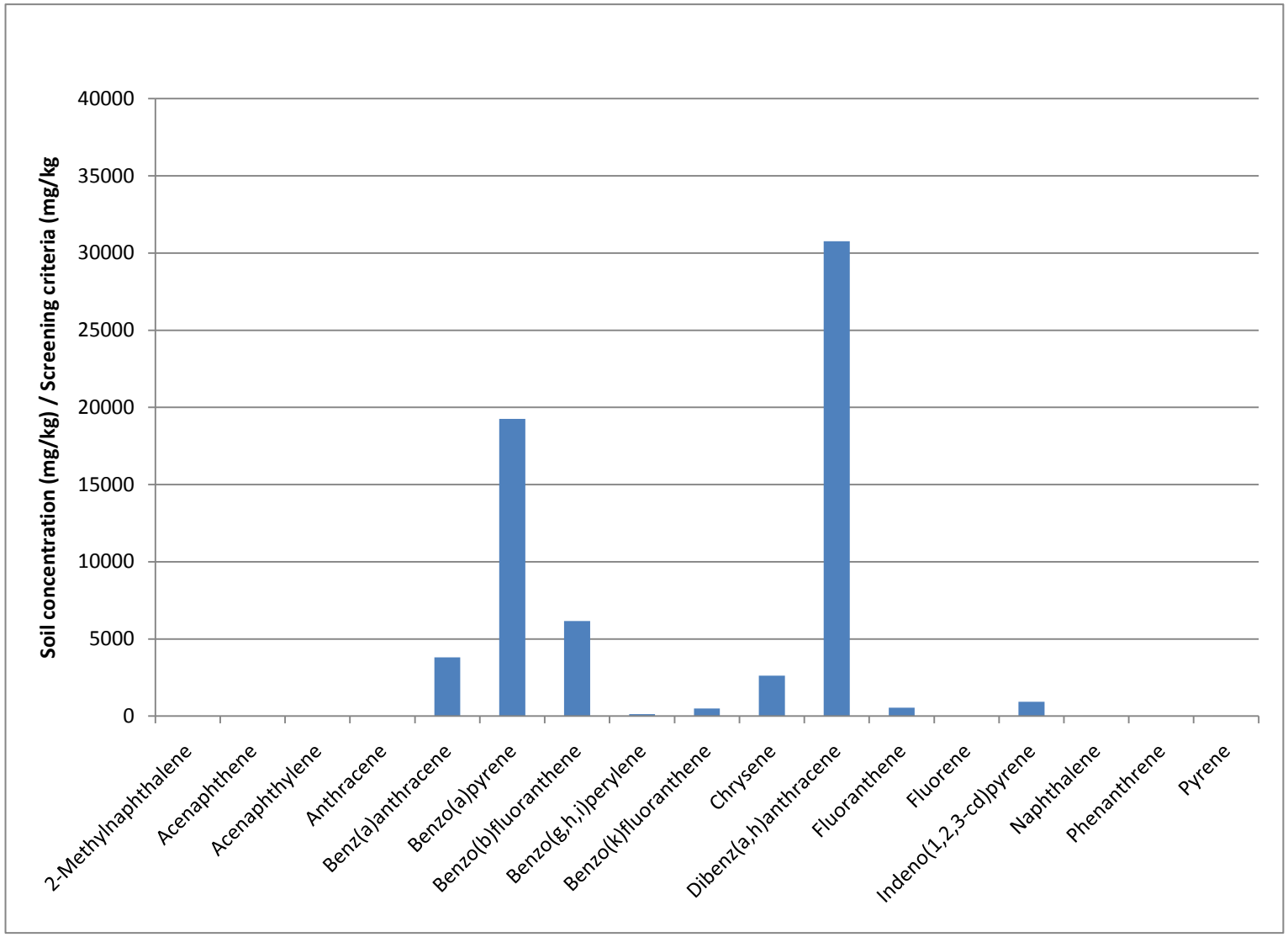


Figure C-6.
Magnitude of Screening Criteria Exceedances for Individual PAHs Using Proposed Screening Criteria for Surface and Subsurface Soils

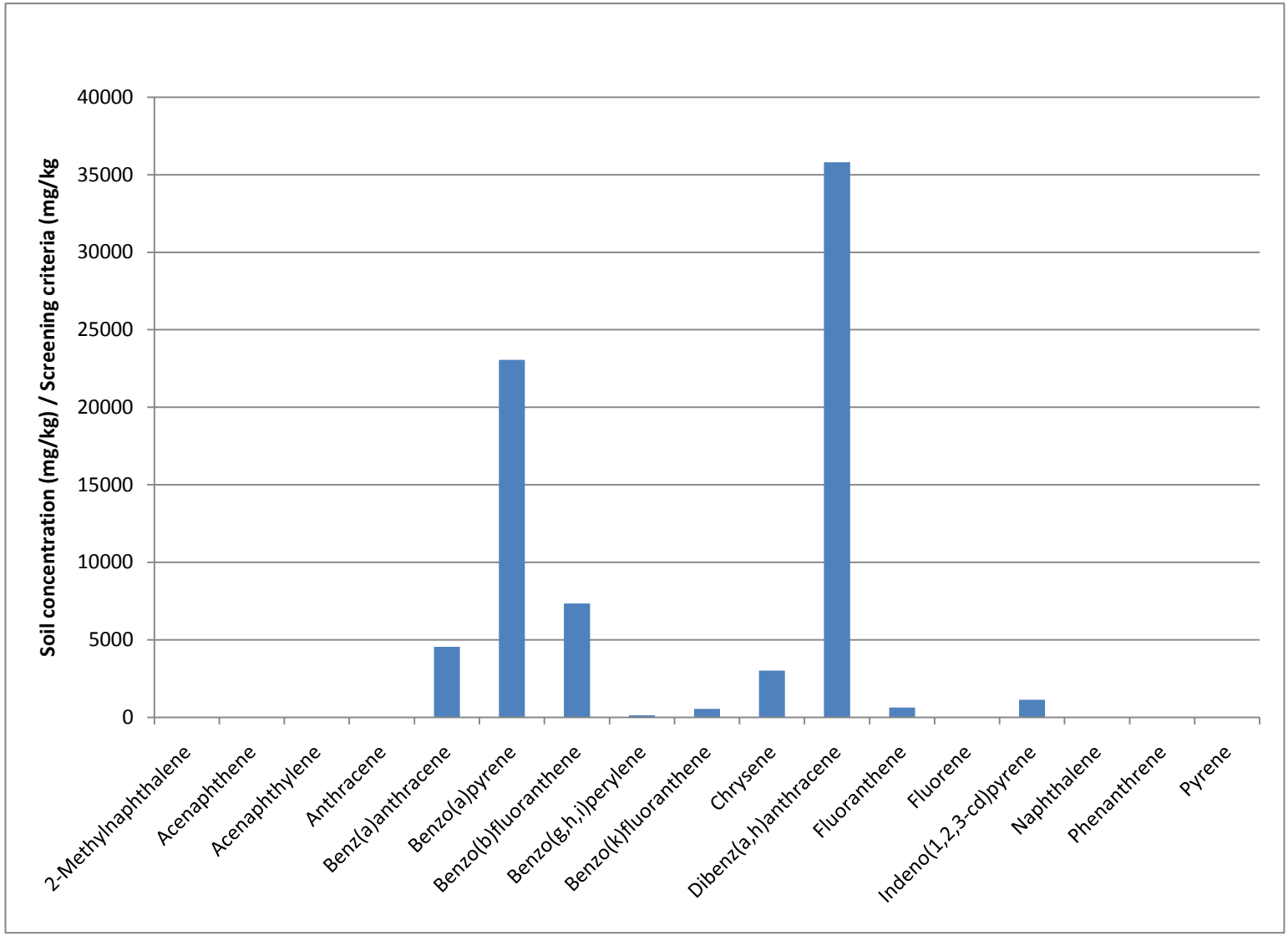


Figure C-7.
Magnitude of Screening Criteria Exceedances for Individual PAHs Using Proposed Criteria for Surface Soils Only

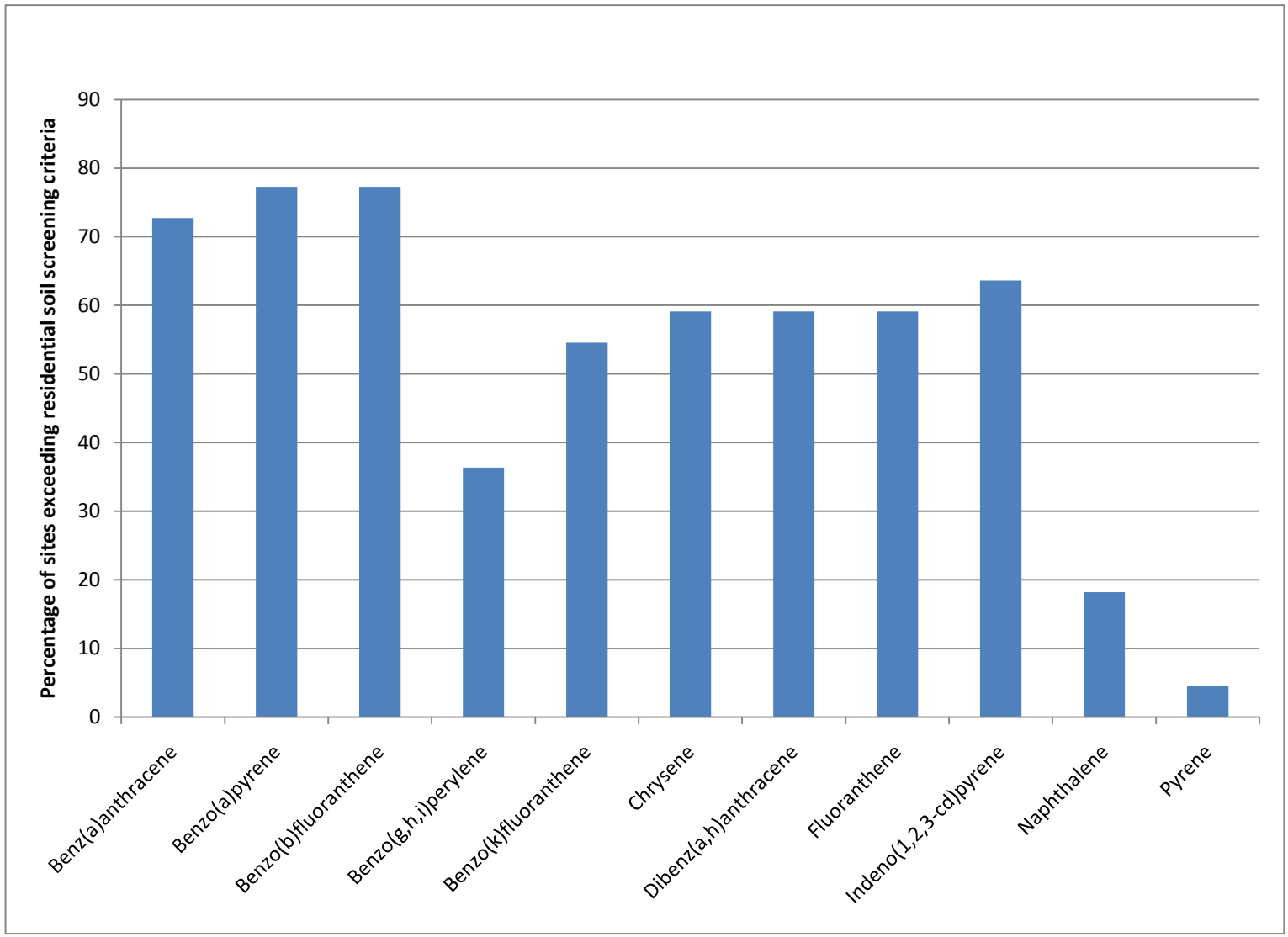


Figure C-8.
Percentage of Sites Exceeding Proposed Residential Screening Criteria for Surface and Subsurface Soils

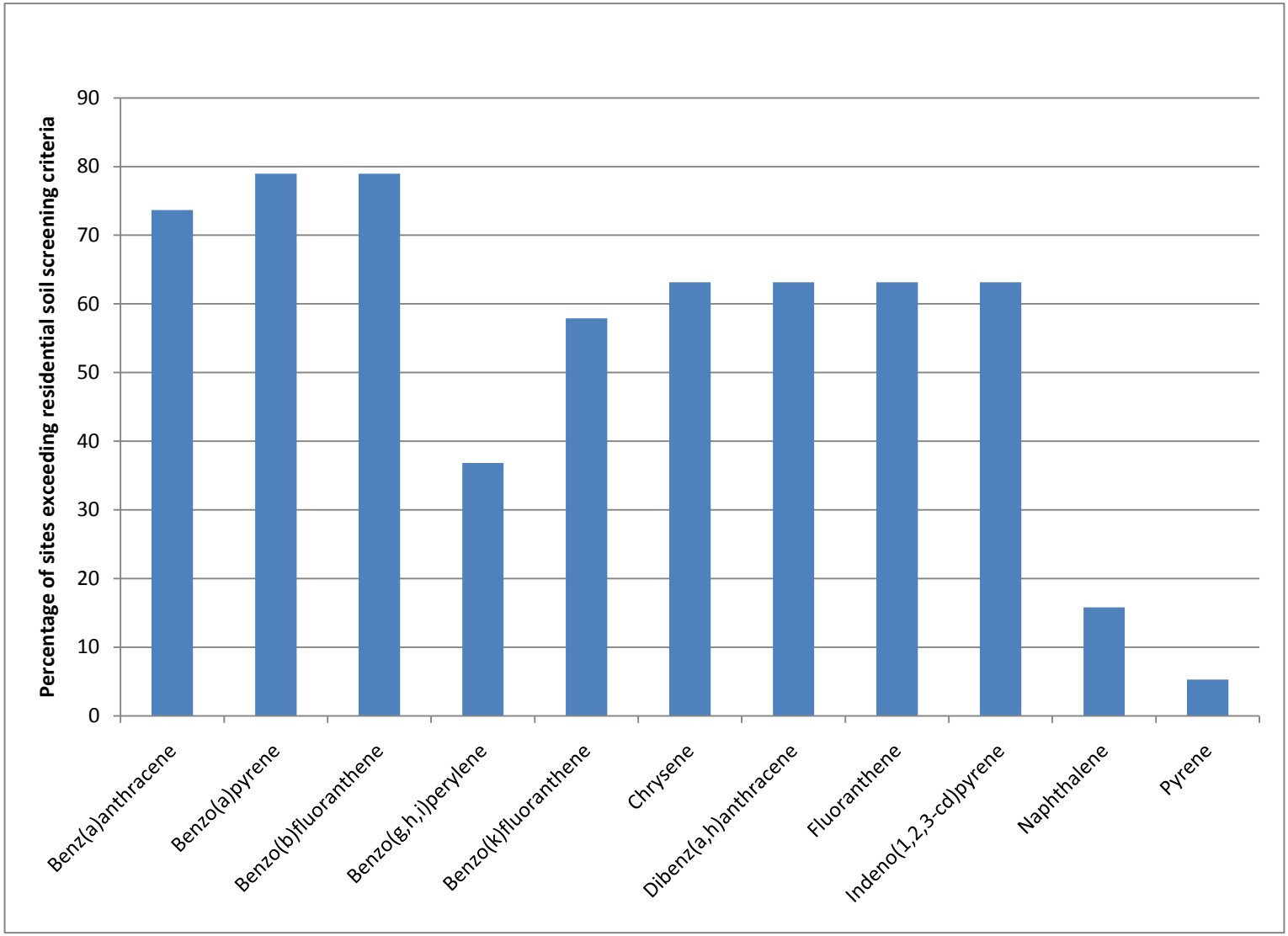


Figure C-9.
Percentage of Sites Exceeding Proposed Residential Soil Screening Criteria for Surface Soils Only

Abstract Number: 1975 — Poster Board Number: 565

Assessment of the Impacts of Changes in Regulatory Toxicology of Polycyclic Aromatic Hydrocarbons on Site Assessments

J. H. Salatas[1] [1] Exponent, Bellevue, WA
M.R. Garry[1] [2] Exponent, Boulder, CO,
Y.W. Lowney[2]

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INTRODUCTION

Evolving regulatory approaches to assessing potential health effects of polycyclic aromatic hydrocarbons (PAHs) in the environment could substantially affect site assessments and the resulting cleanup levels. The U.S. Environmental Protection Agency (EPA) has historically regulated 16 PAHs as priority pollutants, seven of which are considered carcinogenic and have relative potency factors (RPFs). However, EPA is currently considering expanding the list of PAHs with RPFs to include 18 additional PAHs.

This study was completed as part of an ongoing project under the auspices of the U.S. Department of Defense (DoD) environmental research program known as the Strategic Environmental Research and Development Plan (SERDP). The overall project goals are to

- Combine research on soil-PAH chemistry with *in vivo* measures of bioavailability across diverse soil types and contaminant sources
- Better understand PAH sequestration in soil
- Support the development of an inexpensive assay to estimate bioavailability on a site-specific basis.

This poster reports an initial effort to identify the specific PAHs that are currently driving (and historically have driven) human health risks at DOD sites. PAHs that may drive regulatory decision making in the future under EPA's proposed revisions for PAH risk assessment were identified.

METHODS

The process used to identify and review Records of Decision (RODs) for DoD sites is presented in Figure 1. Table 1 presents RPFs for PAHs that are in current use and those proposed by EPA.

Figure 1. Evaluating which PAHs drive remedial decisions

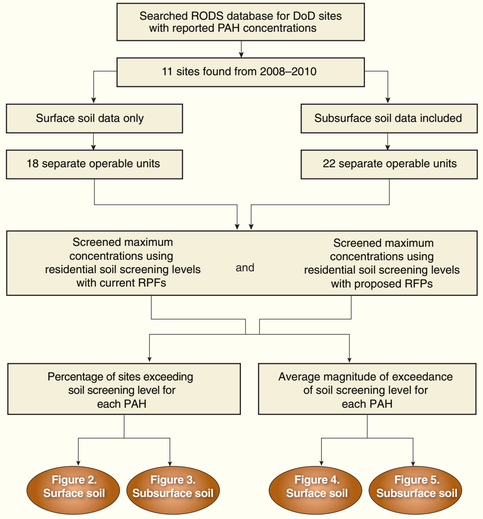


Table 1. Comparison of relative potency factors for PAHs

	U.S. EPA (1993)	CalEPA (2009)	U.S. EPA (2010) Proposed
Anthanthrene	--	--	0.4
Benzo(a)pyrene	1	1	1
Benz[a]anthracene	0.1	0.1	0.2
Benz[b,c]aceanthrylene, 11H-	--	--	0.05
Benzo[b]fluoranthene	0.1	0.1	0.8
Benzo[c]fluorene	--	--	20
Benz[e]aceanthrylene	--	--	0.8
Benzo[g,h,i]perylene	--	--	0.009
Benz[j]aceanthrylene	--	--	60
Benzo[j]fluoranthene	--	0.1	0.3
Benzo[k]fluoranthene	0.01	0.1	0.03
Benz[l]aceanthrylene	--	--	5
Chrysene	0.001	0.01	0.1
Cyclopenta[c,d]pyrene	--	--	0.4
Cyclopenta[d,e,f]chrysene, 4H-	--	--	0.3
Dibenzo[a,e]fluoranthene	--	--	0.9
Dibenz[a,j]acridine	--	0.1	--
Dibenz[a,h]acridine	--	0.1	--
7H-dibenzo(c,g)carbazole	--	1	--
Dibenzo[a,e]pyrene	--	1	0.4
Dibenz[a,c]anthracene	--	--	4
Dibenz[a,h]anthracene	1	0.34	10
Dibenzo[a,h]pyrene	--	10	0.9
Dibenzo[a,i]pyrene	--	10	0.6
Dibenzo[a,l]pyrene	--	10	30
Fluoranthene	--	--	0.08
Indeno[1,2,3-c,d]pyrene	0.1	0.1	0.07
Naphtho[2,3-e]pyrene	--	--	0.3
5-methylchrysene	--	1	--
1-nitropyrene	--	0.1	--
4-nitropyrene	--	0.1	--
1,6-dinotropyrene	--	10	--
1,8-dinotropyrene	--	1	--
6-nitrochrysene	--	10	--
2-nitrofluorene	--	0.01	--
7,12-dimethylbenzanthracene	--	21	--
3-methylcholanthrene	--	1.8	--
5-nitroacenaphthene	--	0.01	--

Notes:

U.S. EPA. 2010. Development of a relative potency factor (RPF) approach for polycyclic aromatic hydrocarbon (PAH) mixtures. Draft. February. EPA/635/R-08/012A

U.S. EPA. 1993. Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons. July EPA/600/R-93/089.

CalEPA. 2009. Technical Support Document for Describing Available Cancer Potency Factors: Methodologies for derivation, listing of available values, and adjustments to allow for early life stage exposures. Appendix A: Hot Spots Unit Risk and Cancer Potency Values. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, May. CalEPA RPFs calculated by dividing the oral slope factor listed for each chemical by the oral slope factor for benzo[a]pyrene.

RESULTS

Figure 2. Percentage of DoD sites with surface soil exceeding residential soil RSLs for individual carcinogenic PAHs

NOTE: Naphthalene has a different mode of action for carcinogenesis than B(a)P and pyrene is not carcinogenic. They are included for comparison and to show that evaluation of some PAHs will not be affected by the proposed RPFs.

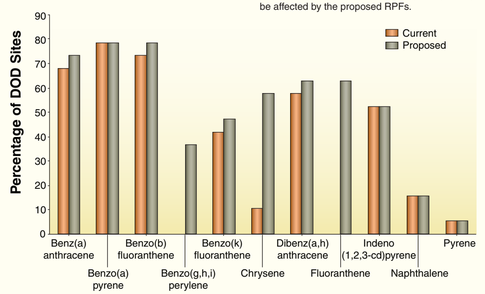


Figure 3. Percentage of DoD sites with subsurface and surface soil RSLs for individual carcinogenic PAHs

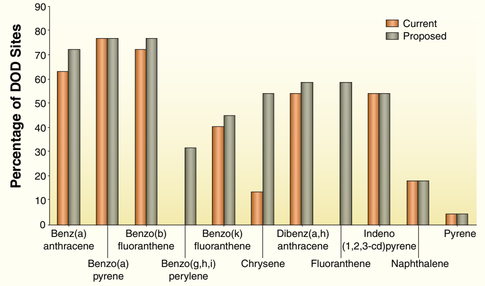


Figure 4. Magnitude of exceedance of residential soil RSLs for individual carcinogenic PAHs in surface soil at DoD sites

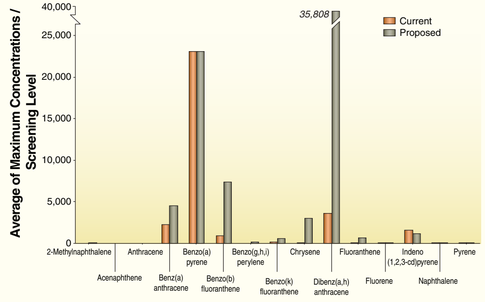


Table 2. PAH soil background concentrations and health-based screening levels derived using relative potency factors (µg/kg)

PAH	Soil Background Concentrations[a]		Residential Soil Screening Level[b]	
	Rural	Urban	Based on U.S. EPA (1993)	Based on U.S. EPA (2010)
Anthanthrene	NA	NA		38
Benzo(a)pyrene	2–1,300	165–220	15	15
Benz[a]anthracene	5-20	169–59,000	150	75
Benz[b,c]aceanthrylene, 11H-	NA	NA	--	300
Benzo[b]fluoranthene	20–30	15,000–62,000	150	19
Benzo[c]fluorene	NA	NA	--	0.75
Benz[e]aceanthrylene	NA	NA	--	19
Benzo[g,h,i]perylene	10–70	900–47,000	--	1,667
Benz[j]aceanthrylene	NA	NA	--	0.25
Benzo[j]fluoranthene	NA	NA	--	50
Benzo[k]fluoranthene	10–110	300–26,000	1,500	500
Benz[l]aceanthrylene	NA	NA	--	3.0
Chrysene	38.3	251–640	15,000	150
Cyclopenta[c,d]pyrene	NA	NA	--	38
Cyclopenta[d,e,f]chrysene, 4H-	NA	NA	--	50
Dibenzo[a,e]fluoranthene	NA	NA	--	17
Dibenzo[a,e]pyrene	NA	NA	--	38
Dibenz[a,c]anthracene	NA	NA	--	3.8
Dibenz[a,h]anthracene	NA	NA	15	1.5
Dibenzo[a,h]pyrene	NA	NA	--	17
Dibenzo[a,i]pyrene	NA	NA	--	25
Dibenzo[a,l]pyrene	NA	NA	--	0.5
Fluoranthene	0.3–40	200–166,000	--	188
Indeno[1,2,3-c,d]pyrene	10–15	8,000–61,000	150	214
Naphtho[2,3-e]pyrene	NA	NA	--	50

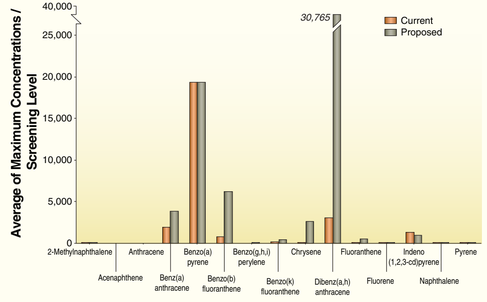
Notes:
NA - background data not available
[a]Background soil concentrations of polycyclic aromatic hydrocarbons measured in the United States (ATSDR 1995).
[b]Residential soil screening levels for PAHs that are based on the a carcinogenic relative potency factor. Screening levels based on U.S. EPA (1993) are taken directly from EPA Regional Screening Levels table. Screening levels based on U.S. EPA (2010) were calculated by dividing the benzo[a]pyrene screening level by the proposed relative potency factor.

ATSDR. 1995. Toxicological Profile for Polycyclic Aromatic Hydrocarbons. Agency for Toxic Substances and Disease Registry. August.

U.S. EPA. 2010. Development of a relative potency factor (RPF) approach for polycyclic aromatic hydrocarbon (PAH) mixtures. Draft. February. EPA/635/R-08/012A.

U.S. EPA. 1993. Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons. July. EPA/600/R-93/089.
EPA Regional Screening Levels available at: http://www.epa.gov/reg3hwmd/risk/human/rb-concentration_table/Generic_Tables/index.htm.

Figure 5. Magnitude of exceedance of residential soil RSLs for individuals carcinogenic PAHs in subsurface and surface soils at DoD sites



CONCLUSIONS

- When screened against current EPA residential soil screening levels for PAHs, BaP was the overwhelming risk driver at the DoD sites included in this study.
- Using the proposed RPFs, the number of sites exceeding screening levels increased for five PAHs: benz[a]anthracene, benzo(b)fluoranthene, benzo[k]fluoranthene, chrysene, and dibenz[a,h]anthracene.
- Two additional PAHs without current RPFs also triggered exceedances using the proposed RPFs: benzo[g,h,i]perylene and fluoranthene.
- The magnitude of exceedance would increase for all sites.

IMPLEMENTATION CHALLENGES

- **Additional analytes—** The draft guidance increases the number of carcinogenic PAHs with RPFs from 7 to 25, including BaP.
- **Treatment of undetected PAHs—** Undetected PAHs could contribute significantly to total PAH risk estimates. If one-half the detection limit is used for undetected chemicals, as is common practice, PAHs that may not be present could potentially drive risk estimates.
- **Background concentrations—** Assessing site-related PAHs is challenging because of the presence of both anthropogenic, non-point-source impacts and "natural" background levels (e.g., fires). Background data exist for a few PAHs, but are generally lacking for most of the PAHs for which EPA has developed new RPFs. In urban areas, background levels of PAHs in soil typically exceed health-based screening levels.

Acknowledgement

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Appendix B

Soil Chemical Interactions

Environmental Science & Technology

Article

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Effect of Polycyclic Aromatic Hydrocarbon Source Materials and Soil Components on Partitioning and Dermal Uptake

Huan Xia, Jose L. Gomez-Eyles,[‡] and Upal Ghosh*

Department of Chemical, Biochemical and Environmental Engineering, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, Maryland 21250, United States

Supporting Information

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 4 orders of magnitude. Source material had the greatest impact on PAH partitioning. Soils containing skeet generally exhibited the highest K_D 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. 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, highlighting the importance of using native field soils in bioavailability and risk assessments.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are often released into soils either as byproducts 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,[1−5] resulting in partition coefficients that are several orders of magnitude higher than sorption to natural organic matter (NOM).[1,6] 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.[1] 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.[4] 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 natural organic matter or adsorption onto BC.[3,7] 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, Stroo et al.[8] demonstrated for lampblack impacted soils that the estimated cancer risk was reduced by 97% when accounting for measured dermal absorption of native PAHs.

Since PAH adsorption to BC such as soot and char is nonlinear, 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.[1,9] Hong et al.[6] found that for oil and 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 the residual oil phase.

While there is general understanding that PAH bioavailability in soil can be influenced by many factors including PAH

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Table 1. Composition of Experimental Soils and Their Respective PAH Concentrations[a] (as target B(a)P levels)

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 nontmorillonite	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	-	-

[a]Concentrations shown above are target values before weathering.

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 4 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 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.

MATERIALS AND METHODS

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[10] and consisted of 10% peat moss (Miracle-Gro, Enriched Sphagnum Peat Moss), 20% kaolin clay (ACROS Organics, CAS:1332-58-7), and 70% 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 adheres to human skin.[11]

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.[12]

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 3 weeks to ensure a homogeneous distribution of PAH within the soils and weathered[13] for 8 weeks as described in the Supporting Information.

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 min (pulsing for 30 s on and 30 s 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%.

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).[14]

Soil/Source Material Aqueous Equilibrium Experiment. The freely dissolved PAH concentrations in each test soil and source material was determined by equilibrating the soils/sources in a sterile aqueous solution (containing 100 mg/L sodium azide) with 76-μm-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).[15] 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 tissue paper, and extracted in an acetone:hexane (50:50) mixture (3 × 24 h, with sequential extracts pooled). The POM extracts were then cleaned up and analyzed in the same way as the soil extracts.

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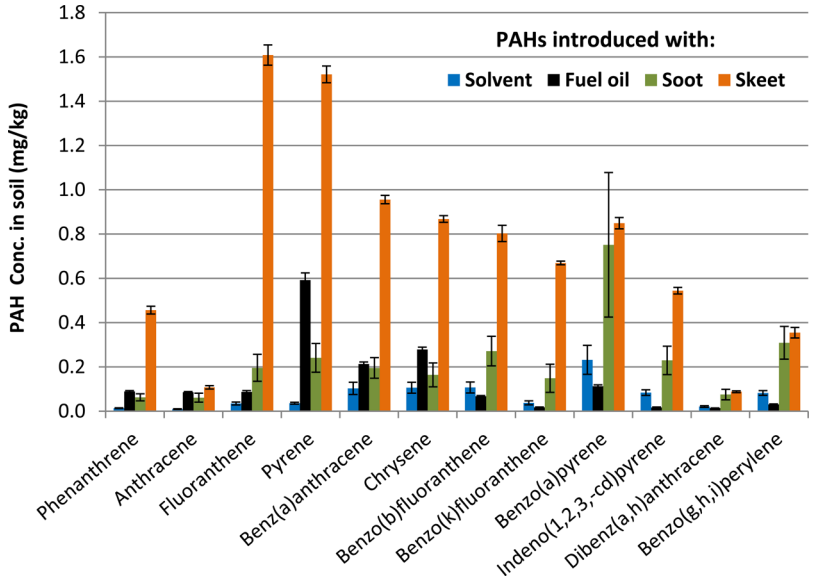

Figure 1. PAH concentrations after weathering in baseline synthetic soil prepared with different PAH source materials targeted to achieve 1 mg/kg B(a)P (n = 3, error bars represent ±1 standard deviation).

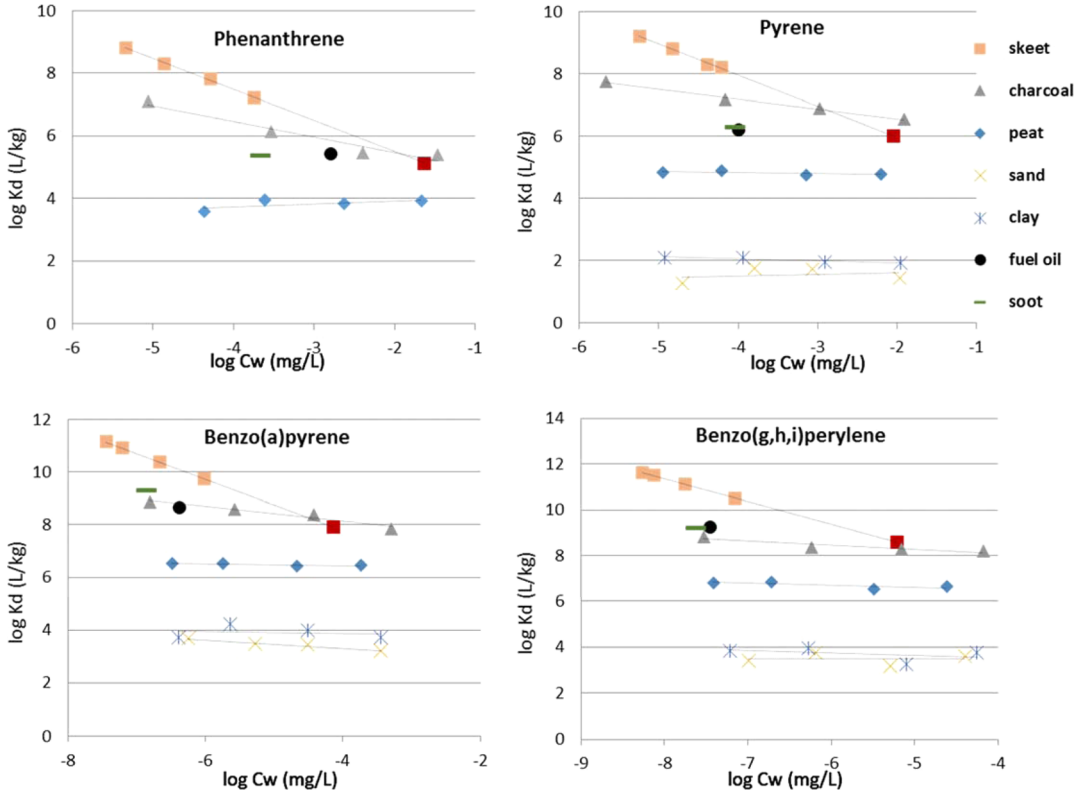

Figure 2. Comparison of measured partition coefficients (log K_D [L/kg]) for soil components and source materials for PHE, PYR, B(a)P, and B(ghi)P. The dark red square represents K_D for skeet measured with minimal depletion (at POM/skeet mass ratio of 0.125), while the orange squares represent K_D measured 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.

Freely dissolved PAH concentrations (C_W) were calculated using experimentally determined PAH partition coefficients for POM (K_{POM}): $C_W = C_{POM}/K_{POM}$;[16] equilibrium partition coefficients (K_D) for each soil were calculated using $K_D = C_{SOIL}/C_W$. The partition coefficients of the source materials (K_{skeet}, K_{soot}, and $K_{fuel\text{-}oil}$) were calculated similarly.

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

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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 cosolvent 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 4 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_i) for each soil component "i" (sand, clay, peat) was 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: $C_{charcoal} = K_{F\text{-}charcoal}\ C_W^n$, where $C_{charcoal}$ is the PAH concentration in charcoal, and Freundlich sorption coefficient $K_{F\text{-}charcoal}$ and n were estimated by fitting the model to the measured adsorption isotherm.

Dermal Uptake of PAHs from Soil. PAH dermal uptake was measured in vitro using pig skin as described in detail in the Supporting Information.

RESULTS AND DISCUSSION

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 ± 134 mg/kg and 197 ± 3 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; Supporting Information 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 both biotic and 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.[7] 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 rehomogenize fully at the scale of samples taken for PAH analysis.

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,[17] 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_{peat} values measured in this study were in line with those reported by Gidley et al. (e.g., log K_{peat} of 3.85 and 4.82 for phenanthrene and pyrene from Gidley et al.).[18] 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 $K_{charcoal}$ = 5.40−7.10 for phenanthrene) were in line with the findings on coal carbon (e.g., log K_{coal} = 6.3−6.8), fusinite charcoal (e.g., log $K_{charcoal}$ = 5.57), and biochar (e.g., log $K_{biochar}$ = 5.38−6.60) in other literature[19−21] but lower than those for activated biochar (e.g., log $K_{biochar}$ = 7.52) and activated carbon (e.g., log K_{AC} = 8.71) reported by Gomez-Eyles et al.[21]

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.[2] 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)[3,7] (Figure 2). These elevated partition coefficients are most likely the result of specific processing and extensive weathering in the field.[3] 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.[22] 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_{OIL} values close to 7.0 for PHE in both oils).[23,24] 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).

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 (Supporting Information Table S4).[18] 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).

Effect of Source Materials on Soil K_D. 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

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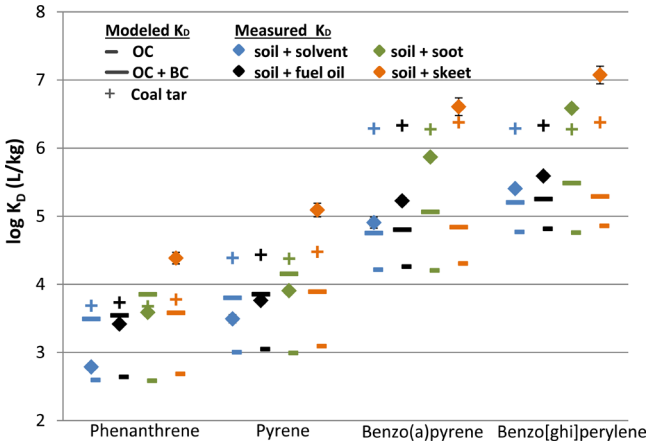

Figure 3. Comparison of measured and predicted soil K_D using a single domain model ($K_D = f_{OC}K_{OC}$; using generic K_{oc}[34] or coal tar K_{oc}[27]) and dual domain model ($K_D = f_{OC}K_{OC} + 35f_{BC}K_{BC}C_w^{n-1}$; using generic K_{BC}[35]). Soils presented in the figure are BSS soils spiked with solvent (blue), fuel oil (black), soot (green), and skeet (orange) at a target B(a)P concentration 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 capacities for black carbons in the literature.[9,25] 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.[3] 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.[3,26] 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.[27] As illustrated in the Supporting Information 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.

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%.[28] 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). The effects of mineral components are expected to be even lower when PAHs are introduced with a strong sorbing source (not studied).

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 (Supporting Information Figure S7). In the fuel oil spiked soils, we hypothesize that the charcoal surface was fouled by the excess oil hydrocarbons rendering it less effective at increasing the K_D as greater fouling effect was observed at higher oil levels (Supporting Information Figure S8). Similar fouling effects of oils on black carbons have been reported previously.[29,30] 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,[4,9] and black carbon amendment has been shown to reduce pollutant bioavailability in soils.[31−33] We show here for the first time that PAH source material sorption capacity influences the observed effectiveness of soil black carbon in increasing partitioning.

Modeling Partitioning Based on TOC and BC. As shown in Figure 3, the model predictions based merely on natural organic carbon (OC) partitioning ($K_D = f_{OC}K_{OC}$)[34] under-predicts sorption in the soils. The BC-inclusive dual domain model ($K_D = f_{OC}K_{OC} + f_{BC}K_{BC}C_w^{n-1}$)[35] 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.,[27] the predicted K_D values are improved for soot and skeet spiked soils, and fall within 1 order of magnitude of the measured K_D (see Supporting 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.

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:

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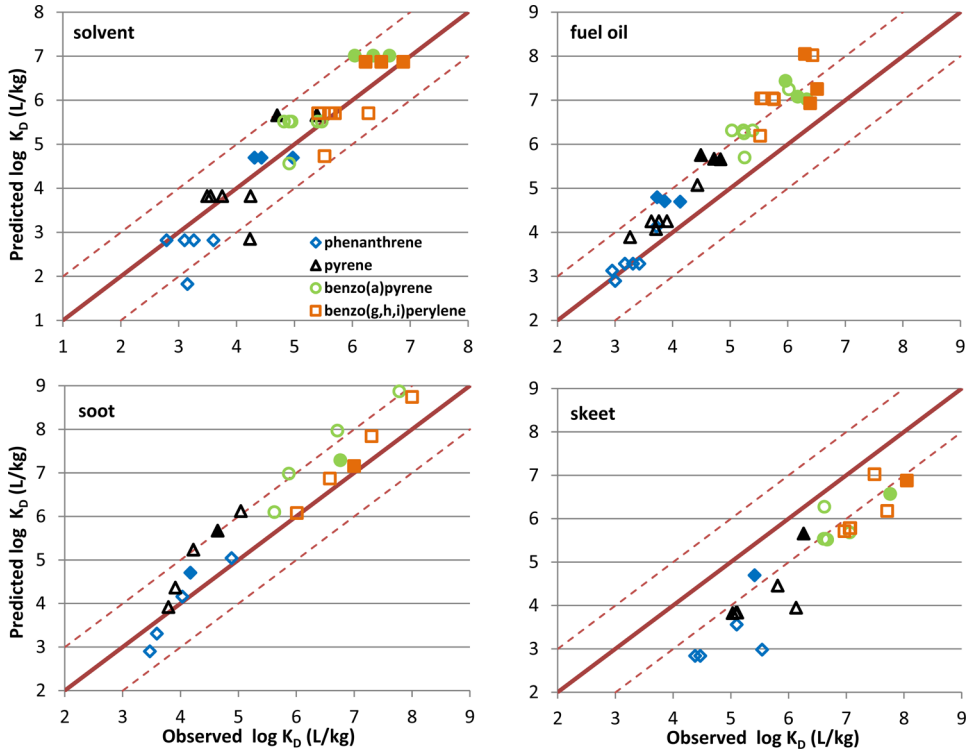

Figure 4. Comparison between predicted K_D from multidomain sorption model and observed K_D for soils with different source materials shown for PHE, PYR, B(a)P, and B(ghi)P). Solid fills for each shape represent soils with addition of 2% charcoal.

$$C_S = f_{clay} K_{clay} C_W + f_{sand} K_{sand} C_W + f_{peat} K_{peat} C_W + f_{charcoal} K_{charcoal} C_W^n + f_s K_s C_W \quad (1)$$

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 eq 1 and the overall soil 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 (Supporting Information 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.[23] 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 (Supporting Information 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). As shown in 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's 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.

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 eq 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

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of PAHs is very unlikely.[36] Ghosh et al.[36] 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_{SKEET} 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 Supporting Information Figure S11, with only 0.3% of B(a)P mass extracted from skeet (at POM:skeet mass ratio of 500), the K_{SKEET} value increased by 3 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.[1,37] 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_{SKEET} values with depletion. The PAH redistribution from skeet to soil is much less after considering the highly nonlinear behavior of K_{SKEET} 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.

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.[38,39] 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 the absorption rate is influenced by PAH sources in soil (see Supporting Information). As shown in Figure 5a, the dermal uptake flux for soils with PAHs 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, most dermal uptake studies relate uptake to concentration in soil and do not measure or correlate to aqueous concentration or partitioning.[40]

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 2 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.[41−45] 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.[8] 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.

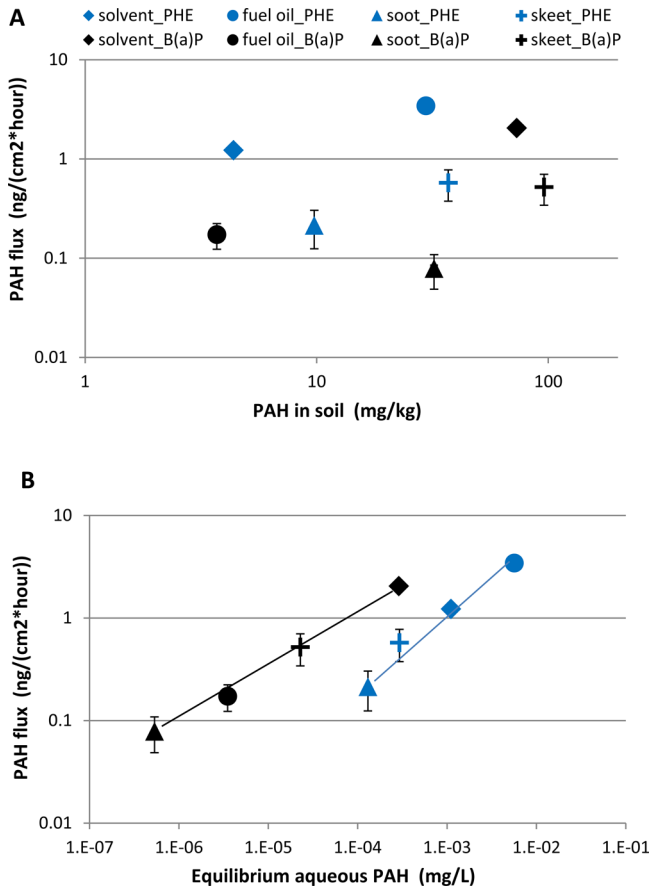

Figure 5. PHE and B(a)P flux through pig skin as a function of concentration in soil (A) and equilibrium aqueous concentration (B).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b06164.

Concentrations of PAHs measured in all soil compositions, K_D calculations and model predictions, and experimental data as described in the manuscript. In addition, data associated with each figure in the manuscript are provided as tables (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*(U.G.) E-mail: ughosh@umbc.edu. Phone: 410-455-8665.

Present Address

‡Integral Consulting Inc., 719 2nd Avenue, Seattle, Washington 98104, United States.

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Notes

The authors declare the following competing financial interest(s): Gomez-Eyles now works for a scientific consulting firm that provide risk assessment services to private and public-sector clients. The remaining authors declare no competing interest.

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Supporting Information

Effect of PAH source materials and soil components on partitioning and dermal uptake

Huan Xia, Jose L. Gomez-Eyles,[‡] and Upal Ghosh[*]

Department of Chemical, Biochemical and Environmental Engineering, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250, U.S.A.

[*]Corresponding author. Email: ughosh@umbc.edu phone: 410-455-8665

[‡] Current address: Integral Consulting Inc., 719 2nd Avenue, Seattle, WA 98104

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Supplementary Table of Contents

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.[1] 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 < 150μm, and stored at 4°C till use.

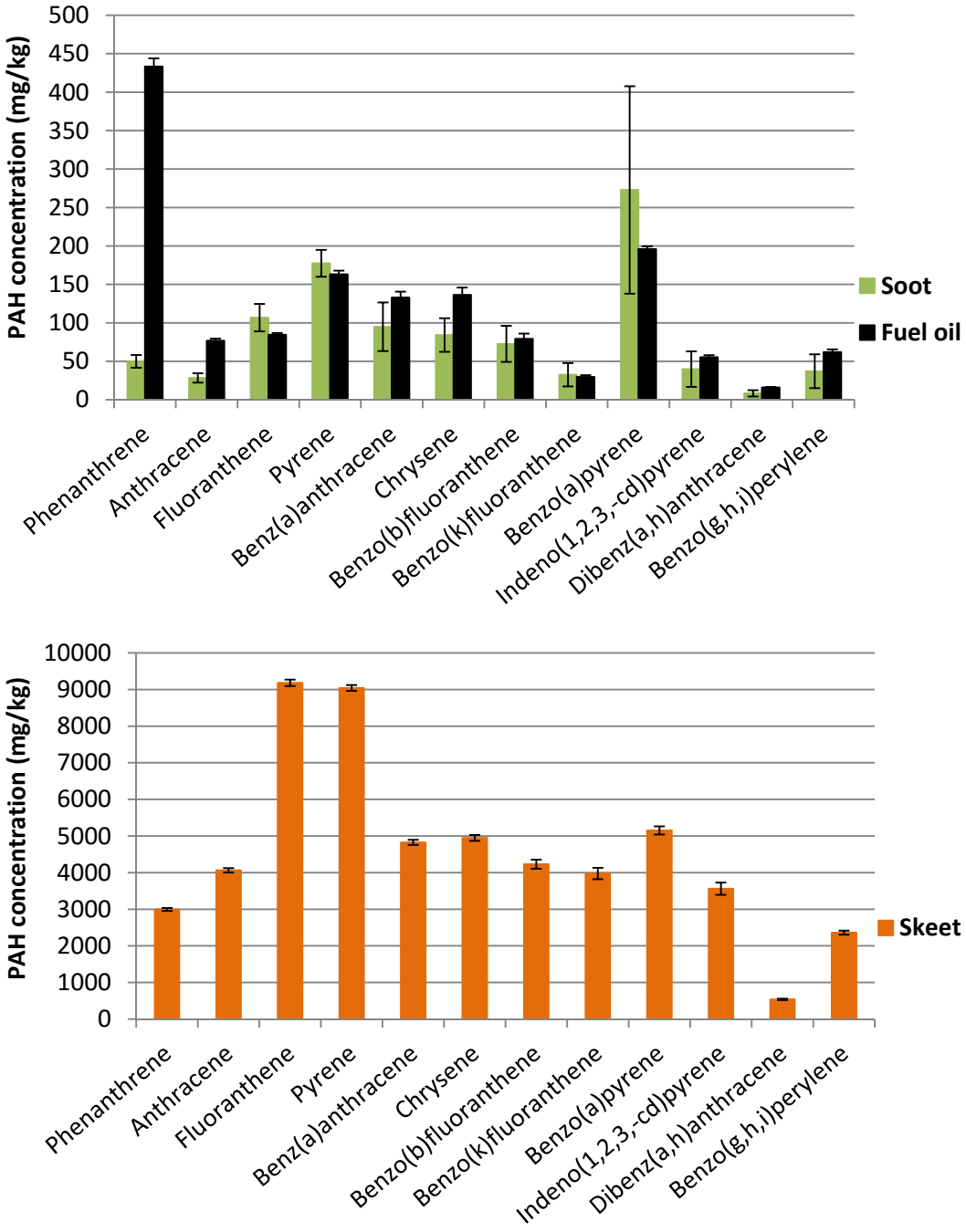

Figure S1. Average PAH concentrations in source materials before introduction into soils (n=3, error bars represent standard error).

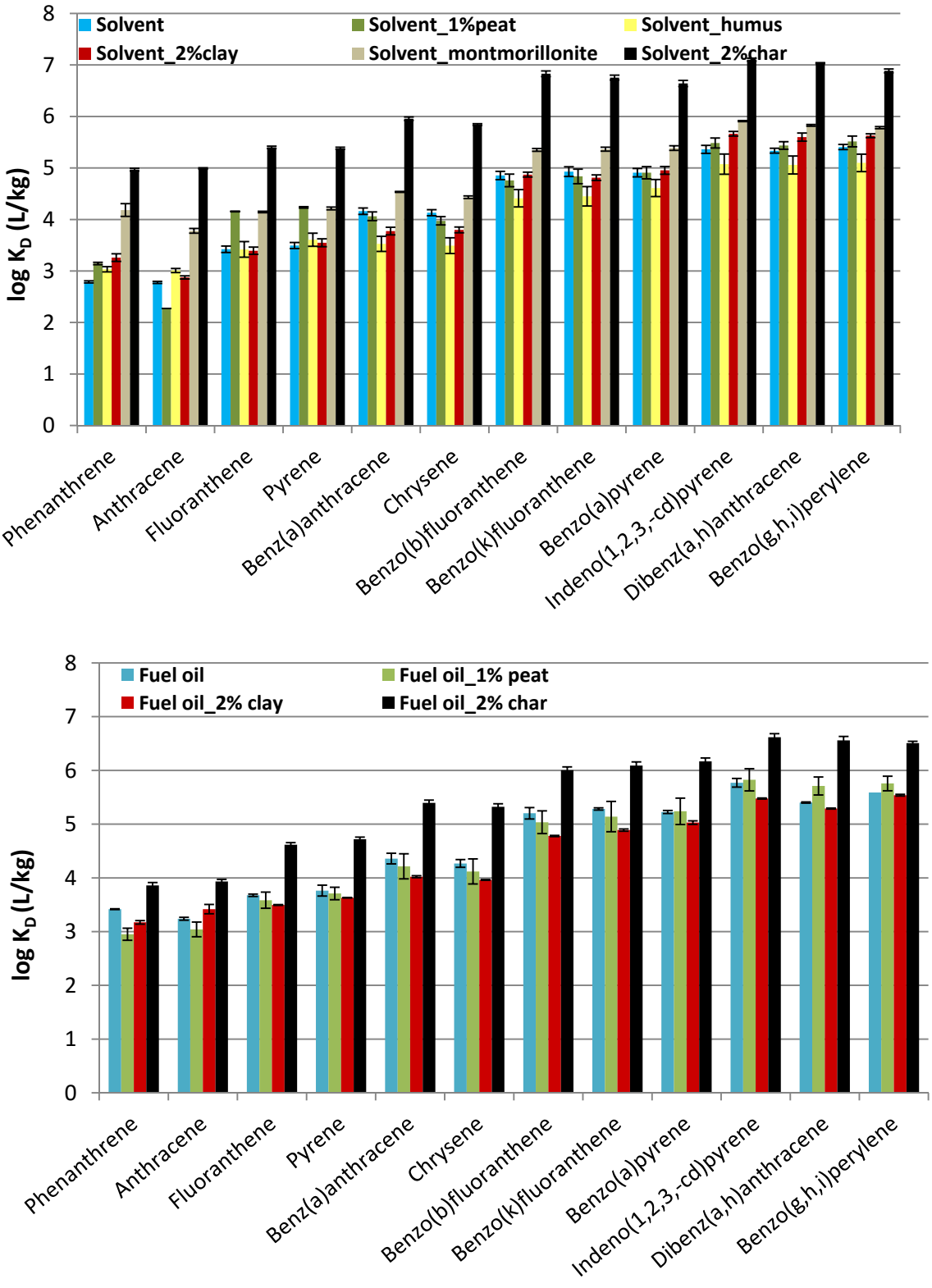

Figure S2. Effect of soil composition on PAH partitioning in solvent and fuel oil spiked weathered soils with 1mg/kg target BaP (n= 3, error bars represent standard error).

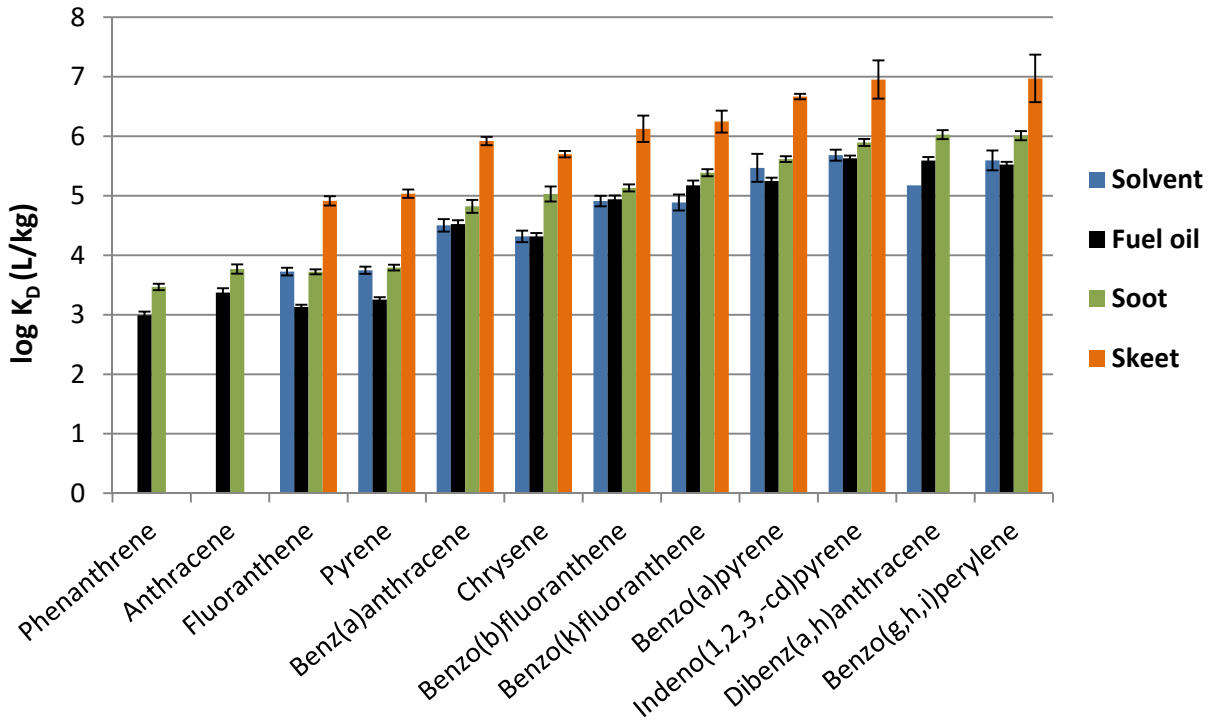

Figure S3. K_D in BSS spiked with different source materials targeted to achieve 0.1 mg/kg target BaP concentration (n= 3, error bars represent standard error).

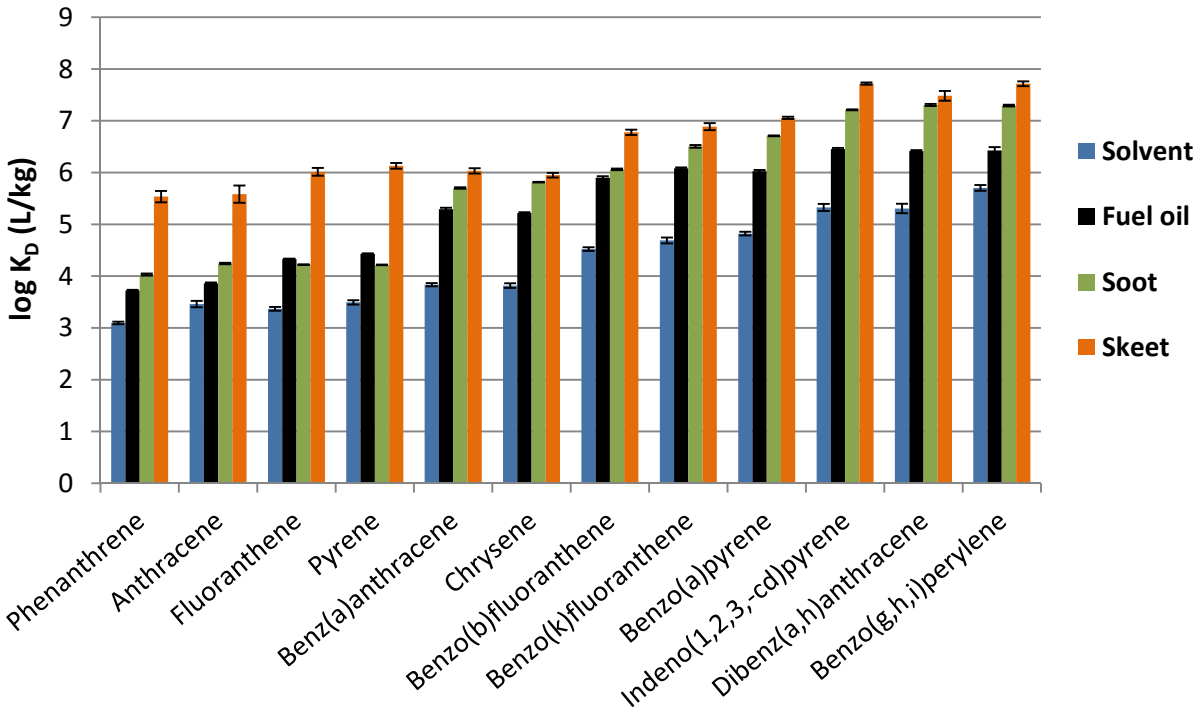

Figure S4. K_D in BSS spiked with different source materials targeted to achieve 10 mg/kg target BaP concentration (n= 3, error bars represent standard error).

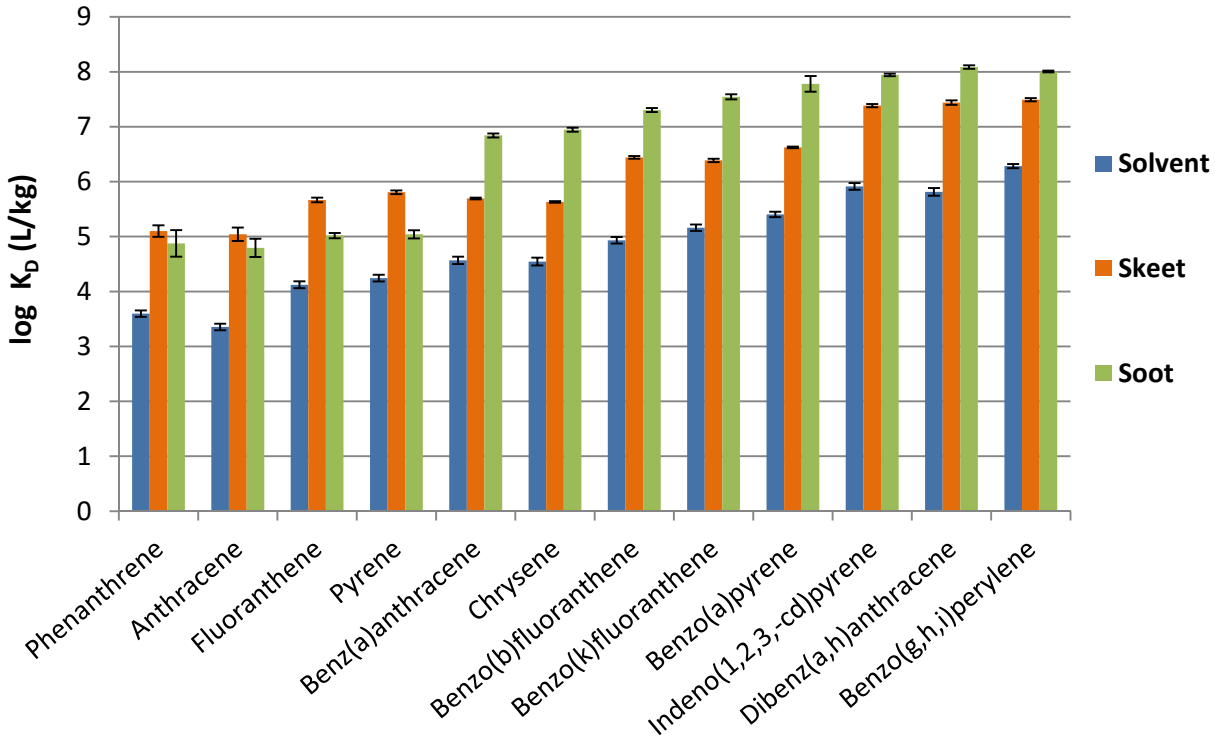

Figure S5. K_D in BSS spiked with different source materials targeted to achieve 100 mg/kg target BaP concentration (n= 3, error bars represent standard error).

Effect of Aging on Soil K_D. 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_D 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_{DS} for phenanthrene and anthracene increased after weathering by a factor of 6 compared to a factor of 1.5 for B(a)P. Very small change in K_D 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 ageing on PAH bioavailability [2], but those studies involved fresh spiking of soils with PAHs in solvent that are likely to be impacted more strongly by weathering processes.

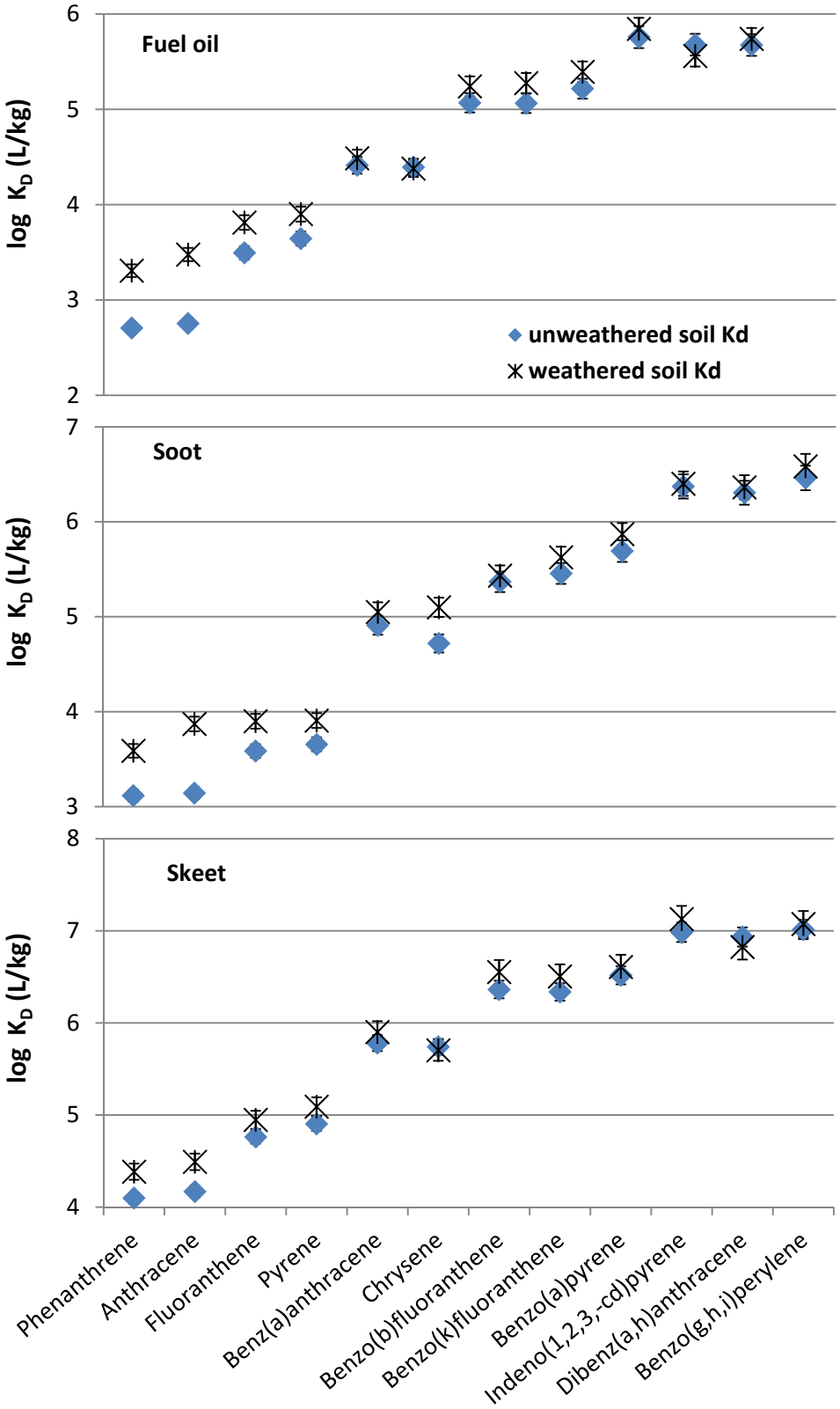

Figure S6. Comparison of K_D between freshly constructed and weathered (two months) soils with 1 mg/kg target BaP (n= 3, error bars represent standard error).

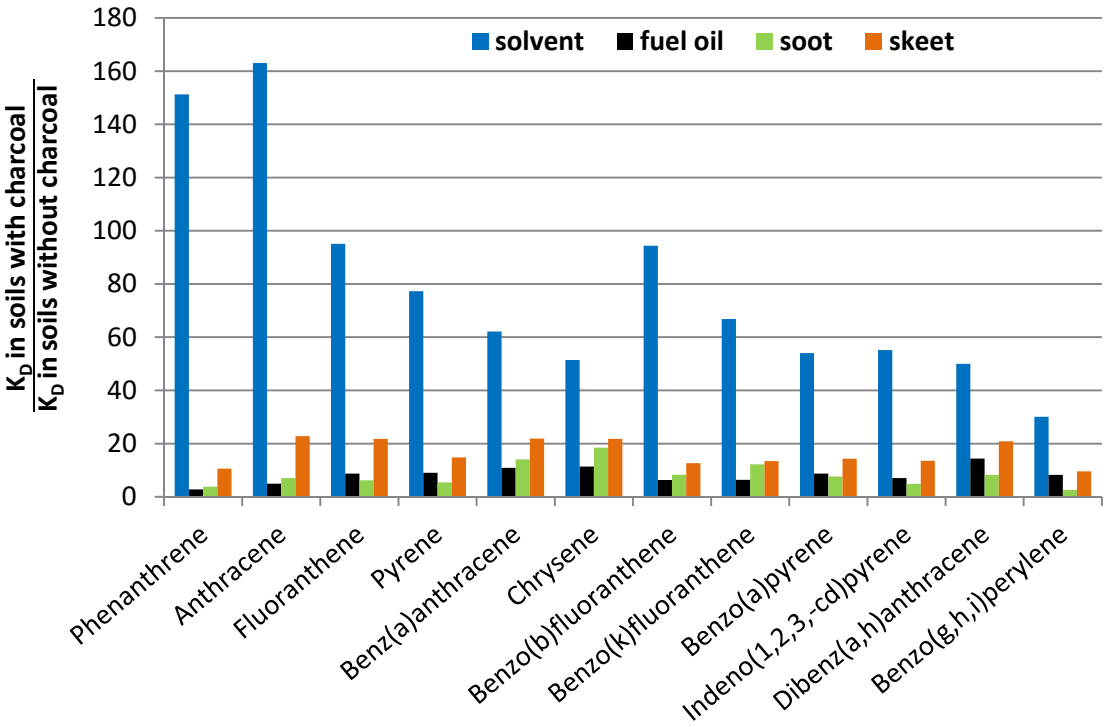

Figure S7. Ratio of K_D in weathered soils with/without 2% charcoal under different source materials of 1mg/kg target B(a)P (n= 3, error bars represent standard deviation).

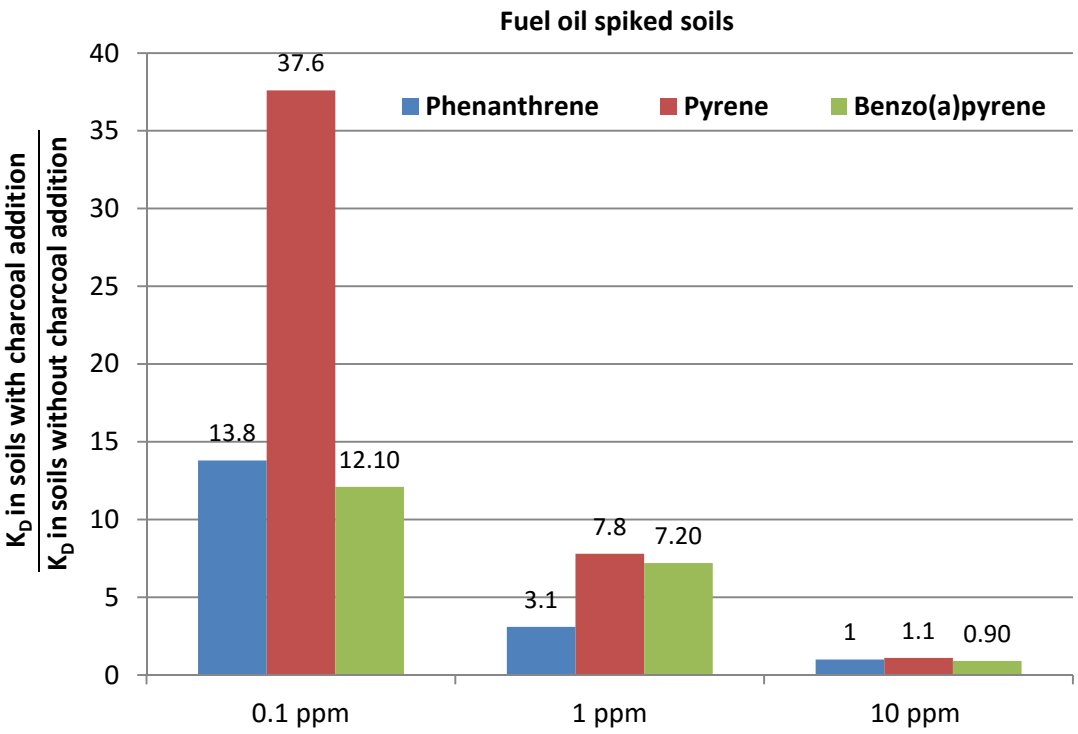

Figure S8. Ratio of K_D in fuel oil spiked soils with/without 2% charcoal addition at different PAH concentrations (as Target BaP).

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 ($K_D = f_{OC}*K_{OC} + f_{BC}*K_{BC}*C_w^{n-1}$) 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 [3, 4]; n is the nonlinearity coefficient [4] 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)[5]; and the foc is the measured TOC content.

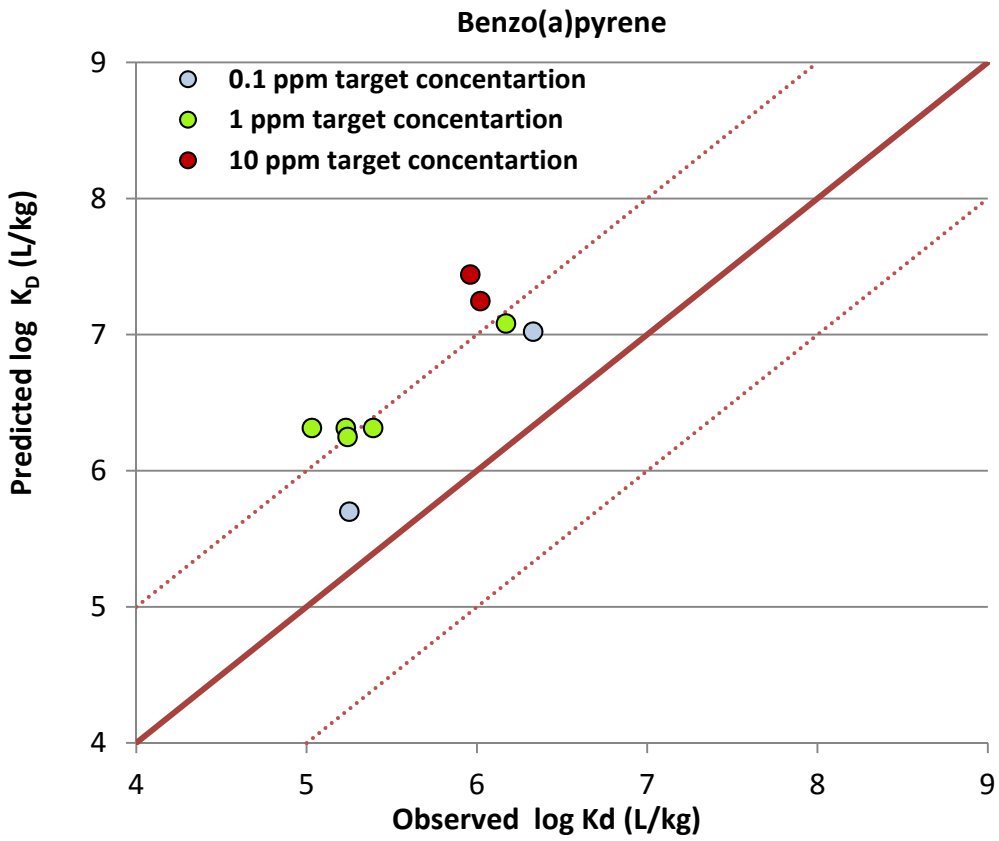

Figure S9. Model predicted K_D against observed K_D in fuel oil spiked soils at different PAH concentrations (as target BaP).

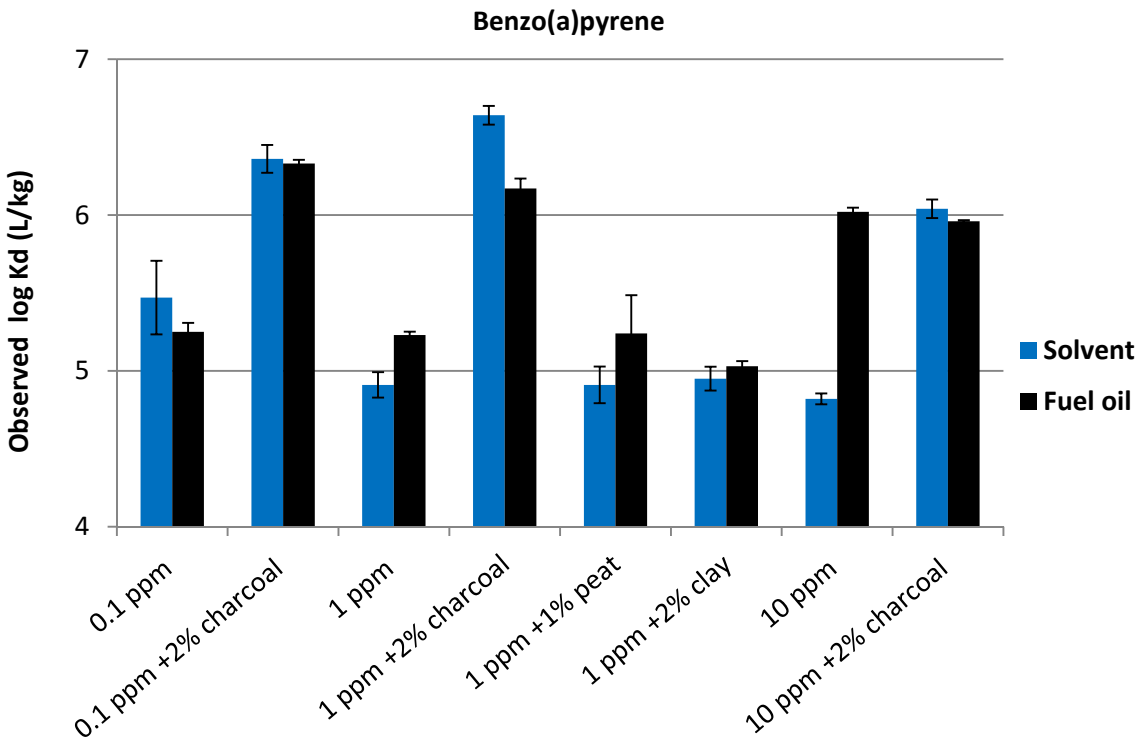

Figure S10. Comparison of K_D of BaP in solvent spiked and fuel oil spiked soils with identical soil compositions and PAH concentration (as target BaP) (n= 3, error bars represent standard error).

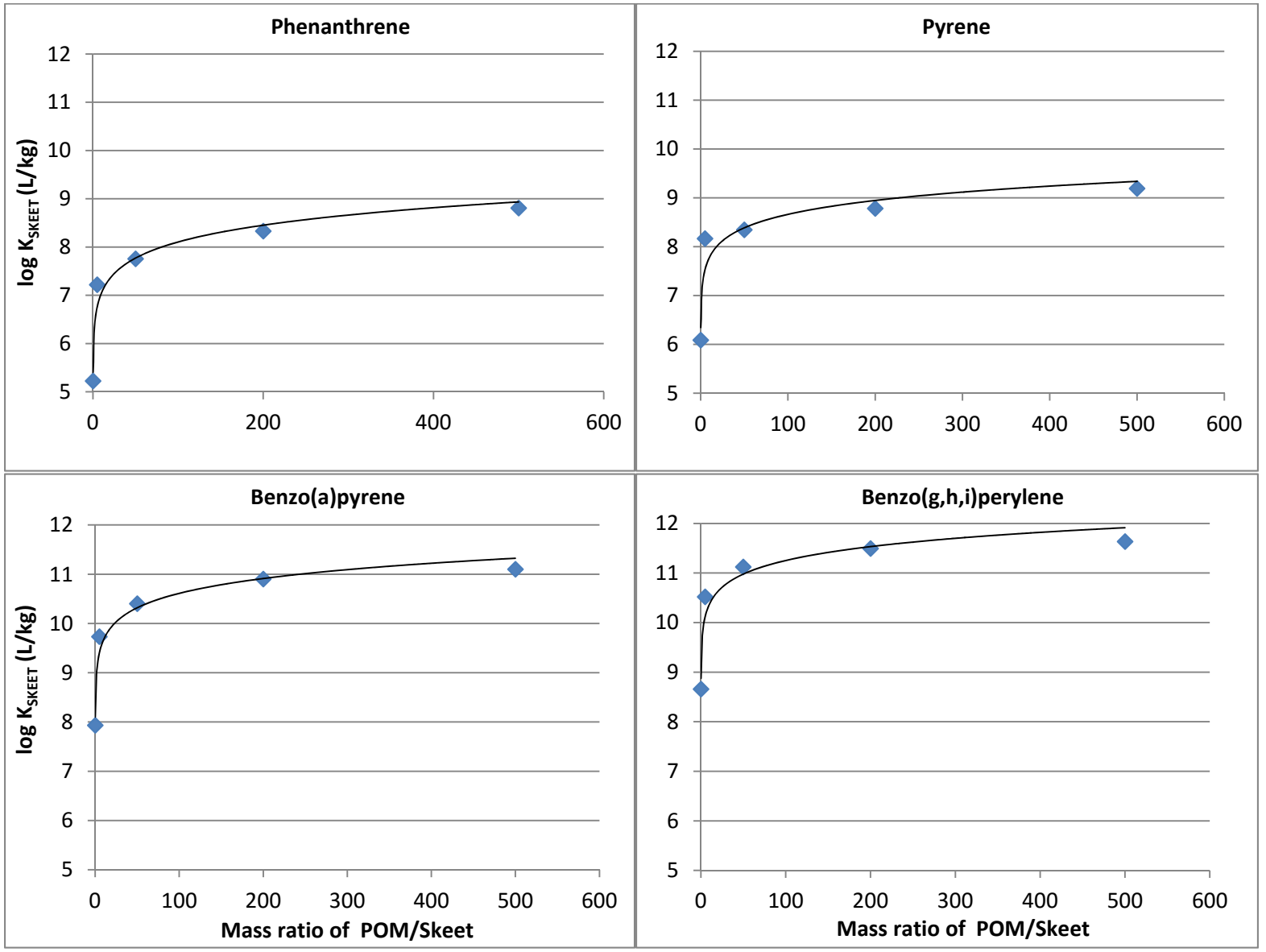

Figure S11. Increase of measured Log K_{SKEET} for different PAHs with increased mass ratio of POM/Skeet (0.125, 5, 50, 200, 500) in a well-mixed system.

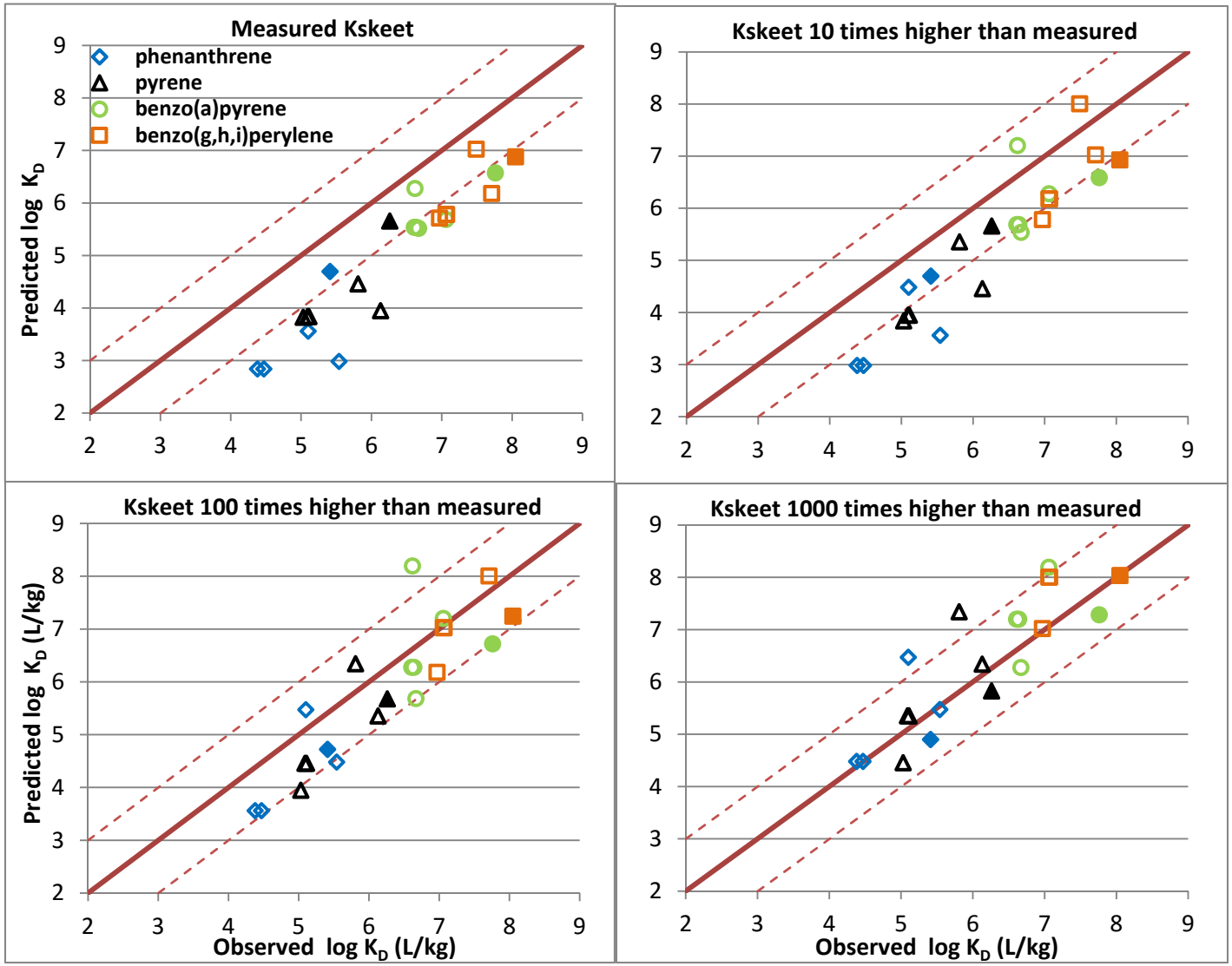

Figure S12. Comparison between predicted K_D from sorption model and observed K_D for skeet spiked soils using K_{SKEET} values orders of magnitude higher than measured (shown for PHE, PYR, B(a)P and B(ghi)P; log-transformed data). Blue diamonds represent PHE; black triangles represent PYR; orange squares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

Table S1. PAH concentrations in stock spiking solution.

PAH	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 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	-	-

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

	K_{PEAT}	K_{CLAY}	K_{SAND}	$K_{CHARCOAL}$ (at Cw =1 ng/L)	K_{FUEL}	K_{FUEL} Raoult's law estimate (MW=800 g/mole)	K_{SOOT}	K_{SKEET} (with minimal depletion)
PAH Compound								
Phenanthrene	3.82	<dl	<dl	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

note:<dl = below the detection limit

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 Source	Target BaP concentration (mg/kg)	Composition	Total Organic Carbon (%) average ± stdv	Black Carbon (%) average ± stdv
Control	0	BSS	2.63 ± 0.01	0.36 ± 0.03
Control	0	BSS+2% charcoal	2.84 ± 0.01	0.41 ± 0
Solvent	0.1	BSS	2.37 ± 0.02	0.24 ± 0.02
Solvent	1	BSS	2.45 ± 0.01	0.31 ± 0.01
Solvent	10	BSS	2.39 ±0.01	0.44 ± 0.01
Solvent	100	BSS	2.48 ± 0.01	0.34 ± 0
Solvent	0.1	BSS+2% charcoal	2.51 ± 0.01	0.34 ± 0.01
Solvent	1	BSS+2% charcoal	2.55 ± 0.01	0.38 ± 0.03
Solvent	10	BSS+2% charcoal	2.51 ± 0.02	0.48 ± 0
Solvent	1	BSS+1% peat	0.33 ± 0	0.02 ± 0
Solvent	1	BSS+2% clay	2.73 ± 0.12	0.35 ± 0.03
Solvent	1	BSS+ clay replaced by Montmorillonite	3.01 ± 0.05	0.26 ± 0.01
Solvent	1	BSS+ peat replaced by Humus	0.42 ± 0.01	0.02 ± 0
Soot	0.1	BSS	2.09 ± 0.01	0.33 ± 0.04
Soot	1	BSS	2.39 ± 0	0.77 ± 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 ± 0.01
Skeet	10	BSS	2.80 ± 0.05	0.40 ± 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 ± 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 ± 0.01
Fuel oil	10	BSS	4.85 ± 0.01	0.64 ± 0.03
Fuel oil	0.1	BSS+2% charcoal	2.61 ± 0.04	0.29 ± 0.05
Fuel oil	1	BSS+2% charcoal	2.95 ± 0.03	0.38 ± 0.01
Fuel oil	10	BSS+2% charcoal	4.46 ± 0.04	0.26 ± 0.03
Fuel oil	1	BSS+1% peat	0.51 ± 0	0.06 ± 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 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(mg/kg)	PAH mass fraction in 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 S6. Average masses of POM and soil used for the soil equilibrium partitioning experiment and their respective percent depletion of PAHs at equilibrium.

PAH Source	Target BaP concentration (mg/kg)	Composition	Mass of soil (g)	Mass of POM (g)	depletion of pyrene	depletion of B(a)P	depletion of total PAHs
Control	0	BSS	40.2	1.003			
Control	0	BSS+2% charcoal	40.1	0.947			
Solvent	0.1	BSS	40.1	0.025	0.33%	0.10%	0.26%
Solvent	1	BSS	40.6	0.026	0.89%	0.88%	0.96%
Solvent	10	BSS	40.3	0.028	0.70%	0.90%	1.09%
Solvent	100	BSS	40.1	0.027	0.19%	0.32%	0.39%
Solvent	0.1	BSS+2% charcoal	40.0	4.953	1.21%	3.18%	2.59%
Solvent	1	BSS+2% charcoal	6.1	1.007	2.84%	4.47%	3.61%
Solvent	10	BSS+2% charcoal	6.0	0.205	3.80%	3.31%	3.80%
Solvent	1	BSS+1% peat	40.3	0.026	0.14%	0.44%	0.44%
Solvent	1	BSS+2% clay	40.1	0.027	0.70%	0.83%	1.10%
Solvent	1	BSS+ clay replaced by Montmorillonite	39.9	0.026	0.16%	0.28%	0.26%
Solvent	1	BSS+ peat replaced by Humus	41.4	0.025	0.44%	0.98%	1.59%
Soot	0.1	BSS	41.8	0.532	5.43%	3.01%	5.29%
Soot	1	BSS	42.2	0.510	3.54%	1.46%	6.33%
Soot	10	BSS	40.7	0.502	3.32%	0.26%	4.85%
Soot	100	BSS	7.2	0.510	1.72%	0.06%	0.80%
Soot	1	BSS+2% charcoal	41.4	1.980	4.19%	0.57%	1.69%
Skeet	0.1	BSS	39.8	0.025	0.02%	0.01%	0.02%
Skeet	1	BSS	40.3	0.026	0.02%	0.02%	0.03%
Skeet	1	BSS_duplicate	39.6	0.025	0.02%	0.02%	0.03%
Skeet	10	BSS	39.7	0.026	0.001%	0.006%	0.01%
Skeet	100	BSS	39.8	0.205	0.03%	0.11%	0.11%
Skeet	1	BSS+2% charcoal	6.0	1.058	0.47%	0.43%	0.52%
Fuel oil	0.1	BSS	39.8	0.026	1.54%	0.40%	0.97%
Fuel oil	1	BSS	39.9	0.025	0.38%	0.32%	0.47%
Fuel oil	1	BSS_duplicate	39.6	0.025	0.26%	0.19%	0.34%
Fuel oil	10	BSS	40.5	0.026	0.09%	0.06%	0.14%
Fuel oil	0.1	BSS+2% charcoal	40.1	5.013	4.67%	3.60%	4.12%
Fuel oil	1	BSS+2% charcoal	40.0	1.031	1.68%	1.25%	7.40%
Fuel oil	10	BSS+2% charcoal	39.8	0.208	4.40%	3.61%	6.53%
Fuel oil	1	BSS+1% peat	40.3	0.027	0.65%	0.64%	1.20%
Fuel oil	1	BSS+2% clay	40.5	0.025	0.51%	0.43%	0.73%

Note: BSS consists of 10% peat moss, 20% kaolin clay and 70% sand.

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

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

Table S8. Percent depletion of PAHs from skeet into POM in source material equilibrium experiment.

Percentage of PAH depleted from skeet into POM					
Mass ratio of POM/Skeet	**0.15**	**5**	**50**	**200**	**500**
Phenanthrene	0.33%	0.82%	1.16%	1.32%	1.38%
Anthracene	0.05%	0.13%	0.17%	0.24%	0.32%
Fluoranthene	0.13%	0.32%	0.48%	0.53%	0.65%
Pyrene	0.23%	0.64%	0.83%	1.02%	1.24%
Benz(a)anthracene	0.12%	0.17%	0.18%	0.20%	0.27%
Chrysene	0.08%	0.10%	0.11%	0.14%	0.19%
Benzo(b)fluoranthene	0.03%	0.08%	0.12%	0.15%	0.21%
Benzo(k)fluoranthene	0.02%	0.03%	0.04%	0.05%	0.07%
Benzo(a)pyrene	0.09%	0.14%	0.18%	0.21%	0.34%
Indeno(1,2,3,-cd)pyrene	0.01%	0.03%	0.04%	0.05%	0.09%
Dibenz(a,h)anthracene	0.02%	0.04%	0.05%	0.06%	0.10%
Benzo(g,h,i)perylene	0.01%	0.03%	0.05%	0.09%	0.15%

Dermal uptake of PAHs

Pig skin was used as a surrogate for human exposure through dermal uptake due to the similarity in histological and physiological characteristics and permeability properties.[6-8] In the past, full-thickness skin from a variety of receptors (e.g. rat, pig and humans) has been applied in percutaneous absorption studies for a diversity of organic contaminants. [7, 9, 10] In this test, 5 cm^2 of full-thickness pig 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 μm,[11] and 100 mg of each dry soil was evenly distributed onto the skin surface to reach a soil loading of 20 mg/cm^2.[12] After spreading the soil evenly, 10 drops of DI water was evenly placed across the skin surface to provide moisture as suggested by Turkall et al,[13] and was subsequently mixed gently with the soil using a spatula. After that, the evaporating dish was sealed with parafilm and placed in the dark at 25 °C for 16 hours.

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.[14] 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 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 cleanup and PAH analysis.

Our measured dermal fluxes for PAHs are in the range of those reported in literature. For example, for phenanthrene the measured flux into skin from solvent spiked soil is 0.28 ± 0.04 $ng/(cm^2*hour)$(normalized to soil concentration) in this study, which is within the range of 0.22 to 0.40 $ng/(cm^2*hour)$(normalized to soil concentration) observed in an in vitro pig skin study by Abdel-Rahman et al.[15] Also, for benzo(a)pyrene, our observed flux of 0.028 ± 0.00 $ng/(cm^2*hour)$ from solvent spiked soils is consistent with the range of 0.024 to 0.22 $ng/(cm^2*hour)$ reported by Wester et al for human and monkey skin. [16]

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Appendix: Raw Data for Figures

Data for 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 ±1 standard deviation)**:**

PAH Source	solvent		fuel oil		soot		skeet	
	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE
Phenanthrene	0.01	0.00	0.09	0.00	0.1	0.02	0.46	0.02
Anthracene	0.01	0.00	0.09	0.00	0.1	0.02	0.11	0.01
Fluoranthene	0.03	0.01	0.09	0.01	0.2	0.06	1.61	0.05
Pyrene	0.04	0.00	0.59	0.03	0.2	0.06	1.52	0.04
Benz(a)anthracene	0.10	0.03	0.21	0.01	0.2	0.05	0.96	0.02
Chrysene	0.11	0.02	0.28	0.01	0.2	0.05	0.87	0.02
Benzo(b)fluoranthene	0.11	0.02	0.07	0.00	0.3	0.07	0.80	0.04
Benzo(k)fluoranthene	0.04	0.01	0.02	0.00	0.1	0.06	0.67	0.01
Benzo(a)pyrene	0.23	0.07	0.11	0.01	0.8	0.33	0.85	0.03
Indeno(1,2,3,-cd)pyrene	0.08	0.01	0.02	0.00	0.2	0.06	0.54	0.02
Dibenz(a,h)anthracene	0.02	0.00	0.01	0.00	0.1	0.02	0.09	0.00
Benzo(g,h,i)perylene	0.08	0.01	0.03	0.00	0.3	0.07	0.35	0.02

Data for 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**:**

	phenanthrene		pyrene		benzo(a)pyrene		Benzo(g,h,i)perylene	
source/soil component	log Cw (mg/L)	log Kd (L/kg)	log Cw (mg/L)	log Kd (L/kg)	log Cw (mg/L)	log Kd (L/kg)	log Cw (mg/L)	log Kd (L/kg)
charcoal	-5.06	7.1	-5.67	7.8	-6.81	8.86	-7.53	8.8
	-3.54	6.1	-4.16	7.2	-5.57	8.61	-6.23	8.4
	-2.40	5.4	-2.98	6.9	-4.41	8.38	-5.16	8.3
	-1.46	5.4	-1.91	6.5	-3.29	7.86	-4.16	8.2
skeet	-1.64	5.1	-2.04	6.0	-4.14	7.9	-5.20	8.6
	-3.74	7.2	-4.21	8.2	-6.02	9.7	-7.14	10.5
	-4.29	7.8	-4.39	8.3	-6.67	10.4	-7.75	11.1
	-4.86	8.3	-4.83	8.8	-7.21	10.9	-8.12	11.5
	-5.34	8.8	-5.24	9.2	-7.44	11.1	-8.26	11.6
sand			-4.70	1.3	-6.25	3.7	-6.99	3.39
			-3.79	1.8	-5.28	3.5	-6.18	3.77
			-3.07	1.7	-4.52	3.4	-5.29	3.16
			-1.97	1.4	-3.45	3.2	-4.39	3.62
clay			-4.93	2.1	-6.39	3.7	-7.2	3.85
			-3.94	2.1	-5.64	4.2	-6.3	3.97
			-2.91	2.0	-4.52	4.0	-5.1	3.24
			-1.95	1.9	-3.45	3.7	-4.3	3.77
peat	-4.36	3.6	-4.94	4.8	-6.48	6.5	-7.4	6.80
	-3.61	3.9	-4.21	4.9	-5.75	6.5	-6.7	6.85
	-2.62	3.8	-3.15	4.8	-4.67	6.4	-5.5	6.51
	-1.67	3.9	-2.20	4.8	-3.73	6.4	-4.6	6.64
fuel	-2.80	5.4	-4.00	6.2	-6.37	8.67	-7.45	9.2
soot	-3.66	5.4	-4.04	6.3	-6.85	9.29	-7.62	9.2

Data for Figure 3. Comparison of measured and predicted soil K_D using both single domain model ($K_D = f_{OC}*K_{OC}$) and dual domain model ($K_D = f_{OC}*K_{OC} + f_{BC}*K_{BC}*C_w^{n-1}$) [4]. 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**:**

Source	solvent	fuel	soot	skeet
		phenanthrene		
measured soil K_D	2.79 ±0.01	3.42 ±0.02	3.59 ±0.03	4.38 ±0.01
K_{OC} model	2.60	2.64	2.58	2.68
$_{OC}+ _{BC}$ model	3.49	3.54	3.85	3.58
		pyrene		
measured soil K_D	3.49 ±0.01	3.76 ±0.01	3.91 ±0.02	5.09 ±0.01
K_{OC} model	3.00	3.05	2.99	3.09
$_{OC}+ _{BC}$ model	3.80	3.85	4.15	3.89
		benzo[a]pyrene		
measured soil K_D	4.91 ±0.02	5.23 ±0.02	5.87 ±0.04	6.61 ±0.01
K_{OC} model	4.22	4.26	4.20	4.30
$_{OC}+ _{BC}$ model	4.75	4.80	5.06	4.84
		benzo[ghi]perylene		
measured soil K_D	5.41 ±0.02	5.59 ±0.02	6.58 ±0.03	7.07 ±0.01
K_{OC} model	4.77	4.81	4.76	4.86
$_{OC}+ _{BC}$ model	5.20	5.25	5.49	5.29

Data for 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**:**

source	target conc.(mg/kg)	composition	estimated log Kd	measured log Kd
		phenanthrene		
Solvent spike	1	BSS	2.82	2.79
Solvent spike	10	BSS	2.82	3.1
Solvent spike	100	BSS	2.82	3.6
Solvent spike	0.1	BSS+2% charcoal	4.70	4.31
Solvent spike	1	BSS+2% charcoal	4.70	4.97
Solvent spike	10	BSS+2% charcoal	4.70	4.43

Solvent spike	1	BSS+1% peat	1.83	3.15
Solvent spike	1	BSS+2% clay	2.82	3.26
		pyrene		
Solvent spike	0.1	BSS	3.82	3.75
Solvent spike	1	BSS	3.82	3.49
Solvent spike	10	BSS	3.82	3.49
Solvent spike	100	BSS	3.82	4.24
Solvent spike	0.1	BSS+2% charcoal	5.66	5.39
Solvent spike	1	BSS+2% charcoal	5.66	5.38
Solvent spike	10	BSS+2% charcoal	5.66	4.7
Solvent spike	1	BSS+1% peat	2.85	4.23
Solvent spike	1	BSS+2% clay	3.82	3.55
		benzo[a]pyrene		
Solvent spike	0.1	BSS	5.52	5.47
Solvent spike	1	BSS	5.52	4.91
Solvent spike	10	BSS	5.52	4.82
Solvent spike	100	BSS	5.52	5.40
Solvent spike	0.1	BSS+2% charcoal	7.01	6.36
Solvent spike	1	BSS+2% charcoal	7.01	6.64
Solvent spike	10	BSS+2% charcoal	7.01	6.04
Solvent spike	1	BSS+1% peat	4.56	4.91
Solvent spike	1	BSS+2% clay	5.51	4.95
		benzo[ghi]perylene		
Solvent spike	0.1	BSS	5.7	5.6
Solvent spike	1	BSS	5.7	5.4
Solvent spike	10	BSS	5.7	5.7
Solvent spike	100	BSS	5.7	6.3
Solvent spike	0.1	BSS+2% charcoal	6.9	6.2
Solvent spike	1	BSS+2% charcoal	6.9	6.9
Solvent spike	10	BSS+2% charcoal	6.9	6.5
Solvent spike	1	BSS+1% peat	4.7	5.5
Solvent spike	1	BSS+2% clay	5.7	5.6
		phenanthrene		
Fuel oil spike	0.1	BSS	2.90	3
Fuel oil spike	1	BSS	3.29	3.42
Fuel oil spike	1	BSS_duplicate	3.29	3.31
Fuel oil spike	10	BSS	4.13	3.72
Fuel oil spike	0.1	BSS+2% charcoal	4.70	4.13
Fuel oil spike	1	BSS+2% charcoal	4.71	3.86
Fuel oil spike	10	BSS+2% charcoal	4.80	3.73
Fuel oil spike	1	BSS+1% peat	3.13	2.95
Fuel oil spike	1	BSS+2% clay	3.29	3.17
		pyrene		

Fuel oil spike	0.1	BSS	3.89	3.26
Fuel oil spike	1	BSS	4.25	3.76
Fuel oil spike	1	BSS_duplicate	4.25	3.9
Fuel oil spike	10	BSS	5.07	4.43
Fuel oil spike	0.1	BSS+2% charcoal	5.66	4.83
Fuel oil spike	1	BSS+2% charcoal	5.67	4.72
Fuel oil spike	10	BSS+2% charcoal	5.75	4.49
Fuel oil spike	1	BSS+1% peat	4.08	3.71
Fuel oil spike	1	BSS+2% clay	4.25	3.63
		benzo[a]pyrene		
Fuel oil spike	0.1	BSS	5.70	5.25
Fuel oil spike	1	BSS	6.31	5.23
Fuel oil spike	1	BSS_duplicate	6.31	5.39
Fuel oil spike	10	BSS	7.25	6.02
Fuel oil spike	0.1	BSS+2% charcoal	7.02	6.33
Fuel oil spike	1	BSS+2% charcoal	7.08	6.17
Fuel oil spike	10	BSS+2% charcoal	7.44	5.96
Fuel oil spike	1	BSS+1% peat	6.25	5.24
Fuel oil spike	1	BSS+2% clay	6.31	5.03
		benzo[ghi]perylene		
Fuel oil spike	0.1	BSS	6.19	5.52
Fuel oil spike	1	BSS	7.04	5.59
Fuel oil spike	1	BSS_duplicate	7.04	5.74
Fuel oil spike	10	BSS	8.02	6.43
Fuel oil spike	0.1	BSS+2% charcoal	6.93	6.39
Fuel oil spike	1	BSS+2% charcoal	7.25	6.51
Fuel oil spike	10	BSS+2% charcoal	8.05	6.30
Fuel oil spike	1	BSS+1% peat	7.02	5.76
Fuel oil spike	1	BSS+2% clay	7.04	5.54
		phenanthrene		
Soot spike	0.1	BSS	2.90	3.47
Soot spike	1	BSS	3.31	3.59
Soot spike	10	BSS	4.16	4.03
Soot spike	100	BSS	5.04	4.88
Soot spike	1	BSS+2% charcoal	4.71	4.17
		pyrene		
Soot spike	0.1	BSS	3.92	3.79
Soot spike	1	BSS	4.37	3.91
Soot spike	10	BSS	5.24	4.22
Soot spike	100	BSS	6.12	5.04
Soot spike	1	BSS+2% charcoal	5.67	4.64
		benzo[a]pyrene		
Soot spike	0.1	BSS	6.10	5.62

Soot spike	1	BSS	6.98	5.87
Soot spike	10	BSS	7.97	6.71
Soot spike	100	BSS	8.87	7.78
Soot spike	1	BSS+2% charcoal benzo[ghi]perylene	7.29	6.76
Soot spike	0.1	BSS	6.1	6.0
Soot spike	1	BSS	6.9	6.6
Soot spike	10	BSS	7.8	7.3
Soot spike	100	BSS	8.7	8.0
Soot spike	1	BSS+2% charcoal phenanthrene	7.2	7.0
Skeet spike	1	BSS	2.84	4.38
Skeet spike	1	BSS_duplicate	2.84	4.47
Skeet spike	10	BSS	2.98	5.54
Skeet spike	100	BSS	3.56	5.1
Skeet spike	1	BSS+2% charcoal pyrene	4.70	5.41
Skeet spike	0.1	BSS	3.82	5.03
Skeet spike	1	BSS	3.84	5.09
Skeet spike	1	BSS_duplicate	3.84	5.11
Skeet spike	10	BSS	3.95	6.13
Skeet spike	100	BSS	4.46	5.81
Skeet spike	1	BSS+2% charcoal benzo[a]pyrene	5.66	6.26
Skeet spike	0.1	BSS	5.52	6.67
Skeet spike	1	BSS	5.54	6.61
Skeet spike	1	BSS_duplicate	5.54	6.64
Skeet spike	10	BSS	5.68	7.06
Skeet spike	100	BSS	6.28	6.62
Skeet spike	1	BSS+2% charcoal benzo[ghi]perylene	6.57	7.76
Skeet spike	0.1	BSS	5.71	6.97
Skeet spike	1	BSS	5.78	7.07
Skeet spike	1	BSS_duplicate	5.78	7.06
Skeet spike	10	BSS	6.18	7.71
Skeet spike	100	BSS	7.02	7.49
Skeet spike	1	BSS+2% charcoal	6.88	8.05

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

Source	flux (ng/(cm2*hour))	Cs (mg/kg)	Cw (mg/L)
		phenanthrene	
solvent	1.23	4.4	0.0011
fuel	3.44	29.6	0.0056
soot	0.21	9.8	0.00013
skeet	0.58	37.0	0.00029
		Benzo(a)pyrene	
solvent	2.05	73.1	0.00029
fuel	0.17	3.7	3.51E-06
soot	0.08	32.1	5.33E-07
skeet	0.52	95.9	0.000023

Effect of Source Material on PAH Bioavailability to Humans and Ecological Receptors

Huan Xia and Upal Ghosh

Dept. of Chemical, Biochemical, and Environmental Engineering, UMBC

Introduction

- Polycyclic aromatic hydrocarbons (PAHs) are a group of organic contaminants that are widely distributed in soils, some of which are potent human carcinogens.
- PAHs are released into soils within different sorption domains (e.g. soot, char, coal tar or NAPLs) (Figure 1), that may alter their bioavailability from soils to ecological and human receptors through different exposure pathways. [1,2]
- Dermal contact and incidental oral ingestion of contaminated soils are two major pathways for human exposure to PAHs from soils.
- Our previous study has demonstrated the prominent and diverse effect of PAH sources on porewater concentrations, and incidental oral bioavailability predicted using a physiologically based in vitro extraction test.
- The impact of typical PAH sources on bioavailability to ecological receptors and human dermal exposure has not been fully addressed.

Weathered Skeet Particles | Soot Carbon | Fuel Oil #6

Typical PAH Source Materials

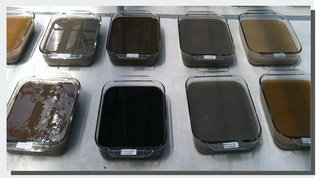
Objectives

- To construct a variety of soils with different PAH source materials and measure the soil partition constants and PAH equilibrium porewater concentration.
- To investigate the effects of PAH source materials on dermal bioavailability to humans and uptake by ecological receptors from soils.
- To explore the effect of carbon amendment on PAH uptake by ecological receptors from soils with different source materials

Methods

I. Soil construction

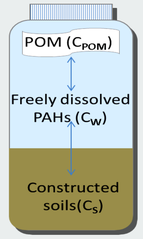
- Baseline ASTM soil constructed with peat, clay and sand (sieved to <150um)
- Control soil (no source) spiked with PAH solvent into baseline soil
- PAH sources (skeet particles, soot and fuel oil) introduced to baseline soil
- 2% charcoal applied to a subset of soils



Two months soil weathering

Composition of test soils		
PAH sources	untreated	treated
PAH solvent	baseline soil	baseline +2%charcoal
Fuel oil	baseline soil	baseline +2%charcoal
Soot	baseline soil	baseline +2%charcoal
Skeet	baseline soil	baseline +2%charcoal

II. Equilibrium partitioning test

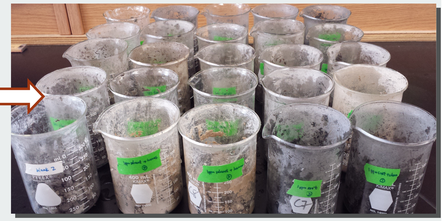


- PAH porewater concentrations measured using Polyoxymethylene (POM) 76um-thickness strips
- 4 weeks end over end mixing
- $C_W = C_{POM}/K_{POM}$, K_{POM} previously determined [3]
- Soil $K_D = C_S/$was calculated Lower K_D

III. PAH bioaccumulation by ecological receptors

- Adult earthworm E. fetida exposed to soils for 30 days [4]
- Moisture maintained at 60% soil water holding capacity
- Worms depurated, saponified , and extracted for PAHs [5]

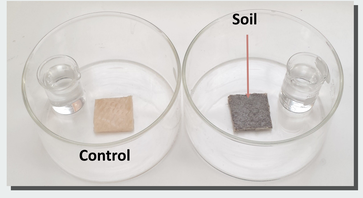
Eisenia fetida



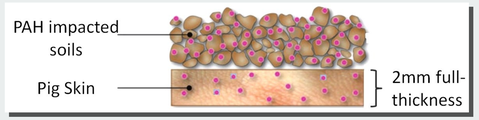
IV. PAH assimilation efficiency (α) into earthworms from soils

- Two groups of worms exposed soils for 2 hours
  - ⇒ One group analyzed for PAH immediately = initially ingested total PAH
  - ⇒ The other depurated till complete egestion and analyzed for PAH= residual PAH
- α= residual PAH in worms /initially ingested PAHs from soils [6]

V. Dermal bioavailability to humans



- Full thickness pig skin [7]
- Area: 5 cm^2, thickness: 2mm
- Soil loading: 20 mg/cm^2
- Skin wetted with DI water [8]
- 16 hours exposure at dark [9]

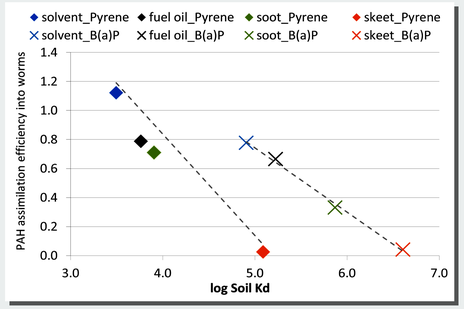


Dermal uptake scheme

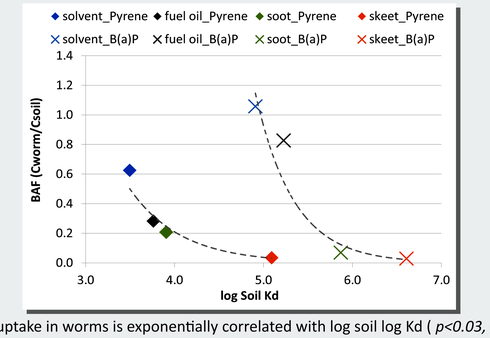
- Skin rinsed, saponified, extracted with hexane for PAH analysis [5]
- PAH flux into skin calculated

Results and discussion

I. Effect of source material on PAH assimilation into worms through ingestion



- Earthworm gut assimilation efficiency is linearly correlated with log soil Kd ($p<0.02$, $R^2>0.97$). Source materials with high Kd has low gut assimilation efficiency.

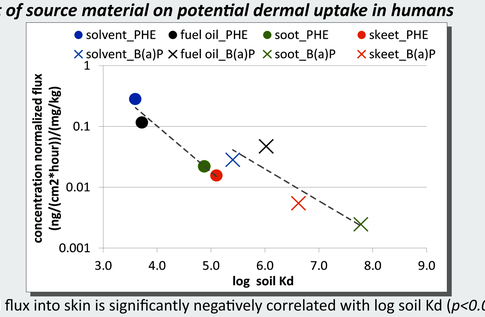


- PAH uptake in worms is exponentially correlated with log soil log Kd ($p<0.03$, $R^2>0.94$): highest uptake in control soils with lowest Kd, followed by fuel oil, soot and skeet soils

PAH sources	Total PAH BAF untreated	Total PAH BAF treated	Reduction in BAF
PAH solvent	11.33	0.34	97%
fuel oil	5.78	1.85	68%
soot	2.07	0.43	79%
skeet	0.55	0.16	70%

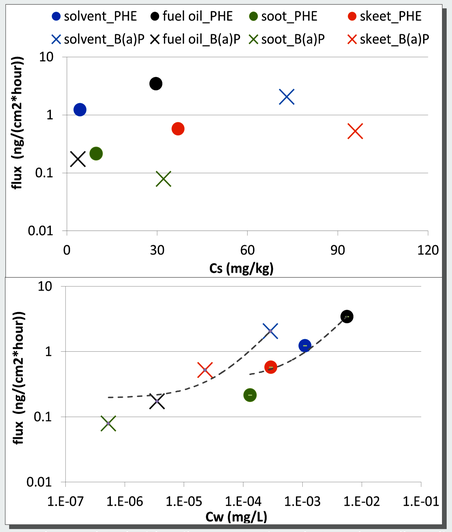
- Effect of charcoal amendment on PAH uptake in earthworms is impacted by source material: higher soil Kd → less amendment efficacy ; carbon may be fouled by fuel oil

III. Effect of source material on potential dermal uptake in humans



- PAH flux into skin is significantly negatively correlated with log soil Kd ($p<0.04$). Solvent and fuel oil spiked soils had higher flux than soot and skeet.

IV. Dermal uptake (pig skin) Vs. soil & porewater concentrations



- Flux is poorly correlated with soil concentrations, but can be well predicted by equilibrium porewater concentration using a linear regression ($p<0.01$, $R^2>0.98$).

Conclusion and future work

- Source materials with high Kd lead to low PAH gut assimilation efficiency and bio-accumulation in earthworms from soils: **uptake in earthworm: solvent> fuel oil > soot > skeet**
- Effect of charcoal amendment on PAH uptake in earthworms is influenced by source materials: **higher Kd → lower amendment efficacy**
- Source materials with high Kd has low potential PAH dermal uptake in humans from soils: **dermal uptake: solvent and fuel oil > soot and skeet**
- Dermal uptake is not significantly affected by soil concentrations, but can be predicted by equilibrium aqueous concentration.

On going work:

1) using Cs, Cw, α, soil Kd and elimination rate to model PAH uptake into earthworms from various soils.
2) measuring skin sorption capacity for PAHs and modeling dermal uptake process based on PAH mass transfer into skin.

Acknowledgement

The soil construction and equilibrium partitioning study were funded by SERDP Project ER-1743.

References

1) Jonker, M. & Koelmans, A. Environmental Science & Technology 2002, 36 (17), 3725-3734.
2) Khalil, M. F et al. Environmental Science & Technology 2006, 40 (18), 5681-5687.
3) Gomez-Eyles, et al. Environmental Science & Technology 2012, 46 (2), 962-969.
4) Gomez-Eyles, et al. Environmental Pollution 2011 , 159(2): 616-622.
5) Hyötyläinen, T. et al. Water Research 2002, 36, (18), 4497-4504.
6) Lu, X. et al. Archives of Environmental Contamination and Toxicology 2004, 47(2): 207-214.
7) Abdallah, M. A.et al. Environment International 2015, 74, 13-22.
8) Turkall, R. M. et al. Proceedings of the Annual International Conference on Soils, Sediments, Water and Energy 2010, 13.

1

PAH Interactions with Soils and Effects on Bioavailability to Humans

Huan Xia, Jose L. Gomez-Eyles, Upal Ghosh – **UMBC**
Mike Ruby – **Integral Consulting, CO**
Yvette Lowney – **Exponent, CO**

Introduction

2

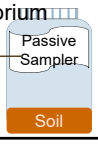
PAHs Distribution and Exposure Pathway from Soils

- PAHs are released into soils within different matrices (e.g. soot, char, or NAPLs)

Altered Bioavailability

- Bioavailability to ecological receptors (e.g. earthworm) is determined by aqueous equilibrium concentration
- Soil characteristics (organic carbon, clay) influence aqueous equilibrium

e.g. Polyoxymethylene (POM) or Polyethylene (PE) strip



Introduction

3

PAHs Distribution and Exposure Pathway from Soils

- For Humans, the main risk driver is the incidental ingestion of contaminated soil
- Physiologically-based extraction tests (PBET) have been used to estimate PAH bioavailability to humans
- Existing PBET data:
  - Different source materials not evaluated
  - Lack of validation against an *in vivo* model

Objectives

4

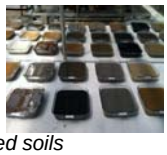
Objectives

- Investigate the effect of different source materials on PAH aqueous partitioning and oral bioavailability
- Examine how different soil compositions affect PAH partitioning and oral bioavailability
- Compare PAH aqueous partitioning with oral bioavailability measured by PBET
- Develop a simple, reproducible, and inexpensive *PBET* that correlates with *in vivo* measures of relative bioavailability

Method

5

Construction of PAH contaminated soils



Pre-weathered soils slurries

2 months of wet and dry cycles

Weathered soils

- Grind peat, clay, sand and sieve down to <150um
- Construct baseline synthetic soil: 70% sand, 20% clay and 10% peat moss
- Introduce amendments for different compositions
- Introduce source materials at different concentrations

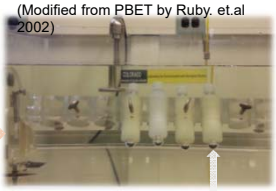
Method

6

Physiologically Based Extraction Test

- Phase one: acidic gastric condition
- Phase two: near-neutral intestinal condition

Extraction Device
(Modified from PBET by Ruby. et.al 2002)



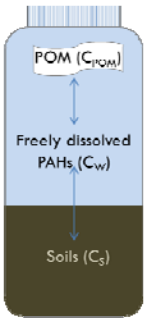
lipid source (sunflower oil), surfactant (bile salts) gut enzymes

Simulated gastrointestinal

Soil Aqueous Partitioning Experiment

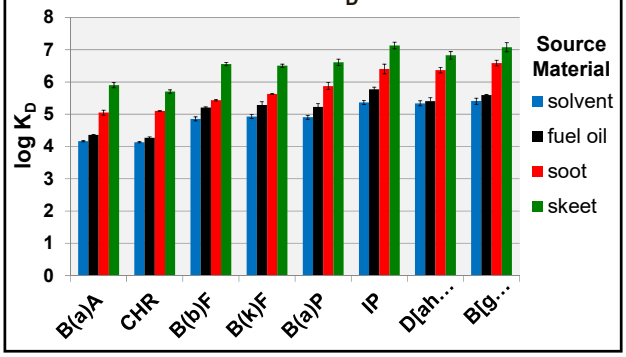
7

- Equilibrium was reached among POM strip, soil and water
- $C_W=C_{POM}/K_{POM,}$ (K_{POM} was previously determined)
- Soil partitioning coefficient, $K_D=C_S/C_W$
- Lower K_D represents higher PAH bioavailability



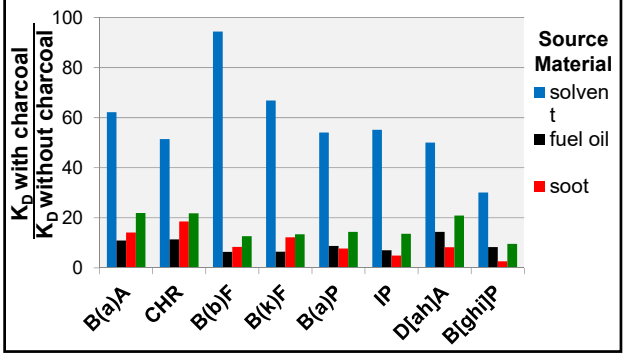
Effect of Different PAH Source Materials on Soil K_D

8



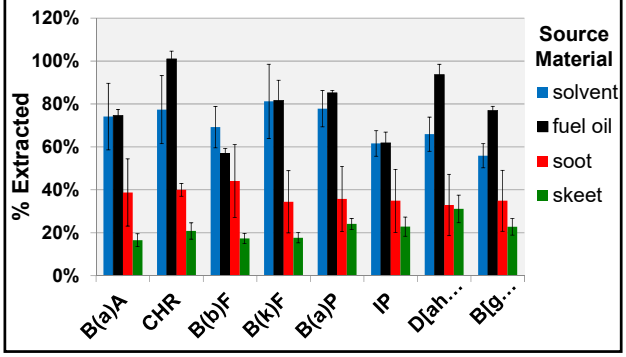
Effect of Addition of 2% Charcoal on Soil K_D

9



Effect of Different Source Materials on PBET

10



Effect of Addition of 2% Charcoal on PBET

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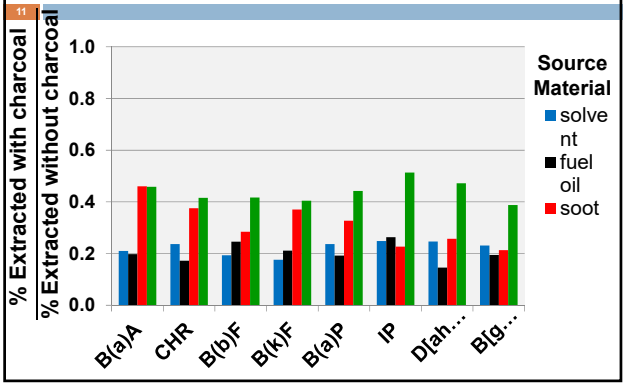
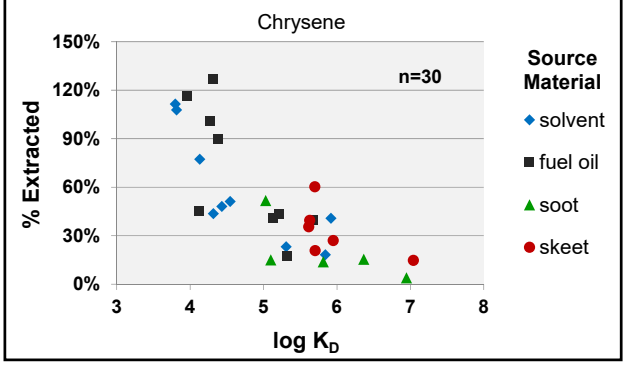


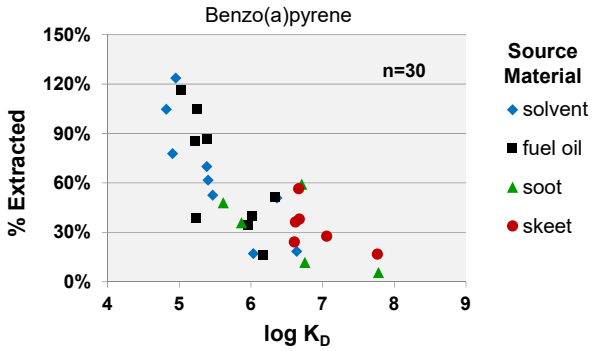
Comparison between Soil K_D and PBET

12



Comparison between Soil K_D and PBET

13



Key Findings

14

- Large variability (1 - 2 orders of magnitude difference) in K_D observed:
  Solvent < fuel oil < soot < skeet
- Source materials also affected PAH extraction by PBET:
  Fuel oil> solvent> soot> skeet
- Charcoal greatly enhanced soil K_D, and dramatically reduced PAH extraction by PBET.
- Soil partition coefficient (K_D) could be a good indicator of PAH bioavailability to humans.
- Activated carbon and biochar application to PAH contaminated soils could reduce risk to humans.

Future Work & Acknowledgements

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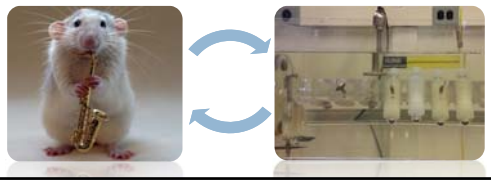
- Data generated by PBET will be compared to in vivo rat data. This will involve an iterative process to optimize PBET.
- This work is part of Project ER-1743, funded by the Strategic and Environmental Research Development Program (SERDP) of the DoD.



Thank you!

16

Effect of Source Material on PAH Partitioning in Soils and Bioavailability to Humans

Huan Xia[1], Jose L. Gomez-Eyles[1,2], Michael V. Ruby[2], Yvette W. Lowney[3], Charles A. Menzie[3], and Upal Ghosh[1]

[1] Dept. of Chemical, Biochemical, and Environmental Engineering, UMBC [2] Integral Consulting, Seatle, WA. [3] Exponent, Boulder, CO.

Introduction

- Polycyclic aromatic hydrocarbons (PAHs) are a group of organic contaminants that are widely distributed in soils, some of which are potent human carcinogens.
- PAHs are released into soils within different sorption domains (e.g. soot, char, coal tar or NAPLs), that may alter their freely dissolved concentrations and bioavailability from soils to humans through different exposure pathways. [1,2]
- Dermal contact and incidental oral ingestion of contaminated soils are two major pathways for human exposure to PAHs from soils.
- Bioavailability through dermal contact can be assessed based on uptake into pig skin [3] and bioavailability through oral ingestion is often estimated using physiologically-based in vitro extraction test (PBET). [4]
- PAH partitioning in soil/sediment has been shown to greatly influence bioavailability to ecological receptors (e.g. earthworms) in previous studies.[5,6]
- Bioavailability to humans may also be impacted by partitioning in soils components and PAH source materials but this has not been evaluated carefully.

Weathered Skeet Particles | Soot Carbon | Fuel Oil #6

Typical PAH Source Materials

Objectives

- Construct a variety of soils with different PAH source materials and measure their respective freely dissolved PAH concentrations and soil partition constants.
- Investigate the effects of PAH source materials (e.g. skeet, soot and fuel oil) and soil components (e.g. charcoal, peat, clay) on PAH partitioning .
- Investigate the effects of PAH source materials on bioavailability to humans through dermal uptake (using pig skin) and oral ingestion (by PBET).
- To explore the effect of soil carbon type on PAH partitioning in soils and bioavailability to humans.

Methods

I. Soil construction

- Baseline ASTM soil constructed with peat, clay and sand (sieved to <150um)
- Control soil (no source) spiked with PAH solvent into baseline soil
- PAH sources (skeet particles, soot and fuel oil) introduced to baseline soil
- 2% charcoal applied to a subset of soils

Two months soil weathering

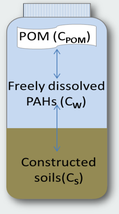
Composition of test soils

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	0.1,1,10mg /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	-	-

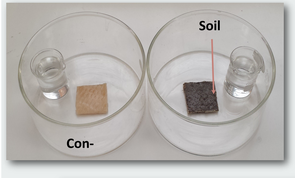
Concentrations shown above are target values before weathering, BSS consists of 10% peat, 20% clay and 30% sand.

II. Equilibrium partitioning test

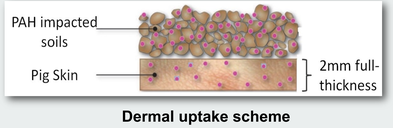
- PAH porewater concentrations measured using Poly-oxymethylene (POM) 76um-thickness strips
- 4 weeks end over end mixing
- $C_W = C_{POM}/K_{POM}$, K_{POM} was previously determined [6]
- Soil $K_D = C_S/C_W$, was calculated
- Lower K_D corresponds to higher sorption capacity and lower freely dissolved concentration



III. Dermal bioavailability to humans estimated using pig skin



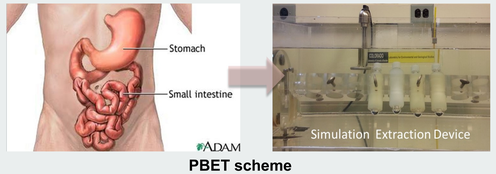
- Full thickness pig skin [7]
- Area: 5 cm^2, thickness: 2mm
- Soil loading: 20 mg/cm^2
- Skin wetted with DI water [8]
- 16 hours exposure at dark



Dermal uptake scheme

- Skin rinsed, saponified, extracted with hexane for PAH analysis [9]
- PAH flux into skin calculated as J = Mass/(Area*Time)

IV. Oral Bioavailability to humans estimated using PBET



PBET scheme

- PBET simulates human gastrointestinal tract.
- Two phase extraction included: acidic gastric phase (1 hour mixing) and adjusted to near-neutral intestinal phase (4 hours mixing)
- Lipids (sunflower oil), surfactants, enzymes etc. added to mimic gut environment
- Solvent extraction of intestinal fluid for PAH analysis

Results and discussion

I. Effect of source material on PAH partitioning in soils

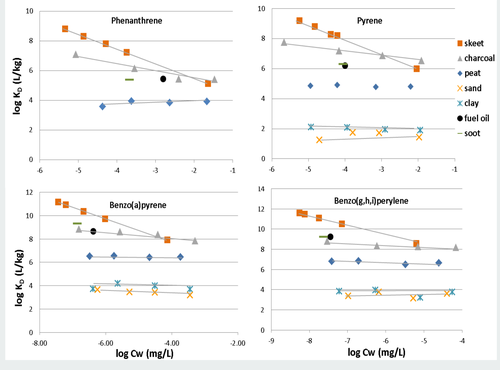


Figure 1. Comparison of log K_D for soil components and source materials for PHE, PYR, B(a)P and B(ghi)P). See ref 13 for details.

- Mineral components (sand and clay) have the weakest sorption capacity
- Sorption nonlinearity observed in charcoal and coal-tar weathered skeet
- Elevation of K_{Skeet} (over 3 log units) observed with increased desorption of PAH from skeet

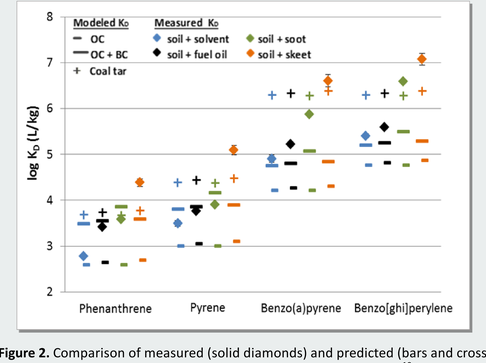


Figure 2. Comparison of measured (solid diamonds) and predicted (bars and crosses) soil K_D using single domain model ($K_D = f_{OC}*K_{OC}$; using generic K_{OC} [10] (short bars) and coal tar K_{OC} [11](crosses)) and dual domain model ($K_D = f_{OC}*K_{OC} + f_{BC}*K_{BC}*C_W^{n-1}$, long bars)[12]. Soils presented in the figure are BSS soils spiked with solvent, fuel oil, soot and skeet at target B(a)P concentration of 1mg/kg. See ref 13 for details.

- Source materials have strong impact on soil K_D: Skeet > soot > fuel oil > solvent
- Same trend also observed for other concentrations: 0.1, 10 and 100 ppm of BaP
- Traditional OC and OC +BC models only predict well for PHE and PYR in solvent/soot/oil soils
- Coal-tar OC model improves prediction for soot and skeet soils

II. Effect of source material on potential dermal uptake

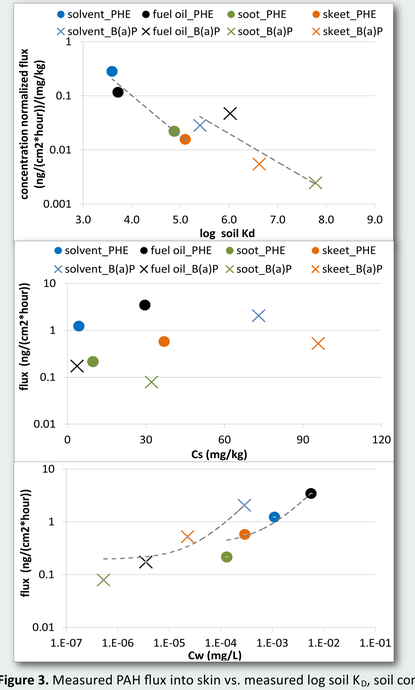


Figure 3. Measured PAH flux into skin vs. measured log soil K_D, soil concentration and freely dissolved concentration C_W under different source materials for PHE, PYR, B(a)P and B(ghi)P). See ref 13 for details.

- PAH flux into skin is strongly negatively correlated with log soil Kd.
- Flux is poorly correlated with soil concentrations, but well predicted by equilibrium porewater concentration .

III. Effect of source material on potential oral bioavailability

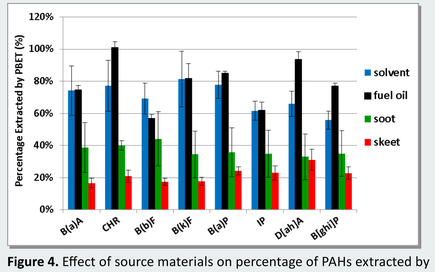


Figure 4. Effect of source materials on percentage of PAHs extracted by PBET at target B(a)P concentration of 1mg/kg.

- Estimated oral bioavailability: fuel oil> solvent> soot> skeet

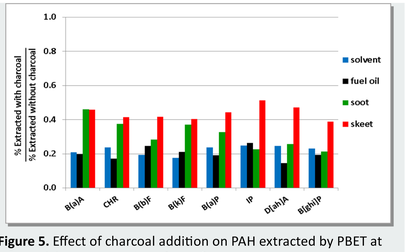


Figure 5. Effect of charcoal addition on PAH extracted by PBET at target B(a)P concentration of 1mg/kg.

- Effect of charcoal on PAH extracted : fuel oil> solvent> soot> skeet

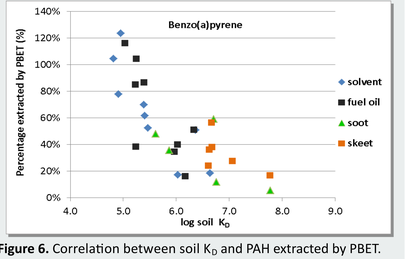


Figure 6. Correlation between soil K_D and PAH extracted by PBET.

- Higher soil K_D leads to lower oral bioavailability estimated by PBET

Conclusions

- Large variability in K_D observed for soils with different sources
- Desorption of PAH from source material can affect source and overall soil K_D
- Different partition models have varying prediction performance for soils with different sources.
- PAH dermal uptake from soil is strongly correlated with, even may be predicted by soil K_D and/or C_W.
- Source materials can also affect PAH extraction by PBET
- Addition of charcoal greatly reduces PAH extraction by PBET.
- Soil K_D may be a good indicator of oral bioavailability to humans.

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Appendix C

Initial Physiologically-Based Extraction Testing

Effect of source materials on PAH bioaccessibility assessed by physiologically based extraction tests

Huan Xia[†], Jose L. Gomez-Eyles[†‡], Michael V. Ruby[#], Yvette W. Lowney[§], and Upal Ghosh[†*]

[†] Department of Chemical, Biochemical and Environmental Engineering, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250, U.S.A.

[#] Integral Consulting Inc., Louisville, Colorado

[§] Exponent, Boulder, Colorado

[*]Corresponding author. Email: ughosh@umbc.edu phone: 410-455-8665

[‡] Current address: Integral Consulting Inc., Seattle, Washington

Introduction

Human exposure to polycyclic aromatic hydrocarbons (PAHs) in soils can occur through incidental soil ingestion and direct dermal contact. Based on the current EPA regulatory paradigm,[1] 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 the focus of several studies since the 1980's. Ortega-Calvo et al. proposes ways to incorporate bioavailability assessments into prospective risk assessment for ecological exposures. [2] As summarized in Ruby et al. [3], the bioavailability of PAHs in soils to human receptors has also been extensively researched.

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 gastrointestinal (GI) tract and eventually absorbed into human systemic circulation. There are a number of animal models developed to predict the PAH oral bioavailability from soils to humans, as summarized in Ruby et al. [3] 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, and associated high cost. Therefore, in vitro extraction tests have attracted attention during the past two decades as a possible alternative to developing data that are useful for understanding bioavailability. In vitro models attempt to predict PAH bioavailability by measuring the dissolved PAH fraction 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 track. 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. pH, incubation time) and understanding how different components of GI fluid (e.g. bile, lipids, etc.) impact PAH bioaccessibility.[4-8] In addition to the gastrointestinal environments, it is also reported that soil properties such as organic matter content can also affect PAH bioaccessibility.[9] However, the role of PAH interaction with different 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 materials in soil

influence PAH bioavailability to ecological receptors.[10-14] Previous research in our lab 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 .[15] 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 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. [4] How the enhanced sorption of PAHs in source materials and soil components influence uptake in a human gut environment is therefore not fully understood.

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 and 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. The details of the soil construction are provided in our previous study. [15] PAH bioaccessibility was measured and compared for different PAH sources, concentrations and soil components, and modeling approaches using partitioning theory (e.g. freely dissolved PAH concentration and partition constants) were tested to explain observed PAH bioaccessibility.

Materials and Methods

Artificially Contaminated Soils. Details for construction of PAH contaminated soils and weathering process were described in Xia et al. [15] 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 tar-based 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 μm prior to soil construction. A control soil was created by spiking PAH stock solution in dimethylchloride into the BSS and allowing solvent dissipation afterwards. All constructed soils were homogenized, weathered at the U.S. Army Edgewood Chemical Biological Center (ECBC, Aberdeen Proving Ground, MD).

Soil PAH Concentrations and Partition Coefficients. PAH concentrations in weathered soils were measured and partitioning coefficients (K_D) for each source material and soil were also determined through aqueous equilibrium experiments using polyoxymethylene passive samplers as described in Xia et al.[15]

Simulated Gastrointestinal Fluid. The in vitro extraction method used in this study was modified from the one reported in Ruby et al. [16] 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 to discourage the formation emulsions during the extraction process. Porcine bile was used in place of bovine bile due to its greater similarity to human, [4] and the bile concentration was reduced from 4g/L to 2 g/L as the latter was proved to be equally effective at mobilizing PAHs from soil. [7] 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 (≥ 65 percent) HCl (Fisher Scientific, USA), 30.0g of glycine (Acros Organics, USA), 1.0g of porcine pepsin (Sigma, USA), 5g 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). After the addition of all components, the volume of the solution was brought to 1 L and the pH of the fluid was then adjusted to 1.50 ± 0.05 by drop-wise addition of concentrated HCl. Then the solution was placed in a water bath at 37°C until the extraction fluid reached 37°C. As described below, the intestinal

phase was simulated by altering the pH of the artificial stomach fluid to 6.5, and addition of porcine bile and porcine pancreatin extract.

Physiologically-Based Extraction Testing (PBET). The PBET used in this study encompassed two phases: simulated stomach extraction and simulated small intestine extraction. For the extraction process, 0.4 g of constructed soils (air-dried and sieved to <150 μm) was dosed into a centrifuge tube with a Teflon-lined screw cap, followed by addition of 40 mL of prepared simulated stomach fluid (soil/liquid ratio 1:100). [17] 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 digestion 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°C, the tube was centrifuged at 1500 x g for 10 minutes to separate the extraction fluid from the soil particles.[18] After that, 30 mL of the supernatant was transferred into a clean 60 mL glass vial with glass Pasteur pipettes and stored at 4°C for further analysis.

Method Development for PAH Solvent Extraction from PBET. Bioaccessible PAHs were then extracted from the physiologically-based fluid three times by hexane solvent on an orbital shaker. Preliminary testing was performed to optimize the extraction of PAHs from the physiologically-based fluid. Different agitation speeds (up to 100 rpm) and contact durations (from 2 to 64 hours) were evaluated to maximize the recovery efficiency (see Figure S1 and S2 for details). Eventually, the optimum method included a maximum of 60 rpm agitation speed (to avoid the formation of emulsions), and three sequential extractions with hexane. Deuterated phenanthrene surrogate was spiked into each container of simulated intestinal fluid at the beginning of the extraction process and 20mL of hexane was introduced into each extraction vial. The first extraction lasted 16 hours at 60 rpm and after that, the solution was allowed to stand for 1 hour for separation of the solvent phase. The hexane was then pipetted into another clean 60 mL glass. The extraction procedure was repeated two more times (24 hours for each) and the three hexane solvent aliquots (total of 60mL) were combined. To remove water from the hexane extract, 10 g of anhydrous sodium sulfate was added and shaken overnight. The volume of the extract was reduced to approximate 2mL using rotary evaporation and nitrogen blow-down. Silica gel cleanup

was performed on the extract following EPA Method 3630C and the final eluate was condensed to approximate 1 ml. Internal standards were spiked and PAHs were 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 prep to isolate and analyze the PAHs from the physiologically-based fluids.

Bioaccessibility Calculation. The PAH bioaccessibility from soil was calculated as ratio of the PAH extracted by the gastrointestinal fluid to the total PAH initially dosed with the soil:

$$\text{bioaccessibility (\%)} = \frac{M_{\text{PAH in GI fluid}}}{M_{\text{initial PAH in soil}}} \qquad \text{eq(1)}$$

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. All extractions were performed in triplicate for each soil sample. The gastrointestinal extraction method recoveries (10 percent frequency) were determined by spiking 100ul 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 extraction method recoveries for 16 PAHs 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 check the consistency of the gastrointestinal extraction procedure. The variability (relative standard deviation) of extractability for the certified reference soil ranged from 30% to 50%. Four internal standards (1-fluoronaphthalene, p-terphenyl-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 extraction and cleanup procedures. The surrogate recoveries varied from 80% to 110 %.

Results and Discussion

Effect of Source Material on Bioaccessibility. PBETs were performed on all of 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. As shown in Figure 1, PAH source material had a great impact on the oral bioaccessibility of PAHs from soils. The soils presented in this figure were all baseline soil spiked with different PAH source materials at a targeted concentration of 1mg/kg target 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 (of around 20%). The higher desorption of PAHs into physiologically-based fluids that was observed for solvent-spiked soils was not unexpected due to the lack of external carbonaceous source from the spike relative to other source materials. Thus, there is a higher tendency for PAHs to desorb from the solid phase (e.g. natural organic matter) into digestion fluid. Interestingly, the observed PAH bioaccessibility from fuel oil spiked soils were 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 simulated GI fluid matrix. 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) and favor the mobilization of the bound PAHs. [4, 7] The residual oil is likely broken up and incorporated into the micellar domains in the simulated GI fluid. This is not surprising as the natural GI fluid is developed by human body to solubilize fats as an important component of the digestion process. Thus, among the common PAH source types PAH bioaccessibility is likely going to be the highest for oils.

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). The extremely high PAH sorption capacity (K_D) associated with lamp-black soot used in this study has been frequently reported, [11,19] which explains the low PAH bioaccessibility observed from soot spiked soils. Similarly low bioaccessibilities (15-50%) of BaP bound soot have been reported by others, even when attempting to enhance the PBET using an extended colon-phase or adding infinite sinks such as silicone rod or sheet to the gastrointestinal fluid. [4, 20, 21] Like soot, high K_D has also been often observed for coal tar derived pitch materials,[12, 22] which explains the low PAH

extractability by digestive fluid from skeet spiked soils. Also, similarly low bioaccessibility (15-41%) was observed in soils from a former tar and pitch work site. [23] 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 that served as one of the source materials in this study has been through decades of field weathering might contribute to its enhanced K_D and reduced bioaccessibility. Additionally, in our previous study we have observed increased K_D for skeet particles by 2-3 orders of magnitude as PAHs were desorbed from the source. Therefore, as PAHs were released into GI fluid, its bioaccessibility would be gradually inhibited by the elevated K_D from skeet.

A similar trend was observed for soils targeted to achieve 0.1mg/kg B(a)P, except the PAH bioaccessibility from fuel oil soil became prominently higher than all other soils, and the difference between soot and skeet spiked soils diminished (Figure S5). When the soil concentration increased to 10 mg/kg, the PAH bioaccessibility from fuel oil soil dropped compared to previous 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 residual fuel oil in the system to serve as a "sink" for the PAHs); or there may be some enhanced form of interaction with the components of the simulated GI fluid 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 started to displace skeet soil and become the most recalcitrant to gastrointestinal extraction (Figure S6). Exactly the same trend was also found in soils with target concentration of 100 mg/kg B(a)P (Figure S7). In our previous partitioning studies, higher K_D 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 K_D could be explained based on the large 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. [15]

Effect of Concentration on Bioaccessibility. During soil construction, the amount of PAH source materials introduced to BSS varied based on the PAH concentration to be achieved: 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 was 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. Additionally, the decrease in bioaccessibility at the highest concentration for the solvent-spiked soil may indicate capacity limitation of the fixed volume of simulated GI fluid.

When investigating BaP bioaccessibility at different concentrations, a study by Sips et al. also found a clear levelling off in percent bioaccessibility in soils spiked with concentrations above 100 mg/kg BaP. [24] This capacity issue has been the focus of recent investigations by Gouliarmou et al.[8, 21] that have pointed out the drawback 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 sink (sunflower oil) was included in our PEBT system and the extraction capacity may have been exceeded. 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.

The 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 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-order 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 ingredients of the GI fluid, such as sorbing the lipid fraction or other key constituents that are critical for the formation of PAH solubilizing micelles.

For skeet spiked soils, the highest PAH bioaccessibility was found in 0.1 ppm soil for all PAHs. However, the trend with increased concentration was different for different PAHs. For the low molecular weight PAHs, there was first a marked decrease in bioaccessibility when the target B(a)P concentration increased to 1 ppm followed by an increase in bioaccessibility with further increase in PAH concentration. For the two PAHs with the highest molecular weight (dibenz(a,h)anthracene and benzo(g,h,i)perylene), the bioaccessibility continually decreased with increase in concentration. This nonlinear trend with PAH concentration is likely caused by the effect of multiple mechanisms that are difficult to de-convolute based on the available results from this study.

Effect of Soil Composition on Bioaccessibility. The effect of different soil compositions on PAH bioaccessibility was only evaluated on solvent and fuel oil spiked soils. As shown for solvent spiked soils in Figure 3, the baseline soil and soil with 2% clay showed the highest PAH bioaccessibility. Based on previous K_D measurement (Figure S4),[15] 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 increased PAH bioaccessibility. Conversely, as expected, soil with addition of charcoal had the lowest bioaccessibility, which was almost certainly due to its strong sorption capacity for PAHs. The soil with clay replaced by montmorillonite had lower PAH bioaccessibility than baseline soils, especially for 4-5 ringed PAHs, which was due to the slightly lower overall soil K_D measured for baseline soils (Figure S8). As for organic matters, humus, a more decomposited type of organic carbon measured a slightly lower K_D, but the soils spiked with which exhibited a lower bioaccessibility compared to its peat spiked soil counterpart. Also interestingly, soils with less peat content (of 2%) had much lower PAH bioaccessibility than baseline soil. In addition, as shown in Figure S9, the same trends were observed for fuel oil soils with reduced peat content: lower bioaccessibility was associated with lower peat content in soil. The contrary would be expected based on how we understand peat influences PAH sorption in soils and this behavior of peat was difficult to explain. One clue we suspected was the non-linear sorption to the peat. Endo et al. suggested that many amorphous organic carbon phases like peat could also be non-

linear.[25] However, the sorption isotherm results from our previous study confirmed the linearity of sorption for the peat we used over a range of aqueous concentrations across up to four orders of magnitude. Thus sorption nonlinearity may not be a key factor.

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 impact from charcoal was 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_D 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. So, for soils with low Kd, the addition of charcoal had the largest effect on bioaccessibility. 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_D increases. [15] Also, the effect of charcoal was very consistent among different PAH compounds. These results are consistent with previous findings that carbonaceous amendments reduce PAH bioavailability to organisms dwelling in soils [26] and sediments [27], and in mammalian research models where added charcoal resulted in decreases in PAH bioavailability from soil following oral exposures[28].

Relation between Soil K_D and Bioaccessibility. 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 estimated by PBET for all three PAHs, with R^2 ranging from 0.65 to 0.74. This strong linear 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. Among all 30 soils investigated, those spiked with soot or skeet which have relatively higher K_D are observed to have much lower PAH bioaccessibility than soils spiked with PAHs in solvent or fuel oil. Also, the lowest bioaccessibility was observed for benzo(g,h,i)perylene with the highest molecular weight and also highest K_D among PAHs. Two key conclusions can be made based on the results shown in Figure 5: first,

bioaccessibility in simulated human GI fluid is strongly influenced by the partitioning characteristics of PAHs in soil. While the solubilization of PAHs in the GI fluid involves much more complex transport processes than simpler 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 that physicochemical processes in soil (e.g. partitioning constants) can impact how PAHs are released in the gut environment, after the study by James et al.[29] Recent work by Gouliarmou et al.[13] attempted to contrast the different in bioaccessibility between field soil and charcoal. However, their study with a modified PBET method did not observe a significant difference in bioaccessibility between the two matrices. The study by James et al. also observed negative linear correlation between soil fugacity (expressed as vapor pressure *K_D*soil particle density) and bioavailability estimated through in vivo swine study.[29] However, in their study, most of the observed correlations were weak and deteriorated with PAH hydrophobicity, with the coefficients of determination (R^2) decreasing from 0.72 to 0.13, from benzo(a)anthracene to benzo(k)fluoranthene.[29] This weak correlation might be caused by the large variance in the detected bioavailability from 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. Similarly decent reproducibility was also observed among other in vitro models such as the Fed Organic Estimation human Simulation Test (FOREhST) with less than 10% relative standard deviation, and Simulator of the Human Intestinal Microbial Ecosystem (SHIME) with less than 20% relative standard deviation.[6] Another possible cause to the weak correlation could be the flaw in their method for determination of C_W: 1) the mixing duration of 14 days might not be sufficient for equilibrium, especially for heavier PAHs, and 2) the C_W was quantified through centrifuging the soil slurry and filtrating the supernatant, which might not successfully remove the dissolved organic carbon in the water phase, resulting in overestimated C_W and underestimated K_D. For example, unreasonably low log K_D values of less than 1 were observed for heavy PAHs (e.g. dibenz(a,h)anthracene and ben(g,h,i)perylene) in soil with organic carbon fraction of 4.6% (soil GW 2).[29] However, despite their flaws in measuring C_W, the strong correlations between K_D and bioaccessibility observed in our study was very consistent with their findings between soil fugacity and bioavailability. Secondly, 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 very distinct. While the elevation of partition constants for skeet relative to solvent-spiked soil (of approximately 2 log unit) 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%). In addition, the relationships are less clear for some source materials when evaluated individually than the overall trend. For example, the measured bioaccessibility from solvent and fuel oil spiked soils generally varied more considerably (from 2% to >100%) than soot and skeet spiked soils, which may be an indication of the higher stability of PAHs within the matrix of soot and skeet.

Overall, the exact mechanistic link between K_D and bioaccessibility is still not clear based on the results from this study alone. Nevertheless, the strong correlation between K_D and bioaccessibility in our study suggested that a simple partitioning approach may suffice to predict bioaccessibility.

Mechanistic Partitioning Model. A mechanistic partitioning model was developed to explain the PAH desorption 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 solute (e.g. enzymes, lipids). Provided the fine size of the soil particles (<150 μm) dosed into GI fluid and the vigorous mixing, it was assumed that PAH desorption from the source 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_{GI}, which was assumed to be constant for a PBET system with specified compositions for simulated GI fluid and environment. It is noteworthy that K_{GI} describes the non-equilibrium partition ratio of PAH concentration in simulated GI solutes to aqueous concentration after 5 hours mixing, which is not a function of soil concentration or soil compositions but PAH properties for a specified PBET system.

Measuring GI Solute Partitioning Constant. The K_{GI} for the PBET system used in this study was experimentally determined with polyethylene passive samplers (that had been pre-impregnated with PAHs) as PAH source, as well as tool for determining PAH aqueous

concentration after 5 hours mixing. The PAH concentration in simulated GI solute was measured by extracting the GI fluid three times with hexane after removing the passive samplers (see supporting information for detailed method).

$$K_{GI}=\frac{C_{GI}}{C_W} \qquad \text{eq (2)}$$

where C_{GI} (in ug/g) and C_W (in ug/mL) are the PAH concentrations in GI solutes and aqueous phase measured from the partitioning test. And based on the previous assumptions, PAH bioaccessibility from soil measured by our PBET system may be predicted using partitioning approach among soil, aqueous and GI solute phases, with experimentally determined K_{GI} and K_D, as well as other in vitro model specified parameters such as D_{GI} and D_S.

$$\text{bioaccessibility (\%)}=\frac{M_{\text{PAH in GI fluid}}}{M_{\text{initial PAH in soil}}}=\frac{K_{GI}D_{GI}C_W}{C_S D_S} \quad \text{eq (3).}$$

where D_S and D_{GI} (both in gram) are the mass of soil particles and overall mass of GI solutes used in our PBET; C_S (in ug/g) is the initial PAH soil concentration and C_W is the previously determined PAH aqueous concentration in different soil slurries at equilibrium from Xia et al.[15] Model projected bioaccessibility results were compared with observed values under different source materials.

As shown in Figure 6, the model performance varied with PAH source materials and overall underestimated bioaccessibility. Our model provided the best bioaccessibility predictions for solvent and fuel oil spiked soils, which was within a factor of 5 for over 99% of the measured values. As for soot spiked soils, over 90% of the predictions fell within a factor of 10 and over 85 % of which fell within a factor of 5. The model gave the poorest prediction for skeet spiked soils with only half of the predictions fell within a factor of 5 and 80% of predictions fell within a factor of 10 of their measured values.

Further work to understand the role of surfactants and the other solubilizing components (e.g. lipid sink) in the simulated GI fluid, and how each of these components may interact with the various soils and PAH source materials may help elucidate the factors that control the bioaccessibility (and bioavailability) of PAHs from soil.

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Appendix D

In Vivo Evaluation of the Relative Oral Bioavailability of PAHs from Soil

Environmental Science & Technology

Article

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Effects of Source and Concentration on Relative Oral Bioavailability of Benzo(a)pyrene from Soil

Stephen M. Roberts,*[,†] John W. Munson,[†] Michael V. Ruby,[‡] and Yvette Lowney[§]

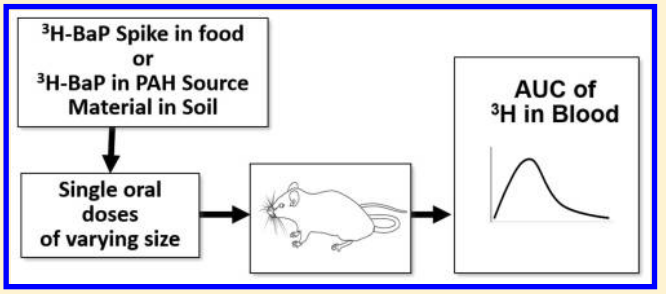
[†]Center for Environmental & Human Toxicology, University of Florida, Gainesville, Florida 32611, United States

[‡]Integral Consulting, Inc., Louisville, Colorado 80027, United States; and

[§]Alloy, LLC, Boulder, Colorado 80302, United Stated

Supporting Information

ABSTRACT: The objective of this study was to examine the influence of soil composition, PAH concentration, and source material type on PAH bioavailability using an approach capable of measuring uptake at low, environmentally relevant PAH concentrations (down to 1 ppm). Contaminated soil samples were constructed using PAHs from three source materials—solvent, soot, and fuel oil—to which ^{3}H-benzo(a)pyrene (^{3}H-BaP; total BaP concentrations of 1, 10, and 100 ppm) was added in a mixture of PAHs. The soils were weathered for 8 weeks using weekly wet−dry cycles. Each soil was administered as a single dose to rats, and blood samples were taken over 6 days. Relative oral bioavailability (RBA) of the BaP from soil was estimated by comparing the area under the curve (AUC) for ^{3}H concentration versus time in blood with the AUC observed from the same PAH mixture dosed in a food matrix. The extent to which BaP RBA was diminished in soil versus food varied among the source materials, but little or no difference was observed among the soil types examined unless carbon amendments were added. These results suggest that the type of PAH source material can have a strong influence on PAH oral bioavailability.



INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment and commonly identified as chemicals of concern in soil at contaminated sites. Several PAHs are considered potential human carcinogens,[1−3] and estimated risk of cancer from PAH exposure typically drives cleanup decisions for these sites. In current exposure models for human contact with soil, the dominant pathway for contaminant intake is through incidental ingestion. In laboratory animals given a PAH such as benzo(a)pyrene (BaP) orally, tumors occur at a variety of sites, including sites away from the gastrointestinal tract.[4] This indicates that PAHs are capable of producing cancer systemically, and that the risk of cancer is therefore related to the amount of PAH that is absorbed after ingestion.

Several studies have shown that the absorption of PAHs from soil is incomplete.[5−11] Although there are limitations in the approaches used in a number of studies,[12] there is ample evidence that determining the bioavailability from soil is important for accurate estimation of potential human exposure. The issue for risk assessment is determining the extent to which PAH absorption from a specific contaminated soil is reduced compared with the absorption that occurred under the conditions of the critical study used to determine the oral cancer potency of PAHs. Currently, the cancer potency estimates used by the U.S. EPA for all carcinogenic PAHs are derived from the cancer potency estimate for the "index" carcinogenic PAH, BaP.[13] The BaP cancer potency estimate, in turn, is based on tumor responses observed in two chronic rodent bioassays in which BaP was given in the diet.[14,15] Thus, the information needed to adjust risk estimates for carcinogenic PAHs in soil is the bioavailability of the PAH from soil relative to its bioavailability from food, that is, its relative bioavailability (RBA).

There are a number of studies that have estimated the RBA of carcinogenic PAHs such as BaP in soil, including studies that investigated the effect of PAH sources and soil characteristics (see Ruby et al. for a recent review).[12] However, an important limitation of all is the use of animal models that require PAH concentrations in the 10s of ppm (or higher) to be able to measure bioavailability. This issue is described more fully in Ruby et al. 2016,[12] and is illustrated by a recent PAH bioavailability study where RBA was not measurable in animals using contaminated site soils (BaP up to 300 mg/kg), and only quantifiable in spiked soils containing BaP concentrations in the range of 1000 or 10 000 mg/kg.[16] In contrast, risk-based cleanup goals for carcinogenic PAHs are often 1 ppm or less. As a consequence, RBA measurements are made at PAH concentrations orders of magnitude higher than concentrations for which regulatory decisions must be made. There is currently

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Table 1. Test Soil Matrix: PAH Sources, Soil Characteristics, and Benzo(a)pyrene Concentrations

PAH source	baseline synthetic soil[a]	synthetic soil added charcoal[b]	synthetic soil reduced peat[c]	synthetic soil reduced clay[d]	synthetic soil montmorillonite in place of kaolinite	synthetic soil humus in place of peat
solvent	1, 10, 100 ppm BaP	10 ppm BaP	10 ppm BaP[e]	10 ppm BaP	10 ppm BaP[e]	10 ppm BaP[e]
soot	1, 10, 100 ppm BaP	10 ppm BaP				
fuel oil	1, 10, 100 ppm BaP	10 ppm BaP	10 ppm BaP	10 ppm BaP		

[a]Baseline synthetic soil, 70% sand, 20% clay, 10% peat. [b]Baseline synthetic soil with 2% charcoal added. [c]Baseline synthetic soil with peat reduced to 1%. [d]Baseline synthetic soil with clay reduced to 2%. [e]Not carried through the study because of evidence for BaP degradation during weathering.

no validation that RBA results obtained from these highly contaminated soils are applicable to more common, environmentally relevant concentrations.

The ability to obtain estimates of PAH RBA at low, environmentally relevant concentrations could conceivably be achieved with a predictive in vitro bioaccessibility test, and an in vitro approach would have additional benefits of providing RBA information more rapidly and at lower cost than an RBA measurement in vivo. However, assessment of the performance of candidate in vitro tests requires a series of soil samples for which the RBA has been determined through in vivo measurement. This suite of soil samples should ideally include different soil types and PAHs in different concentrations from different source materials. The typical approach to assembling a suite of soil samples for this purpose is to collect samples from a limited number of contaminated sites and measure RBA in vivo through conventional means. The disadvantage of this approach is that soil samples are usually selected largely by convenience, limiting the ability to examine the influence of key variables on bioavailability. Additionally, in the case of PAHs, RBA values can only be obtained for soils with relatively high PAH concentrations.

As an initial step in the development of an in vitro PAH RBA estimation method, a different approach was taken in this study, using constructed rather field soils from contaminated sites. This study focused on the index carcinogenic PAH, BaP, and test soils were created to differ independently with respect to three variables potentially affecting BaP bioavailability from soil, viz. BaP concentration, BaP source material, and soil composition. To enable measurement of BaP RBA at concentrations extending down into the range commonly encountered at contaminated sites (i.e., to 1 ppm), a radiolabeled BaP tracer was included. These soils were weathered to simulate conditions that occur in the environment, and RBA was measured relative to food using a rat model. Table 1 shows the "matrix" of factors incorporated into the design of the soil study substrates. In addition to creating benchmark soil samples for in vitro method development, this study provides new information on the effect of BaP source and concentration on its bioavailability from soil. It also raises interesting questions about how RBA information should be applied to PAH concentrations measured at contaminated sites using conventional solvent extraction sample preparation.

MATERIALS AND METHODS

Animal Model. In order to be able to measure BaP bioavailability at low doses, radiolabeled BaP was incorporated into the test soils prior to weathering. Tritium was used as the radiolabel because this material provides a high specific activity allowing bioavailability measurements of BaP in ingested material down to the subppm concentration range.[17] The use of radiolabel also allows both parent BaP and metabolites appearing in blood to be quantified in a straightforward manner. Systemic absorption was determined by measuring the area under the curve (AUC) for the blood concentration versus time profile. Considered the classical method of measuring systemic oral bioavailability, the AUC approach avoids potential problems in estimation of absorption of PAHs that occur with other methods such as measuring PAH metabolites in urine.[12] A critical assumption in relative bioavailability determinations is that the clearance of the substance is the same for the doses that are being compared. BaP and other PAHs are capable of inducing their own metabolism, making this assumption difficult to meet with repeated doses.[12] To avoid this problem, relative oral bioavailability was determined after a single BaP dose. As noted in the Introduction, the cancer potency estimate for BaP developed by the U.S. Environmental Protection Agency is based upon studies in which BaP was administered in food. The appropriate basis for determining RBA of BaP in soil was therefore comparison with bioavailability from food, specifically rodent chow as used in the BaP cancer studies.

The rat was selected as the animal model for this study for a number of reasons. The rat is well established as model for bioavailability studies. In fact, for determining bioavailability of organic compounds in drug development, the rat is second only to humans in frequency of use,[18] and is one of the two species from which the BaP cancer slope factor was derived. The rat is large enough to provide several blood samples of reasonable volume over time from which a blood concentration versus time profile can be obtained, yet small enough so that large amounts of radiolabel are not required and radioactive waste from excreta can be easily managed.

Male Sprague–Dawley rats (approximately 300 g bw) were obtained from Harlan Laboratories (Fredrick, MD). The animals were purchased from the vendor with surgically placed jugular catheters to facilitate blood collection. A polyurethane catheter had been placed in the right jugular vein under ketamine/xylazine anesthesia, and the catheter was tunneled subcutaneously and exteriorized dorsally between the scapulae. The catheter was locked with sterile heparin glycerol solution so as to remain patent. Upon receipt, the animals were housed individually in polycarbonate cages with controlled light/dark cycles of 12 h (08:00–20:00) in temperature- and humidity-controlled animal quarters. Animals were fed a standard diet (Teklad Rodent Diet 8406, Harlan Laboratories) and had free access to water. This study was approved by the Institutional Animal Care and Use Committee and animals were treated according to criteria in the *NIH Guide for the Care and Use of Laboratory Animals*.

Chemicals. ^{3}H-Benzo(a)pyrene (20 Ci/mmol) was obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). Purity of the radiolabeled BaP was verified by

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radiochromatography and was found to be 99%. BaP (unlabeled) was purchased from Sigma-Aldrich (St. Louis, MO). Other polycyclic aromatic hydrocarbons were obtained from Ultra Scientific (Kingstown, RI). Soluene 350, ethanol, hydrogen peroxide (30%), sodium sulfate, dichloromethane, methanol, acetone, ScintiVerse scintillation cocktail, soot, and charcoal were purchased from Fisher Scientific (Pittsburgh, PA). Fuel oil (Fuel Oil No. 6) was provided by Chevron (San Ramon, CA), kaolinite, and montmorillonite clay were from ACROS Organics (through Fisher Scientific, Pittsburgh, PA), sand (SIL-CO-SIL) was obtained from U.S. Silica (Frederick, MD), peat was from Miracle-Gro (Marysville, OH), and humus was from Organic Valley (La Farge, WI).

Food and Soil Sample Preparation. Soils were constructed consistent with an ASTM standard soil developed for the testing of toxicity to terrestrial organisms (ASTM E16-12). This "Baseline Synthetic Soil (BSS)" consisted of 70% sand, 20% kaolinite clay, and 10% peat. Some samples were modified to reduce peat or clay content, or to add charcoal or replace peat with humus (Table 1). Food material was prepared by pulverizing rodent chow pellets to a fine powder. Each constructed soil was thoroughly mixed, air-dried, and sieved to 150 μm. This particle size range was recently identified as the most appropriate soil fraction to assess in understanding human exposures to PAHs in soil.[19] A mixture of PAHs, including BaP, was added to each soil and to food to create a consistent profile, that is, in constant proportions among individual PAHs. Target PAH concentrations in test soils spanned 2 orders of magnitude, with BaP concentrations set at 1, 10, and 100 ppm (Table S1). PAHs were added to soil in a variety of "source materials," including solvent (dichloromethane), soot, or fuel oil, and in solvent to food. The soot and fuel oil naturally contained PAHs (Table S2), and these concentrations were taken into account in determining the amounts of various PAHs added to food and soil to achieve the target concentrations. Some of the added BaP was labeled with tritium (^{3}H) such that each soil and food sample had an initial concentration of ^{3}H-BaP of 50 μCi/g.

Addition of the PAHs to soil and food was as follows. For soils with a solvent source, mixtures of PAHs as shown in Table S1 were prepared in 10 mL dichloromethane and added to a 100 mL glass bottle. The liquid was swirled gently so that the wall of vessel was coated with liquid. Soil (20 g) was immediately added, and the bottle was capped and placed on a roller for 24 h. The bottle was then opened and the soil was allowed to air-dry for 24 h at room temperature. The soil was disaggregated with a spatula until it appeared homogeneous and then mixed on a roller for 24 h. The same procedure was followed for addition of PAHs to food. For soils where soot served as the source of PAHs, a mixture of PAHs in dichloromethane was added to soot, and a slurry containing 0.1, 1, or 10 g of soot was gently swirled in a 100 mL glass bottle, followed by addition of the soil to a total weight of 20 g. The bottle was then capped, rolled, and vented, and the soil air-dried, as described for PAH addition in solvent. The amounts of the PAHs added to soot were determined based upon the concentrations of PAHs already present in the soot (Table S2) such that the target concentrations in Table S1 were achieved, including BaP concentrations of 1, 10, or 100 ppm from addition of 0.1, 1, or 10 g of soot, respectively, to soil. For PAHs added to soil in fuel oil, a similar procedure was followed in which the PAH mixture in dichloromethane was first added to fuel oil, and 0.01, 0.1, or 1.0 g of fuel oil with added PAHs were placed in a glass bottle to which the sand component was added. The bottle was capped, rolled, vented and then after 24 h, the rest of the soil components added. The bottle was again capped, rolled, and vented with the rest of the processing of the soil samples identical to PAHs added in solvent or soot.

Soil Weathering. Each test soil was subjected to an 8-week weathering process consisting of weekly wet/dry cycles. To weather the soils, each week deionized water was added in an amount equal to the water holding capacity of the soil (56% by weight). During the weathering process, soils were kept in open wide-mouthed bottles at room temperature. At the conclusion of the weathering process, each soil was homogenized and aliquots of soil were taken for assessment of ^{3}H-BaP content. Coefficients of variation in radioactivity in triplicate samples from each of the soils used in the study were less than 10%, and all but two were less than 4%, indicating good homogeneity.

Determining the Tritium Content of sSoils. Tritium content of soils after weathering was determined using liquid scintillation counting in two ways. Solvent-extractable tritium content was determined using a modified version of EPA Method 3550. A 200 mg aliquot from each test soil was added to a 20 mL borosilicate glass scintillation vial, along with 6 mL of a 1:1 acetone-dichloromethane mix, and the vial was pulse-sonicated (alternating 30 s on and 30 s off at full power) for 3 min on ice. The sample was centrifuged at 1000g for 5 min at room temperature. The supernatant extract was transferred to a 13 × 100 mm silanized borosilicate glass tube. The soil pellet was re-extracted with another 3 mL acetone-dichloromethane solvent, and after point sonication and centrifugation, the supernatant extract was combined with the original extract then mixed and the volume measured. A 200 μL aliquot of the combined soil extract was added to scintillation fluid (15 mL) and the vials were vortex mixed and allowed to stand for 24 before scintillation counting. Separately, total tritium content of the soil was determined following acid digestion. One hundred μL of concentrated nitric acid was added to a 25 mg aliquot of soil in a 20 mL scintillation vial. The vial was capped and allowed to stand for 24 h. Scintillation fluid (15 mL) was then added, and after another 24 h the sample was counted. In each approach, the amount of radioactivity (in nCi) was determined from dpm with quench correction.

Radiochromatography of Weathered Soil. After weathering, soils were evaluated for the integrity of the tritium label on BaP. An aliquot from each test soil was extracted using the modified Method 3550 procedure described above. The acetone-dichloromethane extract was heated at 50 °C under nitrogen to near dryness. Samples were brought up to 1 mL with methanol, passed through a 0.22 μm filter, and analyzed by HPLC. HPLC separation used an Agilent 1100 HPLC system with fluorescence detection and fraction collection (Agilent, Santa Clara, CA). A C18 column (5 μm packing, 150 mm × 4.6 mm; Grace Corp., Columbia, MD) was used with a programmed change in mobile phase from 50:50 water:-methanol to 100% methanol between 3 and 15 min. Fluorescence detection used an excitation wavelength of 260 nm and an emission wavelength of 430 nm. Injection of a BaP standard was used to determine the BaP retention time. Eluent samples were collected into 6 mL scintillation vials every 30 s for 20 min. Five mL of scintillation fluid was added to each vial, vials were vortexed and allowed to stand for 24 h, and radioactivity was determined using liquid scintillation counting with quench correction. A radiochromatogram for each sample

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was constructed from radioactivity eluted over each 30 s interval.

Dosing and Blood Sampling. Animals were fasted overnight prior to food or soil dosing. Treatment groups consisted of five animals. Each rat was administered 0.5 g (containing 25 μCi ^{3}H-BaP) of food or soil by gavage. Immediately prior to administration, the food or soil dose was prepared as a slurry by adding 1.5 mL deionized water and mixed until the food or soil was dispersed uniformly. The residual dose material remaining in the dead space of the gavage tube after administration was collected by flushing with 150 μL deionized water and radioactivity was measured by scintillation counting. This was subtracted from the nominal 25 μCi dose to determine the actual administered dose for each animal. Two hours after administration of the dose, access to food was restored.

A blood sample (300 μL) was taken via the jugular catheter immediately prior to the dose and 2, 4, 8, 12, 24, 48, 72, 96, 120, and 144 h after the dose. Blood samples were placed into a 20 mL borosilicate glass scintillation vial and stored at 4 °C for up 48 h before determination of radioactive content. One mL of a 1:1 Soluene 350 ethanol mixture was added to each vial, and the vial was capped and vortexed for 60 s. The capped vials were incubated for 2 h at 60 °C and allowed to cool to room temperature. One mL of hydrogen peroxide (30%) was then added dropwise to each vial, and the vials were again incubated at 60 °C for one h and allowed to return to room temperature. Fifteen mL of scintillation cocktail were added and the vials were vortexed for 60 s and allowed to stand for 24 h before counting. Radioactivity in each vial was determined using liquid scintillation counting with quench correction.

Relative Bioavailability Measurement. RBA estimates were based upon the concentration versus time profile for BaP and metabolites in blood quantified by measuring the ^{3}H- label in sequential samples over time. Blood concentrations were expressed as nCi/mL, and the AUC was calculated using the trapezoidal rule. As described above, all soils and food were prepared such that a 0.5 g dose would include 25 μCi, irrespective of the concentration of BaP. Therefore, although the soil concentration and administered dose of BaP varied across the soils, the dose of tritium was held constant. Although the nominal dose of tritium was the same from both food and soil (25 μCi), there were slight differences in doses actually administered, for example, from dose solution retention in the gavage tube. To eliminate this source of error, AUC values for each animal were corrected based on administered dose using the following relationship:

$$\mathrm{AUC}_{\mathrm{corrected}} = \mathrm{AUC}_{\mathrm{observed}} \times \frac{25\mu\mathrm{Ci}}{\mathrm{dose}_{\mathrm{actual}}} \quad (1)$$

As described above, in this study design it is important that each animal be "naive" with regard to PAH exposure to ensure that induced metabolism of the PAHs did not result in differential effects on the AUC for each dose. Consequently, each subject received a single dosing substrate (food or soil containing ^{3}H-BaP) on a single occasion, and as a result RBA estimates could not be derived on an individual animal basis. Instead, RBA estimates for each soil sample were obtained from the mean AUC observed in treated animals and the mean AUC from animals given BaP in food:

$$\mathrm{RBA}_{\mathrm{soil}} = \frac{\mathrm{AUC}_{\mathrm{soil,mean}}}{\mathrm{AUC}_{\mathrm{food,mean}}} \quad (2)$$

RESULTS

Eighteen constructed soils were created that varied in terms of total BaP concentration (1, 10, and 100 mg/kg), PAH source material (solvent, soot, or fuel oil), and soil characteristics (BSS with 70% sand, 20% clay, and 10% peat; soil with reduced proportions of clay or peat, or the addition of charcoal fines) (Table 1). Although the focus of the study was measuring the relative bioavailability of BaP, soils were created with a mixture of PAHs typical of environmental samples, and each soil sample was subjected to weekly wet/dry cycles for 8 weeks before assessment of relative oral bioavailability to simulate natural weathering processes.

Soil Weathering. It was important to verify that the weathering process did not result in degradation of BaP or loss of the tritium label from the BaP molecule. At the completion of weathering, an aliquot was removed from each soil sample and the BaP content was evaluated. Radiochromatography of solvent extracts of the soil samples showed a single peak with an elution time corresponding to BaP in 15 of the 18 soils (Figure S1), indicating that the radiolabel in the sample was present on BaP. Three of the weathered soils (the soils with montmorillonite instead of kaolinite, humus instead of peat, and reduced peat with PAHs added from solvent) showed evidence of BaP degradation. The height of the BaP peak in the radiochromatogram was diminished in these soils, and other tritium peaks with somewhat earlier retention times were observed (Figure S1). Although no attempt was made to identify the labeled compounds responsible for these earlier peaks, their increased hydrophilicity relative to BaP would be consistent with BaP metabolites. Because inclusion of both labeled BaP and BaP degradation products in a soil dose would confound measurement of BaP bioavailability, these three soil samples were not carried forward in the study.

The amount of remaining radioactivity in the soil was initially measured using solvent extraction similar to a standard method used for measurement of PAHs in soil (EPA Method 3550C). In addition to the radiochromatographic analysis described above, extract using this method was added to scintillation fluid and radioactivity was counted directly. Concentrations of radioactivity in the extracts were variable among soils and, in most cases, corresponded to amounts substantially less than what was added before weathering (Table 2). The discrepancy between the amount of radioactivity added to soil before weathering and what was extracted and measured after weathering could be due to loss of ^{3}H-BaP from the soil or to incomplete solvent extraction. To distinguish between these possibilities, additional aliquots of soil were subjected to complete digestion in strong acid. Although this process resulted in destruction of the soil matrix [and BaP], it was possible to determine the total content of radiolabel present (Table 2). The least amount of radioactivity recovered after digestion relative to what was added originally was observed in the three soils with evidence of degradation by radiochromatography. For the other 15 soils, essentially all of the radioactivity that was initially spiked to the soil remained in the soil after weathering (99.8 ± 15.8%; mean ± SD), with variability in results probably within the range of experimental error for this analytical method. To determine the role of weathering on the poor recovery of BaP radiolabel from soil

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Table 2. Benzo(a)pyrene Concentrations in Test Soils After Weathering

soil	source	target concentration (ppm)	postweathering concentrations (% preweathering concentration)	
			^{3}H content following Method 3550C extraction	^{3}H content following acid digestion
BSS	solvent	1	36	105
		10	28	78
		100	60	101
	soot	1	50	98
		10	66	90
		100	43	87
	fuel oil	1	38	93
		10	56	103
		100	52	139
BSS + charcoal	solvent	10	56	96
	soot	10	36	93
	fuel oil	10	64	100
BSS, reduced peat	solvent	10	46	67
	fuel oil	10	85	82
BSS, reduced clay	solvent	10	41	114
	fuel oil	10	41	118
BSS, montmorillonite	solvent	10	28	64
BSS, humus	solvent	10	60	76

[a]BSS = Baseline synthetic soil; "+ charcoal" = 2% charcoal added; "reduced peat" = peat content reduced to 1%; "reduced clay" = clay content reduced to 1%; "montmorillonite" = montmorillonite instead of kaolinite; "humus" = humus instead of peat. [b]BaP concentration determined based upon radioactivity measured and BaP specific activity.

using solvent extraction, a limited experiment was also conducted in which ^{3}H-BaP was added to soil and dried, but not weathered. This soil was extracted and radioactivity was counted after a few days, with minimal opportunity for degradation or decomposition. Under these conditions, extraction of spiked BaP was essentially complete (data not shown), indicating that the lower recovery of BaP in weathered soils was likely due to the formation of sequestered, nonextractable BaP residues associated with the weathering process.

Constant Proportionality between BaP dose and AUC of Radiolabel in Blood. Relative bioavailability studies are most straightforward to conduct and interpret when the measurement end point for absorbed dose (in this case, AUC in blood) is directly proportional to dose over the dose range examined. In a preliminary experiment, rats were given a single oral dose of BaP in food ranging from 0.1 to 100 μg per animal (N = 3 per group). Food was chosen as the dosing medium for this experiment because BaP was expected to have minimal interactions with a food matrix that might confound interpretation of results. As administered, these BaP doses correspond to concentrations in the food of 0.2, 2, 20, and 200 ppm BaP. To facilitate comparison among treatment groups, the specific activity of the ^{3}H-BaP in food was adjusted so that the animals received a consistent dose of 25 μCi ^{3}H-BaP while the total BaP dose was varied. Using this approach, the fraction of dose absorbed could be compared across treatment groups directly from comparison of radioactivity in blood without adjustment. If the fraction of total BaP absorbed was constant among the various BaP doses, the AUCs for the radiolabeled portion of the dose would be the same. If the fraction BaP absorbed was different, the extent of difference would be reflected quantitatively in the radiolabel AUCs.

Following a single gavage dose of food containing BaP to rats fasted overnight, the concentrations of radioactivity in blood, representing BaP and metabolites in the radiolabeled portion of the dose, were followed for 6 days (Figure 1). There were no

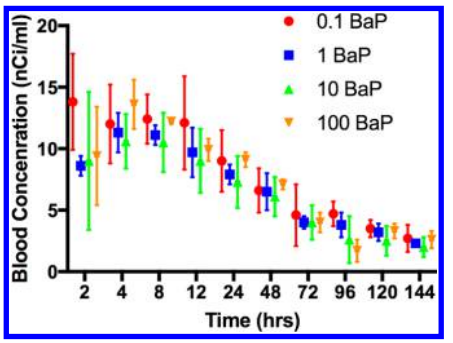

Figure 1. Linear Pharmacokinetics of BaP/^{3}H-BaP in Blood Following Doses Ranging from 0.1 to 100 μg. Rats were administered a single dose of BaP (0.1, 1, 10, or 100 μg) in food by gavage. Each dose contained 25 μCi ^{3}H-BaP. Serial blood samples were taken from 2 to 144 h after the dose and the concentration of radiolabel determined (mean ± SD, N = 5). Blood concentration versus time profiles were similar for all BaP doses and the AUCs derived from these profiles were not significantly different (see Results). The absence of a change in pharmacokinetics indicates linear pharmacokinetics over the dose range examined.

significant differences in the AUCs for radioactivity in blood among any of the BaP doses, indicating consistent absorption regardless the amount of BaP given within the dose range of 0.1 to 100 μg (0.1 μg BaP, AUC = 3598 ± 1019 nCi-hr/mL; 1 μg BaP, 3172 ± 18 nCi-hr/mL; 10 μg; BaP, 2835 ± 979 nCi-hr/mL; BaP 100 μg, 3194 ± 101 nCi-hr/mL; p > 0.05 by ANOVA). The consistent fractional proportionality between dose and blood concentrations and AUCs is indicative of linear pharmacokinetics for the BaP doses and soil and food concentration ranges included in the study.

Effect of BaP Concentration and Source Material on RBA. To establish comparison or benchmark AUCs, BaP was added to food to achieve concentrations of 1, 10, or 100 ppm. These concentrations were selected to match the total BaP concentrations in the test soils. These concentrations corresponded to administered total BaP doses of 0.5, 5, and 50 μg. Although the BaP doses (radioactive and total) were somewhat different from those used in the preliminary experiment presented above, the results were the same in that no significant differences were observed in the AUC for radiolabeled BaP among the three doses (800 ± 227, 800 ± 68, and 850 ± 92 nCi-hr/mL; N = 5 per treatment group; p > 0.05 by ANOVA). In the absence of differences in BaP absorption from food with different BaP doses, data were combined for graphical presentation of blood concentrations over time for BaP from food and the mean of the pooled AUCs from 1, 10, and 100 ppm BaP in food (0.5, 5, and 50 μg total BaP, respectively) (817 ± 28 nCi-hr/mL, N = 15) was used for comparison with AUCs from BaP in soil to derive RBA values.

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RBA values were estimated for each of the 15 soils that did not show evidence of ^{3}H-BaP decomposition. Figure 2 shows

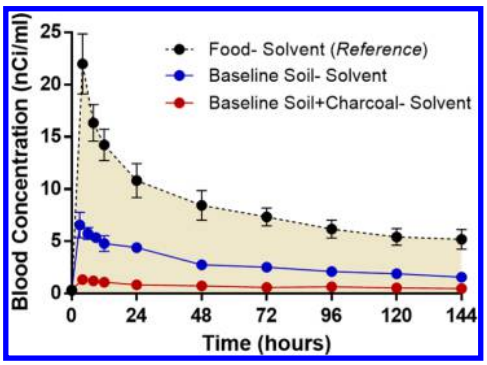

Figure 2. Blood concentrations of ^{3}H following oral administration of BaP/^{3}H-BaP in food and weathered soils. Rats were administered a single dose of either food with BaP (10 ppm; 25 μCi ^{3}H-BaP) or weathered soils (baseline soil and baseline soil with charcoal) BaP (10 ppm; 25 μCi ^{3}H-BaP). The solvent was spiked with BaP/ ^{3}H-BaP and was added to the soil prior to weathering. The bioavailability of BaP from weathered soils was calculated relative to bioavailability of BaP from food. The shaded area represents the food-reference AUC used to calculate the soil RBAs.

example blood concentration versus time profiles for BaP in food compared with BaP (solvent source) in soil and in soil with 2% charcoal. The AUCs for radioactivity in blood for each test soil and for food, and the associated RBA values are summarized in Table 3. RBA values were generally independent of BaP dose when the source was solvent. When the source was soot, BaP RBA was decreased at the highest BaP dose, whereas RBA values generally increased with BaP dose when fuel oil was the source.

Effect of Soil Characteristics on BaP RBA. The elimination of three soils from the study because of BaP degradation during weathering greatly reduced the comparisons that could be made in BaP RBA from soils with different compositions. The addition of charcoal to soil decreased BaP RBA by two-thirds or more regardless the PAH source. Reducing the clay or peat content of the soil had little or no effect on BaP RBA.

DISCUSSION

This study differs from previous efforts to assess the oral bioavailability of a PAH in soil in that it includes examination of potential influences of PAH concentration, source material, and soil characteristics on BaP RBA at lower, arguably more environmentally relevant, soil concentrations. Through the use of constructed, weathered soils, the influence on oral bioavailability of these potential key factors could be examined in a controlled, systematic manner, and the use of a radiolabeled tracer allowed measurement of bioavailability at soil concentrations much lower than previously possible.

Effect of BaP Concentration and Source Material on RBA. An important observation made possible by this approach is that the absorption of BaP was linear over the wide range of BaP concentrations and doses of interest in this study. When BaP was added to food in concentrations that resulted in doses of BaP spanning 3 orders of magnitude (0.1−100 μg), the *fraction* of BaP reaching the systemic circulation, as reflected in the AUC, was unchanged (i.e., the amount absorbed was directly proportional to the dose across the dose range). From a biological perspective, this suggests that there are no capacity-limited or saturable uptake processes affecting absorption of accessible BaP from the GI tract for doses of greatest interest for risk assessment.

Similar RBA values were also observed when BaP (in combination with other PAHs) was added in solvent to soil in doses of 0.5, 5, and 50 μg (resulting in concentrations of 1, 10, or 100 ppm)—0.56, 0.51, and 0.52, respectively. This indicates that, at least for this baseline soil, both biological and geochemical processes affecting bioavailability were linear throughout this BaP concentration range. While other studies have reported RBA values in this approximate range for spiked soils, the study design used in this other research did not directly assess the effect of varying concentrations for the same soil type.[21] When the source was soot, the RBA of BaP was decreased at the highest BaP concentration, while RBA values generally increased with BaP concentration when fuel oil was the source. In interpreting these observations, it is important to note that increasing concentrations of BaP in the test soils were accompanied by increased concentrations of the source materials. For example, the 100 ppm BaP soil from fuel oil was created by adding 10-times more fuel oil to soil than the 10 ppm BaP soil, and 100-times as much as the 1 ppm soil. Consequently, the fuel oil test soil with the highest BaP concentration also has the highest concentrations of other constituents of fuel oil, some of which may act to enhance the bioavailability of BaP from soil. Similarly, the test soil with 100 ppm BaP from soot has substantially more soot than the test soils with lower BaP concentrations, and in fact was

Table 3. Relative Oral Bioavailability of BaP from Soils

matrix	BaP, ppm (μg dose)	solvent		source material			
				soot		fuel oil	
		AUC[a]	RBA	AUC[a]	RBA	AUC[a]	RBA
baseline synthetic soil	1 (0.5)	455 ± 30	0.558 ± 0.082	442 ± 27	0.542 ± 0.074	529 ± 3	0.648 ± 0.008
	10 (5)	413 ± 8	0.505 ± 0.021	550 ± 26	0.673 ± 0.072	785 ± 97	0.961 ± 0.267
	100 (50)	426 ± 65	0.552 ± 0.078	192 ± 13	0.235 ± 0.035	869 ± 119	1.064 ± 0.325
baseline synthetic soil + charcoal	10 (5)	103 ± 16	0.126 ± 0.020	171 ± 4	0.210 ± 0.013	249 ± 24	0.305 ± 0.066
baseline synthetic soil−reduced clay	10 (5)	335 ± 49	0.410 ± 0.060	NA[b]	NA[b]	698 ± 40	0.854 ± 0.111
baseline synthetic soil−reduced peat	10 (5)	—[c]	—[c]	NA[b]	NA[b]	621 ± 70	0.761 ± 0.191
food	[d]	817 ± 28					

[a]nCi-h/mL; mean ± SD, N = 5. [b]NA = not determined per experimental design; see Table 1. [c]Not evaluated due to evidence of BaP degradation during weathering. [d]Average of AUCs obtained from animals receiving BaP in food in concentrations of 1, 10, and 100 ppm, corresponding to doses of 0.5, 5, and 50 μg total BaP.

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approximately 50% soot by weight. Black carbon materials like soot have a high affinity for PAHs,[20] which may act to diminish the bioavailability of BaP from soil. Thus, with increasing BaP concentration, the higher mass of carbon in soot could serve as a sorption matrix for the BaP. This is confirmed by the results from soil to which charcoal, another form of black carbon, was added; the presence of charcoal in the soil substantially decreased the RBA of 10 ppm BaP soil, whether the BaP was from solvent, soot, or fuel oil (Table 3). The point illustrated by these observations is that the source of BaP and the nature of the geosorbents within the soil can potentially have a strong effect on its RBA. Stated another way, the RBA for BaP in soil at a site could be markedly different depending upon how the BaP got there, independent of site-specific soil characteristics. This observation on the effect of source material and black carbon on RBA is consistent with observations of bioavailability to aquatic organisms (e.g., refs 20, 22−24), and investigations into the soil-chemical interactions between soil and PAHs,[25,25] which established that the source material has a major impact on the partitioning behavior of PAHs in soil.

Effect of Soil Characteristics on BaP RBA. Our investigation also included a limited evaluation of the potential effect of soil characteristics on the RBA of BaP. Unfortunately, BaP degradation during weathering precluded the inclusion of soils with montmorillonite instead of kaolinite, humus instead of peat, and reduced peat (with BaP addition in solvent) in the study. The reason why degradation occurred in these soils and not the others is unknown. The constructed soils were not sterilized, and the differences could reflect differences in microflora introduced into the various soils. The modifications to soil composition that remained in the study, elimination of clay or peat from the soil, produced little change in the observed RBA.

Solvent Extractability of BaP and Its Implications for Estimating and Using RBA Values. The RBA values derived in this study are based on the total BaP radioactivity present in the soil doses given to the rats. At the conclusion of the weathering of soil, it was found that some radioactivity present in the soil was intractable to extraction using acetone-dichloromethane with a modified Method 3550C. This method was used in this study because it is among the most common for preparation of environmental soil samples for PAH analysis. Among the soils for which RBA was measured, there was no evidence from radiochromatography of biodegradation of the BaP or loss of the label; essentially all of the radioactivity in extracted material was confined to a single peak corresponding to BaP. This led to the conclusion that the radioactivity remaining in weathered soil after solvent extraction was sequestered, nonextractable BaP. The formation of nonextractable bound residues is a well-documented process during the weathering and aging of PAHs[27] and other organic compounds in soils[28]

The sequestration of BaP and other PAHs that occurs during weathering raises the question of the most relevant way to quantify administered dose—the total amount present in soil that is ingested or only the amount liberated by standard EPA methods? The RBA values presented in Table 3 were derived based upon total BaP present in soil, which could be directly measured in this study because radiolabeled BaP was used. Other methods that fail to capture a sequestered fraction would result in different findings. For example, as shown in Table 2, the solvent (acetone-dichloromethane) extractable fraction of total BaP in the test soils was as low as 30%. Other studies have also shown variable and, in some cases, poor extraction using solvent extraction with sonication for PAHs in soil (see Lau et al. for a review).[29] Jonker and Koelmans[30] compared several solvents for extraction of PAHs from soot and sediment and found dichloromethane to have the worst recovery, with as little as 16% recovered compared with other solvents.

If the solvent-extractable fractions in Table 2 are used as the basis for determining the administered BaP doses, and these BaP doses are used with the AUCs presented in Table 3 to calculate RBA values, substantially higher RBA values are obtained. In fact, this approach would give the improbable result that over 50% of the test soils in this study have an RBA of 1.0 or more, with some as high as 2.1. A plausible explanation for these RBA values is that the relevant dose is, or is much closer to, the total BaP content. These observations also suggest that for at least some of the test soils the extent of gastrointestinal extraction of BaP by the animals is more complete than a modified Method 3550C solvent extraction.

The study here demonstrates that the RBA of BaP in soil can be significantly lower than the default assumption of 100%, and that the specific RBA will depend significantly on the source of the BaP in the soil, and to some extent soil characteristics, especially the content of black carbon. Clearly, development of in vitro methods to estimate the RBA of BaP and other PAHs from soil will need to ensure that these influences are accurately reflected in any proposed test. Despite the many advantages of using laboratory weathered, constructed soil samples with radiolabeled BaP in exploring critical factors that influence bioavailability, it is possible that some differences from contaminated site soils may exist. Thus, it will be important to confirm these findings, to extent possible, with PAH contaminated site soils.

The results also illustrate that consideration of bioavailability needs to be addressed in concert with other aspects of site characterization and risk assessment, particularly illustrated by the fact that different methods for soil sample preparation and analysis can yield very different estimates of PAH concentrations in soil. Therefore, understanding the implications of the different analytical methods for characterizing PAH contaminated soil is relevant to accurate determination of the bioavailable concentration or bioavailable fraction. Additional research is warranted to better address these implications as well. Ref 26.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b01534.

One table showing the total PAH composition of the test soils at the three benzo(a)pyrene target concentrations (1, 10, and 100 ppm); one table with the PAH composition of the soot and fuel oil source materials; and one figure showing solvent extract radiochromatograms from 18 test soils after 8 weeks of weathering (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (352) 294-4514; fax: (352) 392-4707; e-mail: smroberts@ufl.edu.

Notes

The authors declare no competing financial interest.

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Supporting Information

Effects of Source and Concentration on Relative Oral Bioavailability of Benzo(a)pyrene from Soil

Stephen M. Roberts, [*†] John W. Munson,[†] Michael V. Ruby,[§] and Yvette Lowney[††]

[†] Center for Environmental & Human Toxicology, University of Florida, Gainesville, FL 32611, United States;

[§] Integral Consulting, Inc., Louisville, CO 80027, United States; and

[††] Alloy, LLC, Boulder, CO 80302, United Stated

* Corresponding author email: smroberts@ufl.edu

Two tables and one figure; four pages total.

Table S1. PAH Composition of Test Soils

PAH	Target Concentrations (ppm)		
Benzo(a)pyrene	**1**	**10**	**100**
Naphthalene	0.01	0.10	1.0
Acenaphthylene	0.00	0.00	0.0
Acenaphthene	0.02	0.18	1.8
Fluorene	0.01	0.12	1.2
Phenanthrene	0.28	2.80	28.0
Anthracene	0.18	1.77	17.7
Fluoranthene	0.63	6.26	62.6
Pyrene	0.46	4.61	46.1
Benz(a)anthracene	0.37	3.67	36.7
Chrysene	0.36	3.59	35.9
Benzo(b)fluoranthene	0.41	4.14	41.4
Benzo(k)fluoranthene	0.17	1.73	17.3
Indeno (1,2,3-c,d)pyrene	0.30	2.97	29.7
Dibenz(a,h)anthracene	0.09	0.94	9.4
Benzo(g,h,i)perylene	0.36	3.57	35.7

Table S2. PAH Composition of Soot and Fuel Oil Source Materials*

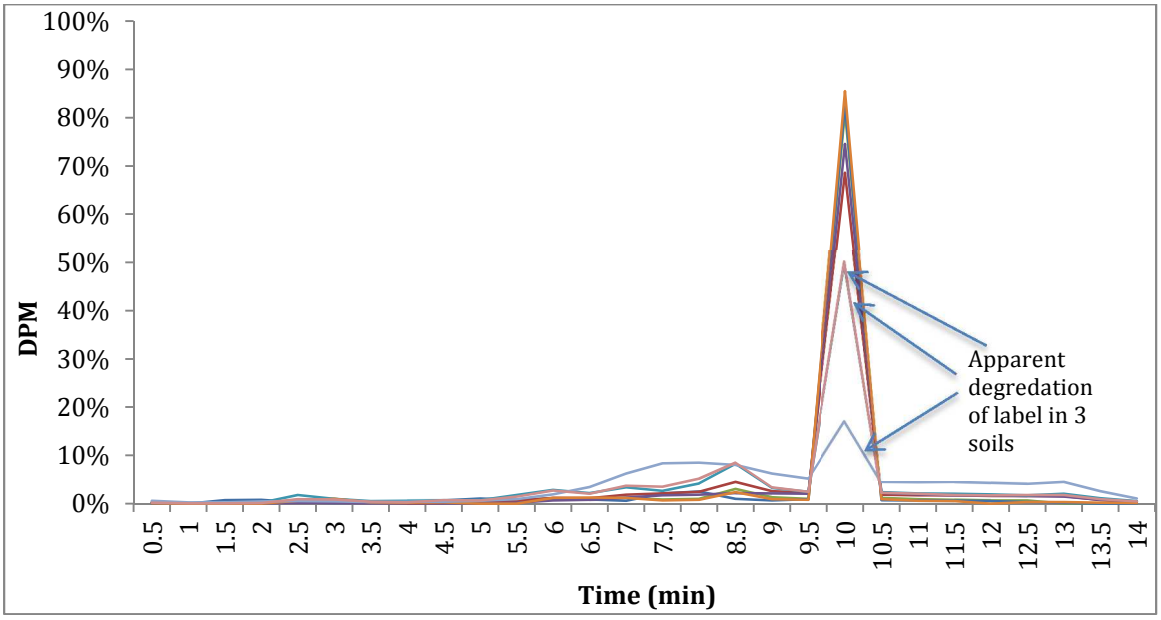
PAH	Soot	Fuel Oil No.6
Benzo(a)pyrene	2.31	28.7
Naphthalene	13.6	389.4
Acenaphthylene	ND	38.1
Acenaphthene	0.48	116
Fluorene	1.48	137
Phenanthrene	6.31	374
Anthracene	8.86	40.9
Fluoranthene	37.8	27.4
Pyrene	306	147
Benz(a)anthracene	ND	36.6
Chrysene	ND	60.0
Benzo(b)fluoranthene	8.99	26.1
Benzo(k)fluoranthene	ND	3.8
Indeno (1,2,3-c,d)pyrene	ND	12.5
Dibenz(a,h)anthracene	0.57	2.5
Benzo(g,h,i)perylene	0.97	ND

ND = Not detected

*These represent concentrations of PAHs in the source materials as received at the lab and prior to laboratory spiking

Figure S1. Composite Radiochromatogram of Test Soils Following 8-Weeks of Weathering

Extracts from 18 test soils weathered for 8 weeks were separated by HPLC with eluent collected and counted at 30 s intervals. The resulting radiochromatogram showed essentially superimposable profiles for 15 of the test soils, with a single peak with the same retention time as BaP standard (ca. 10 min.). Three test soils (reduced peat, humus instead of peat, and montmorillonite instead of kaolinite) had a reduced primary peak at 10 min, with smaller peaks at earlier retention times.



PAH Interactions with Soil and Effects on the Bioavailability to Humans

integral consulting inc. M. Ruby

Exponent® Y. Lowney, C. Menzie

UMBC U. Ghosh, J. Gomez-Eyles

UF UNIVERSITY of FLORIDA S. Roberts

SERDP DOD • EPA • DOE

SCHOOL OF PUBLIC HEALTH UNIVERSITY of WASHINGTON J. Kissel

COLORADOSCHOOLOFMINES EARTH • ENERGY • ENVIRONMENT A. Bunge

Introduction

This poster discusses an ongoing research program, funded by SERDP, to assess the oral and dermal bioavailability of polycyclic aromatic hydrocarbons (PAHs) to humans at U.S. Department of Defense (DOD) installations. PAHs have emerged as one of the most important contaminants driving remedial decisions for soils at DOD sites. Because the results of this research will allow for more accurate human health risk assessments at DOD sites, they will have a direct effect on PAH cleanup goals.

Over the last 30 years, substantial research efforts have focused on PAH bioavailability from solid matrices. This body of work includes investigations into the chemistry of PAH interactions with soil and sediments, the oral and dermal uptake of PAHs into ecological and human receptors, and attempts to develop extraction methods that are predictive of bioavailability measures. Most of this past research has focused on a single site, a particular source of PAH contamination, or a small range of PAH compounds. In contrast, this project will employ a broad-based approach, encompassing the range of contaminant sources and mixtures of PAHs most commonly found in soils at DOD facilities, and tying together the different strands of bioavailability research into an integrated understanding of PAH bioavailability from soil.

Objectives

This project's technical tasks are designed to develop a better understanding of the factors influencing the bioavailability of PAHs in soils to people. Specific objectives are as follows:

- Understand which specific PAHs, exposure pathways, and contaminant sources drive risk estimates and remedial decisions for PAH-contaminated soils at DOD sites
- Develop an understanding of the mechanisms by which PAHs are sequestered in different soil and solid matrices
- Develop and demonstrate an animal model to provide quantitative values of the relative oral bioavailability of PAHs from soil
- Generate a database of information using the animal model to understand oral bioavailability across a diversity of contaminant sources of relevance to DOD
- Develop a simple, reproducible, and inexpensive *in vitro* extraction test that correlates with *in vivo* measures of relative bioavailability
- Assess the dermal absorption of PAHs for a range of test soils.

Identification of Relevant PAH Sources and Exposure Pathways

A review of PAH exposure mechanisms and risk assessments indicates that only the oral and dermal exposure pathways are significant for PAHs in soil. The inhalation pathway and ingestion of PAHs in garden vegetables were determined to be insignificant pathways and, therefore, are not included as topics of study in this project. Using U.S. Environmental Protection Agency (EPA) default exposure values, oral exposure accounts for 73 percent, and dermal exposure accounts for 23 percent of risks from direct contact with PAHs in soil.

A review of records of decision (RODs) for DOD sites from 2009 and 2010 (11 sites, which included 24 exposure units) indicated that the primary human health risk drivers are (Figure 1):

- Benzo(a)pyrene
- Benz(a)anthracene
- Benzo(b)fluoranthene
- Indeno(1,2,3-cd)pyrene
- Dibenz(a,h)anthracene.

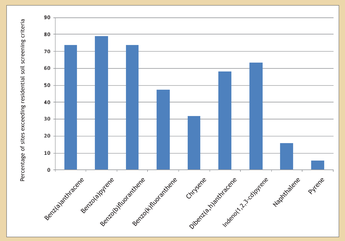
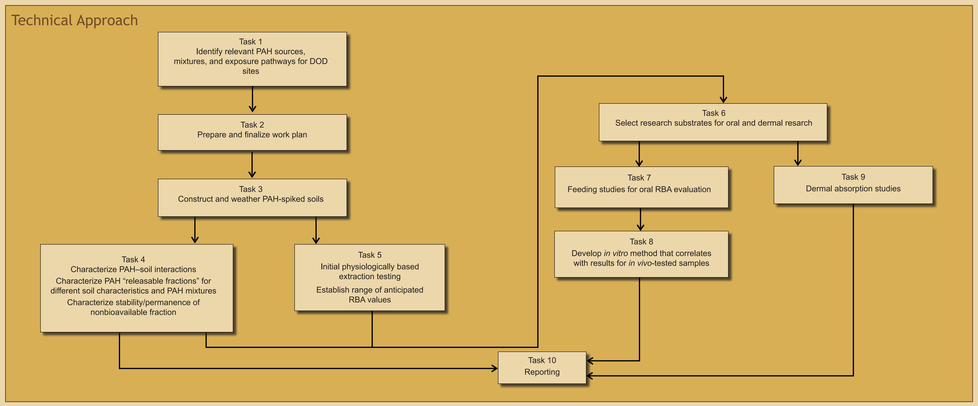


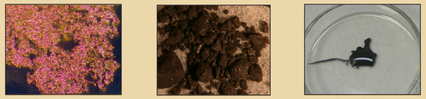
Figure 1. Percentage of Sites Exceeding Current Residential Soil Screening Criteria

Technical Approach



Based on the analysis of RODs and discussions with DOD personnel, the following PAH source materials were selected for this study (Figure 2):

- A mixture of PAHs dissolved in dichloromethane
- Soot
- Weathered skeet particles
- Fuel oil #6.



Soot Carbon | Weathered Skeet Particles | Fuel Oil #6

Figure 2. PAH Source Materials

Construction of PAH-Contaminated Soils

A broad study of PAH sources and the soil properties that influence PAH bioavailability requires the ability to control key variables so that results can be interpreted and hypotheses tested. From a practical standpoint, this level of control can be achieved only by using a library of constructed soil samples, which is the approach that has been selected for this project. Table 1 summarizes the spiked soils that will be constructed and weathered. Weathering will consist of two months of weekly alternating hydrating and air-drying cycles in a greenhouse.

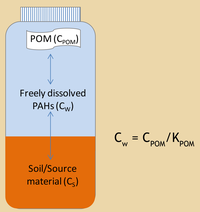
Table 1. Test Soil Matrix Showing Composition and Concentration of Benzo(a)pyrene (BaP) in Each Test Soil

PAH Sources	Negative Control	Mixture of PAHs in Dichloromethane[a]	Soot	Skeet Particles	Fuel Oil #6
Synthetic soil[b]	No PAHs	0.1, 1.0, and 10 mg/kg BaP	0.1, 1.0, and 10 mg/kg BaP	0.1, 1.0, and 10 mg/kg BaP	0.1, 1.0, and 10 mg/kg BaP
Synthetic soil with 2 percent charcoal fines	No PAHs	0.1, 1.0, and 10 mg/kg BaP	1.0 mg/kg BaP	1.0 mg/kg BaP	0.1, 1.0, and 10 mg/kg BaP
Synthetic soil with peat content reduced to 1 percent	—	0.1 and 1.0 mg/kg BaP	—	1.0 mg/kg BaP	1.0 mg/kg BaP
Synthetic soil with kaolinite content reduced to 2 percent	—	0.1 and 1.0 mg/kg BaP	—	1.0 mg/kg BaP	1.0 mg/kg BaP

[a]PAH mixture will simulate the relative concentrations of PAHs in skeet particles.
[b]Synthetic soil is the standard OECD medium for earthworm toxicity testing and consists of 70 percent sand, 20 percent kaolin clay, and 10 percent peat moss.

Characterization of PAH–Soil Chemical Interactions

This portion of the project will characterize the interactions between PAHs from different source materials and various soil components (e.g., spiked organic carbon forms and clay) and the effect these interactions have on the oral bioavailability of PAHs. Measurements on the source materials and spiked soils will include PAH concentrations, total organic carbon and black carbon, and equilibrium partitioning tests that will assess the tendency of PAHs to leave their binding matrix and enter the aqueous phase (Figure 3). An equilibrium partitioning model accounting for the major sorbent components in the spiked soil substrates will be developed and its predictive capability for PAH partitioning in complex soil mixtures will be tested.



$C_w = C_{POM} / K_{POM}$

Figure 3. Aqueous Equilibrium Experiments

In Vivo Oral Bioavailability Assessment

The objective of the *in vivo* studies is to develop an animal model for *in vivo* measurement of relative oral bioavailability of PAHs that is suitable for use with both constructed and field soil samples. The study will be conducted in two phases. Phase 1 will evaluate a number of bioavailability measurement methods in comparison with a definitive benchmark for absorption, the area-under-the-curve (AUC) for radiolabeled PAHs in blood (Figure 4).

These endpoints will be evaluated for ^{14}C-BaP, ^{14}C-indeno(1,2,3-cd)pyrene, and ^{14}C-fluoranthene, which were selected to provide a range of chemical characteristics (number of rings, log K_{ow}) for carcinogenic PAHs.

In Phase 2, the bioavailability measurement method(s) identified during Phase 1 will be applied to 15 constructed soils (created as described above).

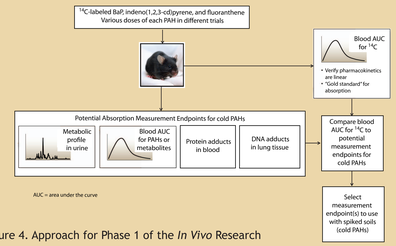


Figure 4. Approach for Phase 1 of the *In Vivo* Research

In Vitro Method Development and Validation

One goal of this research program is to develop a simple, reproducible *in vitro* extraction test that correlates with *in vivo* measures of relative bioavailability. Such a test would provide an efficient and inexpensive method to predict the relative bioavailability of PAHs from soil on a site-specific basis. Ultimately, this test may be either a physiologically based extraction test (PBET) or a simple chemical or solid-phase extraction.

The initial *in vitro* work on this project will be based on a PBET that has been used previously to evaluate the bioaccessibility of PAHs from soil. This test consists of two phases, an acidic gastric phase followed by a neutral small-intestinal phase, which are modeled after the composition of human gastrointestinal fluid. This test will be used to screen soils for evaluation in the in vivo component of the project. Ultimately, results will be compared against *in vivo* relative bioavailability measurements for the same soil samples. This approach has been used previously to develop an *in vitro* to *in vivo* correlation for lead in soil, which has been accepted by EPA for site-specific risk assessment (Figure 5). Depending on the results from this comparison, refinements may be made to the test or new *in vitro* tests will be selected for evaluation until a method with satisfactory predictive capability is developed.

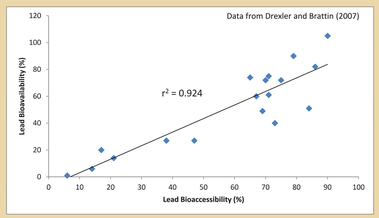
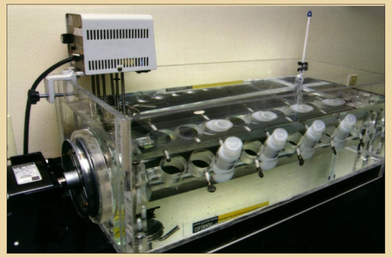


Figure 5. Correlation Between Lead Bioavailability from EPA's Swine Model and Lead Bioaccessibility from *In Vitro* Test



Dermal Absorption Assessment

Currently, EPA's default value for dermal absorption of PAHs from soil is 13 percent. Use of this default value leads to dermal exposure accounting for about 23 percent of estimated risks from direct contact with PAHs in soil (assuming that relative oral bioavailability is 100 percent). However, if relative oral bioavailability is demonstrated to be less than 100 percent, then the relative importance of dermal exposure will increase.

Under this task, ^{14}C-labeled benzo(a)pyrene and fluoranthene will be spiked and weathered into four different soils (same weathering procedure as for the oral bioavailability test soils) and tested for dermal penetration into and through human cadaver skin using *in vitro* diffusion cells (Figure 6).

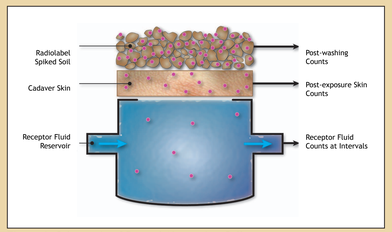


Figure 6. Schematic of Dermal Absorption Cell

Conclusions

Previous work indicates that current default methods of evaluating human exposures to PAHs in soils likely overestimate actual exposures, and associated risks, by up to ten-fold. This project will address uncertainties in the existing default exposure evaluation methods and provide tools for more accurate assessment of the bioavailability of PAHs from soils on a site-specific basis.

M. Ruby
Integral Consulting Inc., Louisville, CO
Y. Lowney
Exponent Inc., Boulder, CO
C. Menzie
Exponent Inc., Alexandria, VA
U. Ghosh
University of Maryland, Baltimore County, Baltimore, MD
J. Gomez-Eyles
University of Maryland, Baltimore County, Baltimore, MD
S. Roberts
University of Florida, Gainesville, FL
J. Kissel
University of Washington, Seattle, WA
A. Bunge
Colorado School of Mines, Golden, CO

PAH Interactions with Soil and Effects on the Bioavailability to Humans

Introduction

This poster discusses an ongoing research program, funded by the Strategic Environmental Research and Development Program, to assess the oral and dermal bioavailability of polycyclic aromatic hydrocarbons (PAHs) to humans at U.S. Department of Defense (DOD) installations. PAHs have emerged as one of the most important contaminants driving remedial decisions for soils at DOD sites. Because the results of this research will allow for more accurate human health risk assessments at DOD sites, they will have a direct effect on PAH cleanup goals.

Over the last 30 years, substantial research efforts have focused on PAH bioavailability from solid matrices. This body of work includes investigations into the chemistry of PAH interactions with soil and sediments, the oral and dermal uptake of PAHs into ecological and human receptors, and attempts to develop extraction methods that are predictive of bioavailability measures. Most of this past research has focused on a single site, a particular source of PAH contamination, or a small range of PAH compounds. In contrast, this project will employ a broad-based approach, encompassing the range of contaminant sources and mixtures of PAHs most commonly found in soils at DOD facilities, and tying together the different strands of bioavailability research into an integrated understanding of PAH bioavailability from soil.

Objectives

This project's technical tasks are designed to develop a better understanding of the factors influencing the bioavailability of PAHs in soils to people. Specific objectives are as follows:

- Understand which specific PAHs, exposure pathways, and contaminant sources drive risk estimates and remedial decisions for PAH-contaminated soils at DOD sites
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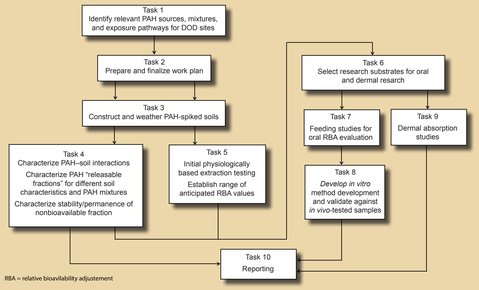
- Develop and demonstrate an animal model to provide quantitative values of the relative oral bioavailability of PAHs from soil
- Generate a database of information using the animal model to understand oral bioavailability across a diversity of contaminant sources of relevance to DOD
- Develop a simple, reproducible, and inexpensive *in vitro* extraction test that correlates with *in vivo* measures of relative bioavailability
- Assess the dermal absorption of PAHs for a range of test soils.

Technical Approach



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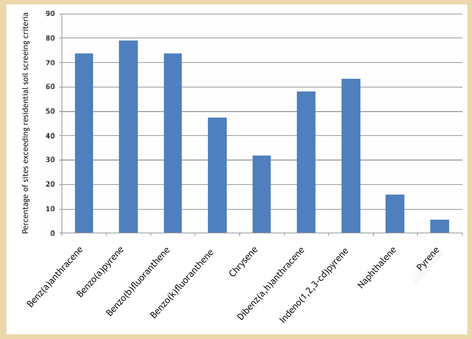
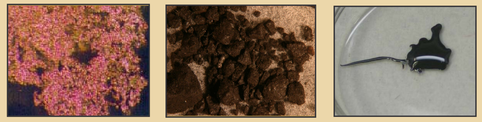


Figure 1. Percentage of Sites Exceeding Current Residential Soil Screening Criteria

Based on the analysis of RODs and discussions with DOD personnel, the following PAH source materials were selected for this study (Figure 2):

- A mixture of the 16 priority pollutant PAHs dissolved in dichloromethane
- Soot
- Weathered skeet particles
- Fuel oil #6.



Soot Carbon | Weathered Skeet Particles | Fuel Oil #6

Figure 2. PAH Source Materials

Construction of PAH-Contaminated Soils

A broad study of PAH sources and the soil properties that influence PAH bioavailability, such as envisioned for this study, requires the ability to control key variables so that results can be interpreted and hypotheses tested. From a practical standpoint, this level of control can be achieved only by using a library of constructed soil samples, which is the approach that has been selected for this project. Table 1 summarizes the spiked soils that will be constructed and weathered (2 months of weekly alternating hydrating and air-drying cycles in a greenhouse).

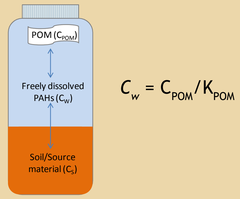
Table 1. Test Soil Matrix Showing Composition and Concentration of Benzo(a)pyrene (BaP) in Each Test Soil[a]

PAH Sources	Synthetic Soil[b]	Synthetic Soil with 2 Percent Charcoal Fines	Synthetic Soil with Humus Content Reduced to 0.5 percent	Synthetic Soil with Clay Content Reduced to 2.5 percent
Negative control (no PAHs added)	Test	Test	—	—
Mixture of 16 priority pollutant PAHs in dichloromethane[c]	0.1, 1.0, and 10 mg/kg BaP	0.1, 1.0, and 10 mg/kg BaP	0.1 and 1.0 mg/kg BaP	0.1 and 1.0 mg/kg BaP
Soot	0.1, 1.0, and 10 mg/kg BaP	1.0 mg/kg BaP	—	—
Skeet particles	0.1, 1.0, and 10 mg/kg BaP	1.0 mg/kg BaP	1.0 mg/kg BaP	1.0 mg/kg BaP
Fuel oil #6	0.1, 1.0, and 10 mg/kg BaP	0.1, 1.0, and 10 mg/kg BaP	1.0 mg/kg BaP	1.0 mg/kg BaP

[a]The concentration of the other PAHs in the spiked soil will be dictated by the relative concentrations of BaP to those other PAHs in the various source materials.
[b]Synthetic soil consists of 70 percent sand, 25 percent clay, and 5 percent humus.
[c]PAH mixture will simulate the relative concentrations of PAHs in skeet particles.

Characterization of PAH—Soil Chemical Interactions

This portion of the project will characterize the interactions between PAHs from different source materials and various soil components (e.g., spiked organic carbon forms and clay) and the effect these interactions have on the oral bioavailability of PAHs. Measurements on the source materials and spiked soils will include PAH concentrations, total organic carbon and black carbon analysis, and equilibrium partitioning tests, which will measure the tendency of PAHs to leave their binding matrix and enter the aqueous phase (Figure 3). An equilibrium partitioning model accounting for the major sorbent components in the spiked soil substrates will be developed and its predictive capability for PAH partitioning in complex soil mixtures will be tested.



$C_w = C_{POM}/K_{POM}$

Figure 3. Aqueous Equilibrium Experiments

In Vivo Oral Bioavailability Assessment

The objective of the *in vivo* studies is to develop an animal model for *in vivo* measurement of relative oral bioavailability of PAHs from soil that is suitable for use with both constructed and field soil samples. The study will be conducted in two phases. Phase 1 will evaluate a number of bioavailability measurement methods in comparison with a definitive benchmark for absorption, the measurement of radiolabeled PAHs in blood (Figure 4).

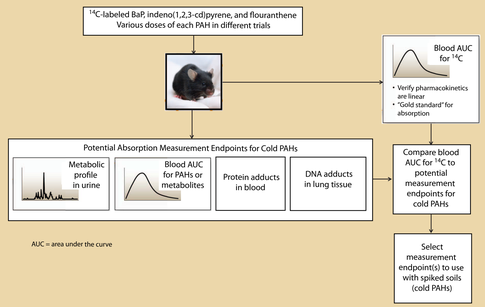


Figure 4. Approach for Phase 1 of the *In Vivo* Research

These endpoints will be evaluated for ^{14}C-BaP, ^{14}C-indeno(1,2,3-cd)pyrene, and ^{14}C-fluroanthene.

In Phase 2, the bioavailability measurement method(s) identified during Phase 1 will be applied to 15 constructed soils (created as described above).

In Vitro Method Development and Validation

One goal of this research program is to develop a simple, reproducible *in vitro* extraction test that correlates with *in vivo* measures of relative bioavailability. Such a test would provide an efficient and inexpensive method to predict the relative bioavailability of PAHs from soil on a site-specific basis. Ultimately, this test may be either a physiologically based extraction test (PBET) or a simple chemical or solid-phase extraction.

The initial *in vitro* work on this project will be based on a PBET that has been used previously to evaluate the bioaccessibility of PAHs from soil. This test consists of two phases, an acidic gastric phase followed by a neutral small-intestinal phase, which are modeled after the composition of human gastrointestinal fluid. This test will be used to screen soils for evaluation in the in vivo component of the project. Ultimately, results will be compared against *in vivo* relative bioavailability measurements for the same soil samples. Depending on the results from this comparison, refinements may be made to the test or new *in vitro* tests will be selected for evaluation until a method with satisfactory predictive capability is developed.

Dermal Absorption Assessment

Currently, EPA's default value for dermal absorption of PAHs from soil is 13 percent. Use of this default value leads to dermal exposure accounting for about 23 percent of estimated risks from direct contact with PAHs in soil (assuming that relative oral bioavailability is 100 percent). However, if relative oral bioavailability is demonstrated to be less than 100 percent, then the relative importance of dermal exposure will increase.

Under this task, ^{14}C-labeled benzo(a)pyrene and fluoranthene will be spiked and weathered into four different soils (same weathering procedure as for the oral bioavailability test soils) and tested for dermal penetration into and through human cadaver skin using *in vitro* diffusion cells (Figure 5).

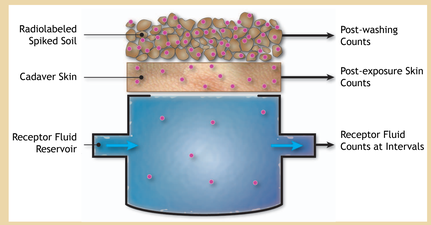


Figure 5. Schematic of Dermal Absorption Cell

Conclusions

Previous work indicates that current default methods of evaluating human exposures to PAHs in soils likely overestimate actual exposures, and associated risks, by up to five-fold. This project will address uncertainties in the existing default exposure evaluation methods and provide tools for more accurate assessment of the bioavailability of PAHs from soils on a site-specific basis.

Estimating the Relative Oral Bioavailability of Polycyclic Aromatic Hydrocarbons (PAHs) from Soil at Environmentally Relevant Concentrations

J.W. Munson[1], Y.W. Lowney[2], M.V. Ruby[3], S.M. Roberts[1]

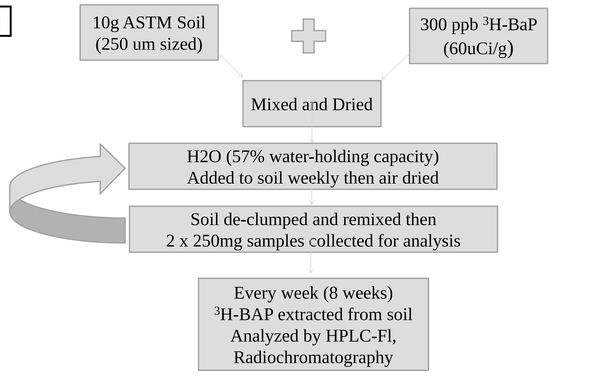
1-Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL
2-Exponent, Inc., Boulder, CO
3-Integral Consulting, Inc., Louisville, CO

Introduction

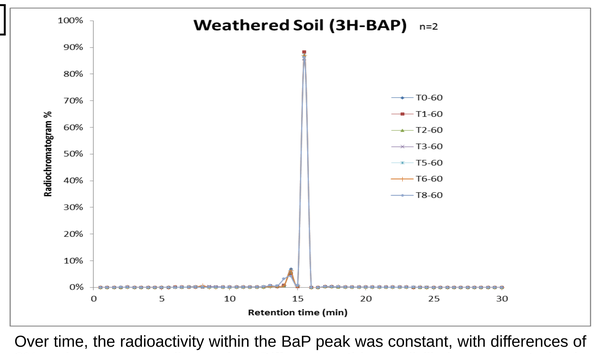
- The risk from exposure to polycyclic aromatic hydrocarbons (PAHs) in soil is driven primarily from incidental ingestion of carcinogenic PAHs, and as with many other soil contaminants, is dependent in part upon their oral bioavailability.
- Most previous studies attempting to understand PAH bioavailability from soil have used PAH concentrations well above the range of greatest environmental interest. We report here a reliable and quantifiable method by which the relative oral bioavailability (RBA) of ^{3}H-benzo[a]pyrene (^{3}H-BaP) in vivo in constructed, weathered soils can be measured for BaP in the ppb range. Using tritiated BaP provides the highest activity at the lowest concentration to allow this possibility.
- One objective was to spike a soil with ^{3}H-BaP, allowed it to weather for up to two months, then determine if any loss of BaP or ^{3}H label occurs over time.
- Developing AUC dose response curves as a reasonable endpoint for determining RBA generated from ^{3}H-BaP in food is explored.
- This work is part of a larger effort to characterize the influence of soil-chemical interactions on the oral and dermal absorption of PAHs from soil.

Stability of ^{3}H-BaP in Weathered Soil

Method



Results



Over time, the radioactivity within the BaP peak was constant, with differences of 3% or less among soil samples. Differences this small likely represent simple experimental error. After 8 weeks, the chromatograms are essentially superimposable and show no appearance of any new peaks over time. These results indicate that the label is not being lost from the BaP molecule.

PAH Relative Oral Bioavailability

Method

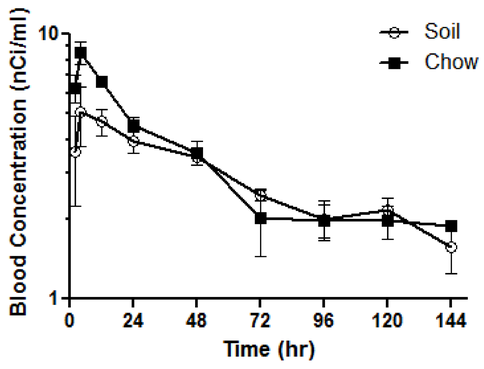
Rats were administered a single dose of un-weathered soil or PAH source material by gavage, and blood samples were taken via jugular cannula. To demonstrate relative oral bioavailability from soil, the appropriate basis of reference for comparison is against food. The area under the curve (AUC) for total radioactivity in blood was used to indicate absorbed BaP dose for each animal, and the ratio AUCs for soil versus food doses was used to estimate RBA.

Either food or soil was administered by gavage as a water slurry, mixed in the dosing syringe. One group of four rats received ^{3}H-BaP in food (20.81 μCi/animal; 87.5 ng total BaP) and another group of four rats received the same ^{3}H-BaP dose from soil (22.18 μCi/animal; 93.2 ng total BaP). Blood collections were made from jugular cannulated SD rats.

Using AUC as an indicator of RBA requires an assumption of linear kinetics; that the AUC increases in direct proportion to the absorbed dose. Other possible endpoints (urinary or fecal metabolite excretion, bile sampling, and others) were explored and rejected as less representative for bioavailability determination.

Results from individual animals were reasonably consistent, and the timing and number of blood samples were adequate to capture the AUC. The number of radioactivity counts in blood samples, even at the late time points, were well above background and more than sufficient to quantify BaP and metabolites remaining in blood.

Results



Concentration of radioactivity in blood following administration of food or soil containing 0.2 ppm ^{3}H-BaP (n=4, Results are mean±SE). The AUC from 0-144 hours was on average 448 nCi-hr/ml for chow and 406 nCi-hr/ml for soil yielding an RBA at 90% (406/448). Much lower RBAs should be expected in weathered soils

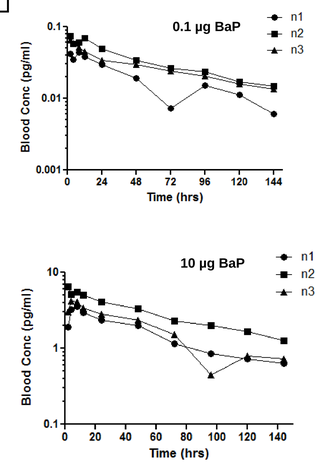
Dose Response

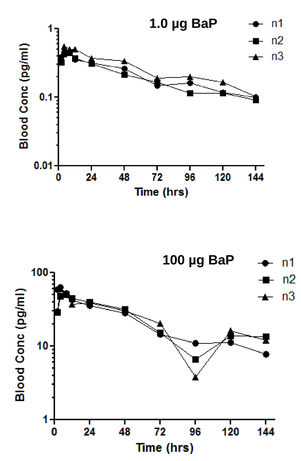
Method

For evaluation of RBA at environmentally relevant doses, it is important to determine whether any non-linearity in kinetics occurs in the dose range of interest.

In this experiment, rats (n=3) were administered the same amount of ^{3}H-BaP in food, but with markedly different total BaP concentrations: 0.2 ppm (0.1 μg BaP per animal); 2 ppm (1.0 μg BaP per animal); 20 ppm (10 μg BaP per animal and 200 ppm (100 μg BaP per animal). Blood samples were taken over time as in the previous experiment and total radioactivity in blood was measured. If the kinetics of the BaP doses are similar, then essentially the same total radioactivity in blood versus time profile should be observed for each of the animals.

Results



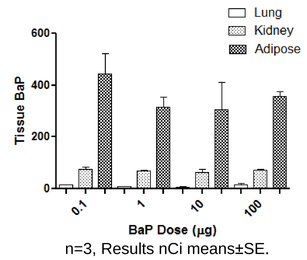


Blood AUCs

0.1 μg	1 μg	10 μg	100 μg
2511	2996	2136	3078
4531	2765	3953	3239
3753	3755	2415	3265

No statistical differences observed between or within groups using one-way ANOVA.

Tissue Deposition



n=3, Results nCi means±SE.

Acknowledgments and Perspective

Special thanks is directed to Roxana Weil, Georgia Hinkley, Marianne Kozuch and Cullen Roberts (University of Florida), for their assistance with lab and animal procedures.

This work is supported in part by a grant from the Strategic Environmental Research and Development Program (SERDP). The work presented here represents the method development for a project targeted at characterizing soil-chemical interactions for PAHs in the range of relevance to health-based remediation goals. This method will now be applied to evaluate the relative oral bioavailability of PAHs from a diversity of soils, including those with PAH contamination from different source materials (e.g., soot, fuel oil) and with varied soil characteristics (e.g., TOC, black carbon, clay).

Conclusions

- 3H-BaP was stable in soil during eight weeks of weathering. There was no evidence of loss of radiolabel or breakdown of BaP.
- Through comparisons of radiolabel AUC in blood, relative bioavailability of BaP in soil could be measured at BaP concentrations as low as 0.2 ppm.
- Blood AUCs and tissue levels increased in proportion to dose over four orders of magnitude suggesting linear pharmacokinetics within this dose range.

Exposure to PAHs in Soil: State of the Science and Future Research

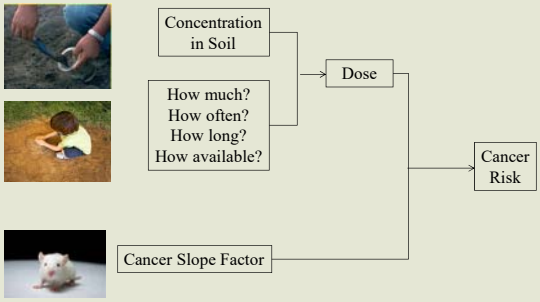
Priscilla Tomlinson
Michael Ruby

March 20, 2013

Outline

- Human exposures to PAHs in soil
- Chemical interactions with soil
- Oral bioavailability
- Dermal absorption
- Regulatory toxicology
- Future research

Fundamentals of Risk Assessment: Calculating Cancer Risk for a Chemical in Soil



Toxicity of PAHs

- Sir Percivall Pot and the London chimney sweeps (1770s)
- Benzo(a)pyrene (BaP)
- Oral cancer slope factor (CSF) based on dosing of mice and rats with BaP in feed
- Use relative potency factor (RPF) approach for other carcinogenic PAHs

Exposure Pathways for PAHs in Soil

Relative Magnitude of Exposure Pathways

- Incidental soil ingestion – 73% of risk (does not consider pica for soil)
- Dermal absorption – 27% of risk
- Ingestion of particulates on vegetables – small contributor
- Inhalation of particulates – negligible

Sources of PAHs to Soils

Type	PAH Source	Primary PAH-bearing Materials
Natural	Forest fires	Soot, char
	Grass fires	Soot, char
	Volcanic eruptions	Soot, char
	Oil seeps	Weathered crude oil
Industrial	Manufactured gas plants	Coal tar, pitch, coal, char, soot
	Coking operations	Coal tar, coal, coke, soot
	Aluminum production	Coal tar pitch (making and disposing of anodes)
	Foundries	Coal tar pitch, creosote, fuel oil (used in making sand casts), soot
	Wood treating	Creosote
	Refineries	Soot, various NAPLs (crude oil, fuel oil, diesel, etc.)
	Carbon black manufacture	Soot, oil tar
	Fuel spills and/or disposal	Various NAPLs (crude oil, fuel oil, waste oil, diesel)
Non-industrial Sources	Skeet	Coal tar pitch or bitumen (used as binder in targets)
	Asphalt sealants	Coal tar
	Landfills	Creosote (treated wood), soot, char
	Incinerators (municipal, hospital)	Soot
	Open burning	Soot, char
	Fire training	Soot
	Fires	Soot, char
	Auto/truck emissions	Soot



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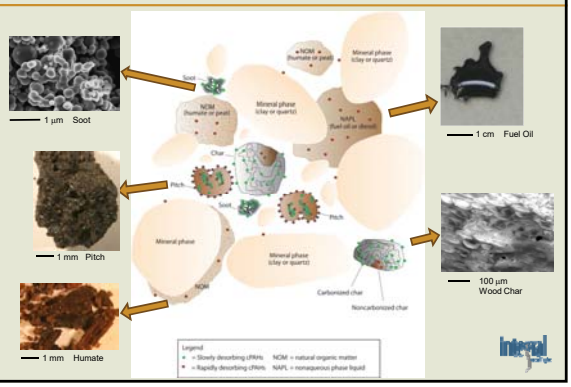
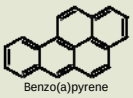
Current Paradigm for Chemical Controls on PAH Availability

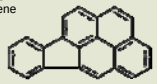


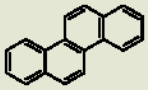
Health Risk is Dominated By Carcinogenic PAHs in Soil

Benzo(a)anthracene,
Benzo(a)pyrene,
Benzo(b)fluoranthene,
Benzo(k)fluoranthene,
Chrysene,
Dibenzo(a,h)anthracene,
Indeno(1,2,3-cd)pyrene

All these PAHs are 5- or 6-ring compounds, with the exception of chrysene (4 rings)


Benzo(a)pyrene


Indeno(1,2,3-cd)pyrene


Chrysene

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Oral Bioavailability of BaP from Soil

- *In vivo* studies required to establish relative bioavailability (RBA)
- Multiple models: mice, rats, swine
- Multiple measurement endpoints
  - Urinary excretion of metabolites
  - DNA adducts in lung and forestomach tissue
  - Parent PAHs in blood or feces
- RBA estimates for BaP range from 8 to 56%


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Oral Bioavailability of BaP (cont.)

- EPA's default assumption is 100% relative bioavailability from soil
- Only one *in vivo* study (Goon et al. 1991) that provides definitive measure of absorption
- Lack of validated *in vivo* endpoint
- Lack of *in vivo* studies at environmentally relevant concentrations

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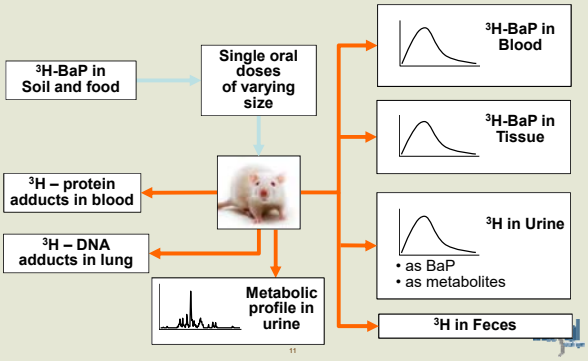
Design of In Vivo Model



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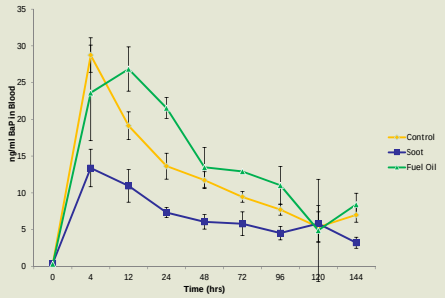
Oral Bioavailability of 3H-BaP in Rats

Doses in rat chow as 3H-BaP in solvent (control), or spiked into soot or fuel oil



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Matrix of Materials for In Vivo Testing

Test Soil Matrix (ASTM synthetic soil; 70% sand, 20% clay, 10% peat)				
PAH Sources	**Synthetic Soil**	**Synthetic Soil with 2 Percent Charcoal Fines**	**Synthetic Soil with Peat Content Reduced to 1 Percent**	**Synthetic Soil with Kaolinite Content Reduced to 2 Percent**
Mixture of PAHs in Dichloromethane	0.1, 1.0, 10, and 100 mg/kg BaP	0.1, 1.0, and 10 mg/kg BaP	0.1 and 1.0 mg/kg BaP	0.1 and 1.0 mg/kg BaP
Soot	0.1, 1.0, 10, and 100 mg/kg BaP	1.0 mg/kg BaP	—	—
Fuel oil	0.1, 1.0, 10, and 100 mg/kg BaP	0.1, 1.0, and 10 mg/kg BaP	1.0 mg/kg BaP	1.0 mg/kg BaP

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Dermal Absorption of PAHs

- Current EPA default assumption is 13% dermal absorption of BaP
- Based on Wester et al. (1990) study in primates
- Study limitations:
  - Soil sieved to 180 to 320 µm (fine to medium sand)
  - No weathering of BaP/soil mixture
  - Dose uncertainty

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Dermal Absorption of PAHs

- Dermal study limitations to address
  - Soil fraction includes fines (<63 µm or <150 µm)
  - Weathering of PAH in soil
  - Continuous contact ensured
  - Use monolayer soil loading (or more) and report flux
  - Concentration of PAH less than soil saturation (ideally at environmentally relevant concentrations)

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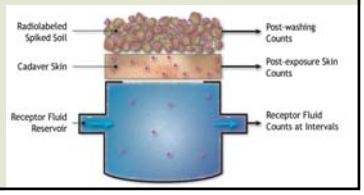
Ongoing Dermal Absorption Study for BaP in Soil

- Testing four soils with a range of TOC and BC content (14C-BaP weathered into soil)
- In vitro method using human cadaver skin
- Preliminary results presented at SOT in 2014



EPA's Proposed Changes to Toxicity Values

- Draft Toxicological Review (EPA/635/R13/138a)
  - Published August 2013
  - Public comment period extended to November 21
- Cancer toxicity values
  - Lower oral cancer slope factor (less conservative)
  - New inhalation unit risk
  - **New dermal cancer slope factor**
- Noncancer toxicity values
  - New reference dose
  - New reference concentration

Evaluating Dermal Exposure Pathway

Current Approach	Proposed New Approach
• **Adjust oral slope** factor to represent absorbed dose	• Use **dermal slope factor**
• Estimate **dose absorbed through skin**	• Estimate **dose in contact with skin**
• Estimate risk of **systemic cancer** due to absorption through skin	• Estimate risk of **cancer at point of contact**

BaP causes non-melanoma skin cancer at the point of contact

Impacts of Proposed Changes on Risk Estimates and Cleanup Levels (CULs)

Proposed Change	Impact on Risk Estimates		Impact on CULs	
Lower oral cancer slope factor	Decreased risks for ingestion	⇩	Significantly reduced CULs	⇩
New inhalation unit risk	Can now calculate risks for inhalation	✚		
New dermal slope factor	Significantly increased risks for dermal contact	⇧		
New oral reference dose and inhalation reference concentration	Can now calculate noncancer hazards	✚	None	–

The Future

- Known knowns
  - PAH risk assessments will get more complex
  - Exposure estimates will get more accurate
  - Toxicity estimates will get more accurate
  - PAH cleanup goals will likely decrease
- Known unknowns
  - Mechanism(s) of PAH mixture carcinogenesis
- Unknown unknowns
  - I don't know

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Questions?

Estimating the Relative Oral Bioavailability of Polycyclic Aromatic Hydrocarbons (PAHs) from Soil at Environmentally Relevant Concentrations

J.W. Munson[1], Y.W. Lowney[2], M.V. Ruby[3], S.M. Roberts[1]

1-Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL
2-Exponent, Inc., Boulder, CO
3-Integral Consulting, Inc., Louisville, CO

Abstract

Several reports in the literature suggest that interactions between polycyclic aromatic hydrocarbons (PAHs) and soil diminish PAH bioavailability and thus reduce risk from incidental ingestion of PAH- contaminated soil. Various animal models and approaches have been used to estimate PAH bioavailability from soil. To facilitate PAH measurement, most of these studies were conducted using PAH concentrations that only occur at heavily contaminated sites. The objective of this study was to explore the influence of soil composition, PAH concentration, and source material type on PAH bioavailability using an approach capable of measuring uptake at low, environmentally-relevant PAH concentrations (down to 1 ppm or less). Contaminated soil samples were constructed using PAHs from three source materials (PAHs in solvent, soot, and fuel oil) to which 3H-benzo(a)pyrene (3H-BaP; total BaP concentrations of 1, 10, and 100 ppm) was added and weathered for eight weeks using weekly wet-dry cycles. Each soil was administered as a single dose to rats, and blood samples were taken over six days. Relative bioavailability (RBA) of the BaP from soil was estimated by comparing the area under the curve (AUC) for ^{3}H concentration versus time in blood with the AUC observed from the same material dosed in a food matrix for comparison. Food was used for comparison because the cancer slope factor for BaP is derived from studies in rats in which the exposure medium was diet. The extent to which BaP RBA was diminished in soil versus food varied among the source materials. Differences were also observed among soils of different composition, suggesting that the nature of the soil as well as the type of PAH source material can influence bioavailability. These data will be informative both in understanding soil-PAH interactions that affect bioavailability and as a basis for the development of in vitro approaches to estimate PAH bioavailability at contaminated sites.

Study Objectives

- Construct weathered soils that vary with respect to PAH source material, BaP concentration, organic carbon type, and clay type and proportion (Table 1). Specific source materials examined include organic solvent, soot, and fuel oil.
- These soils will be used to develop in vitro methods to assess relative bioavailability (RBA) of PAHs.
- Measure the RBA of BaP in the test soils to establish reference values for comparison with results obtained from in vitro approaches in future experiments.
- Incorporate ^{3}H-BaP in the weathered soils in a way that allows measurement of BaP RBA at low, environmentally relevant concentrations.

Table 1. Test Soil Matrix: PAH Sources, Soil Characteristics, and BaP Conc

PAH Sources	Synthetic Soil (70% sand, 20% clay, 10% peat)	Synthetic Soil + 2 % Charcoal	Synthetic Soil Peat Reduced to 1%	Synthetic Soil Clay Reduced to 2%	Synthetic Soil Montmorillonite in place of kaolinite	Synthetic Soil Humus in place of peat
Mixture of PAHs in:						
Solvent	1.0, 10, 100 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg
Soot	1.0, 10, 100 mg/kg	10 mg/kg	—	—	—	—
Fuel oil	1.0, 10, 100 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg	—	—

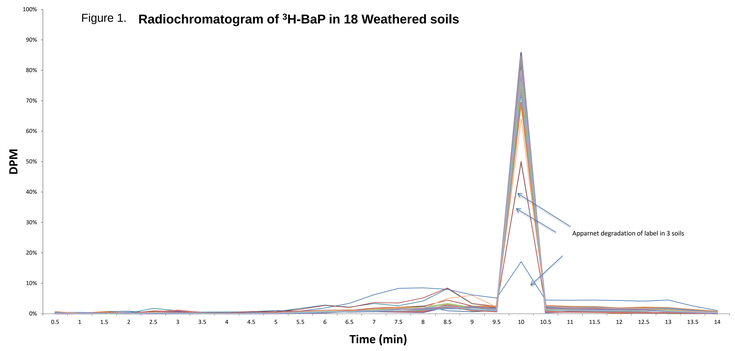
- The Baseline Synthetic Soil (BSS) consisting of 70% sand, 20% Kaolinite clay and 10% peat was constructed and then mixed with source materials: solvent, soot or fuel oil (these matrices were spiked with ^{3}H-BaP and homogenized prior to being added to the BSS).
- Composition of the soil was varied to evaluate the effect of 1) organic carbon type (ex: peat vs. humus), 2) clay type (kaolinite vs. mortmorillonite) and 3) BaP concentration (1, 10 and 100 ppm BaP) on the RBA.
- Test soils were also constructed with 2% charcoal fines added to evaluate the effect of a strong geosorbent on BaP RBA values.

^{3}H-BaP Stability in Weathered Soil

- Constructed soil samples were weathered for 8 weeks following PAH addition through weekly wet-dry cycles.
- 3H-BaP was added to the soil prior to weathering so that it was included in the weathering process.
- It was important to verify stability of the 3H-BaP during the 8- week weathering process (i.e., the absence of significant degradation of BaP or loss of the 3H label from the BaP molecule).
- For 15 of 18 weathered soils, stability of the 3H-BaP during weathering was indicated by: 1) recovery of 90% or more of the radiolabel after weathering; and 2) virtually all of the 3H extracted from the weathered soil co-eluted with (see figure below).
- For three soils (reduced peat, humus instead of peat, and Montmorillonite instead of kaolite soils, see Table 1) evidence of BaP degradation and/or loss of label was observed and these soils were excluded from analysis.

^{3}H-BaP Stability in Weathered Soil (cont.)

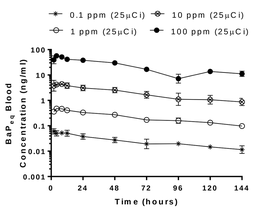
Figure 1. **Radiochromatogram of ^{3}H-BaP in 18 Weathered soils**



Measurement of BaP Relative Bioavailability

- In a preliminary experiment we found that the oral absorption of BaP from food was liner over 4 orders of magnitude, i.e., the fraction absorbed was independent of BaP dose (Fig. 2). Consequently, it was not necessary to administer the same BaP dose in food and soil to measure RBA.

Figure 2. Linear Kinetics of BaP_{eq} Blood Concentrations Following Administration of BaP/^{3}H-BaP in Food. Rats (n = 3/ treatment; results show mean ± SEM) were administered BaP (0.1, 1, 10 or 100 ppm)/ ^{3}H-BaP(25 µCi) in food. The BaP_{eq} (parent compound and metabolites) concentration was calculated based on the concentration of total radioactivity in blood.



- In all treatments the dose of tritiated BaP was the same (25µCi), so, differences in absorption are indicated directly by differences in AUC.
- The RBA of BaP from weathered soil was determined relative to BaP added to food in adult male SD rats (n=5-7) according to the following formula:

$$\textbf{RBA} = \frac{AUC_{[Soil]} \times {}^3\text{H-BaP Dose}_{[Food]}}{AUC_{[Food]} \times {}^3\text{H-BaP Dose}_{[Soil]}}$$

Where: AUC is the area under the ^{3}H- Blood Concentration/ Time Curve

Figure 3. Blood Concentrations of ^{3}H Following Oral Administration of BaP/^{3}H-BaP in Food and Weathered Soils. Rats (n = 5/ treatment; results show mean ± SEM) were administered a single dose of either food with BaP (100 ppm)/ ^{3}H-BaP(25 µCi) or weathered soils (baseline soil and baseline soil with charcoal) BaP(10ppm)/ ^{3}H-BaP(25 µCi). The solvent was spiked with BaP/ ^{3}H-BaP and was added to the soil prior to weathering. The bioavailability of BaP from weathered soils was calculated relative to bioavailability of BaP from food. The shaded area represents the food-reference AUC used to calculate the soil RBAs.

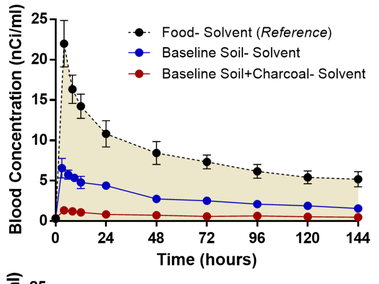
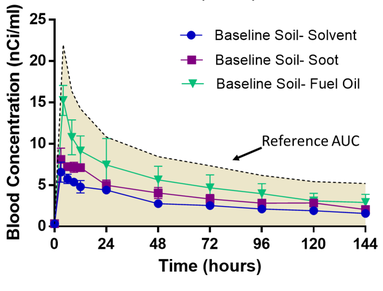


Figure 4. Blood Concentrations of ^{3}H Following Oral Administration of BaP/^{3}H-BaP in Weathered Baseline Soil with Solvent, Soot or Fuel oil as the PAH Source Materials. Rats (n = 5/ treatment; results show mean ± SEM) were administered a single dose of BaP (10 ppm)/ ^{3}H-BaP(25 µCi) in weathered baseline soils containing either solvent, soot or fuel oil as the source material. The source materials were spiked with BaP/ ^{3}H-BaP and added to the soil prior to weathering. The bioavailability of BaP from weathered soils was calculated relative to bioavailability of BaP from food. The shaded area represents the food-reference AUC used to calculate the soil RBAs.



In vivo Absorption of BaP con't

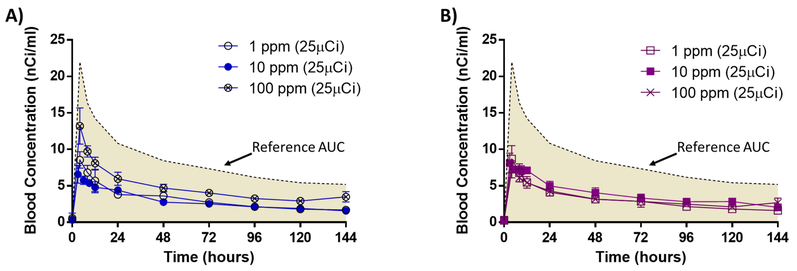


Figure 5. Blood Concentrations of ^{3}H Following Oral Administration Different BaP/^{3}H-BaP Doses in Weathered Baseline Soil with Solvent and Soot. Rats (n = 5/ treatment; results show mean ± SEM) were administered a single dose of BaP (1, 10, or 100 ppm)/ ^{3}H-BaP(25 µCi) in weathered baseline soils containing either solvent (A) or soot (B) as the source materials. The source martials were spiked with BaP/ ^{3}H-BaP, and were added to the soil prior to weathering. The bioavailability of BaP from weathered soils was calculated relative to bioavailability of BaP from food. The shaded area represents the food-reference AUC used to calculate the soil RBAs.

Table 2. BaP Relative Bioavailability

		Source Material					
		Solvent		**Soot**		**Fuel Oil**	
Matrix	**BaP Dose (ppm)**	*AUC (nCi hr/ml)*	*RBA*	*AUC (nCi hr/ml)*	*RBA*	*AUC (nCi hr/ml)*	*RBA*
Baseline Soil (BSS)	1	444 ± 30	**0.37**	439 ± 30	**0.37**	—	—
	10	411 ± 17	**0.35**	548 ± 27	**0.46**	763 ± 99	**0.8**
	100	664 ± 18	**0.56**	462 ± 19	**0.39**	—	—
BSS +Charcoal	10	102 ± 7	**0.09**	—	—	—	—

Effects of weathered soils on C_{max}, AUC and RBA with respect to dose, source material and matrix. Results from across individual animals were reasonably consistent, and the timing and number of blood samples were adequate to capture the AUC.

Conclusions

- ^{3}H-BaP was stable in all but 3 of the test soils during the eight week weathering period. Both label integrity and recovery of ^{3}H-BaP approached target levels throughout the weathering process.
- Using ^{3}H-BaP in soil dosed to cannulated rats allows measurement of RBA at environmentally relevant levels of BaP in soil (down to 1 ppm).
- Relatively similar RBA values (approximately 0.4 to 0.6) were obtained for BaP from solvent or soot at BaP concentrations from 1 to 100 ppm.
- Limited data available to date suggest a higher RBA for BaP from fuel oil and a substantial reduction of BaP RBA in the presence of charcoal.
- Future work will include characterizing RBA for a larger suite of weathered soils. Ultimately, these soils will support the development of a in *in vitro* model to predict RBA

Acknowledgments

Special thanks is directed to Georgia Hinkley, Roxana Weil Ph.D., Marianne Kozuch Ph.D., Cullen Roberts, Ahmed Alaliewe and Kevin Kircheval (University of Florida), for their assistance with lab and animal procedures.

This work is supported in part by a grant from the Strategic Environmental Research and Development Program (SERDP).

Relative Oral Bioavailability of Radiolabeled Benzo(a)pyrene from Constructed Soils: Effect of PAH Source, Concentration, and Soil Characteristics.

J.W. Munson[1], Y.W. Lowney[2], M.V. Ruby[3], S.M. Roberts[1]

1-Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL
2-Exponent, Inc., Boulder, CO
3-Integral Consulting, Inc., Louisville, CO

Abstract

Several reports in the literature suggest that interactions between polycyclic aromatic hydrocarbons (PAHs) and soil diminish PAH bioavailability and thus reduce risk from incidental ingestion of PAH- contaminated soil. Most animal studies, however, were conducted using high PAH concentrations that only occur at heavily contaminated sites. We wish to examine the influence of soil composition, PAH concentration, and source material type on PAH bioavailability using an approach capable of measuring uptake at low, environmentally-relevant PAH concentrations (down to 1 ppm or less). Contaminated soil samples were constructed using PAHs from three source materials (PAHs in solvent, soot, and fuel oil) to which 3H-benzo(a)pyrene (3H-BaP; total BaP concentrations of 1, 10, and 100 ppm) was added and weathered for eight weeks using weekly wet-dry cycles. Each soil was administered as a single dose to rats, where blood samples were taken over six days after which adipose tissue was collected. Relative bioavailability (RBA) of the BaP from soil was estimated by comparing the area under the curve (AUC) for 3H concentration versus time in blood with the AUC observed from the same material dosed in a food matrix for comparison. Adipose RBAs were also examined for each of the different soils types. The extent to which BaP RBA was diminished in soil versus food varied among the source materials. Differences were also observed among soils of different composition, suggesting that the nature of the soil as well as the type of PAH source material can influence bioavailability. Continued weathering of these soils reveal further reduction of RBAs as expected. These data will be informative both in understanding soil-PAH interactions that affect bioavailability and as a basis for the development of in vitro approaches to estimate PAH bioavailability at contaminated sites.

Study Objectives

- Construct weathered soils that vary with respect to PAH source material, BaP concentration, and soil characteristics including organic carbon type, and clay type and proportion (Table 1). Specific PAH source materials examined include organic solvent, soot, and fuel oil.
- Incorporate ^{3}H-BaP in the weathered soils in a way that allows measurement of BaP RBA at low, environmentally relevant concentrations.
- Measure blood and adipose RBA of BaP in the constructed, weathered soils to evaluate the influence of PAH source and soil characteristics on BaP bioavailability *in vivo*.

Table 1. Test Soil Matrix: PAH Sources, Soil Characteristics, and BaP Conc

PAH Sources	Synthetic Soil (70% sand, 20% clay, 10% peat)	Synthetic Soil + 2 % Charcoal	Synthetic Soil Peat Reduced to 1%	Synthetic Soil Clay Reduced to 2%
Mixture of PAHs in:				
Solvent	1.0, 10, 100 mg/kg	10 mg/kg	—	10 mg/kg
Soot	1.0, 10, 100 mg/kg	10 mg/kg	—	—
Fuel oil	1.0, 10, 100 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg

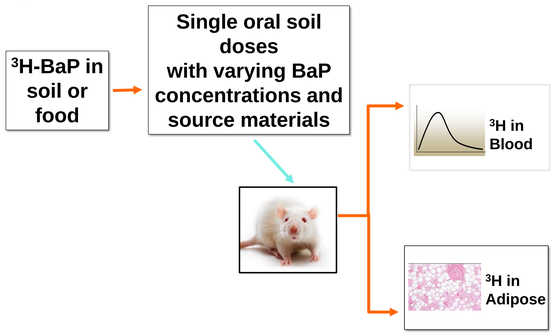
- The Baseline Synthetic Soil (BSS) consisting of 70% sand, 20% Kaolinite clay and 10% peat was constructed and then mixed with source materials: solvent, soot or fuel oil (these matrices were spiked with ^{3}H-BaP and homogenized prior to being added to the BSS).
- Composition of the soil was varied to evaluate the effect of 1) organic carbon type (peat vs. humus), 2) clay type (kaolinite vs. montmorillonite), 3) BaP concentration (1, 10 and 100 ppm BaP) and 4) PAH source on the RBA.
- Test soils were also constructed with 2% charcoal fines added to evaluate the effect of a strong geosorbent on BaP RBA values.

^{3}H-BaP Stability in Weathered Soil

- Constructed soil samples were weathered for 8 weeks with weekly wet-dry cycles following PAH addition.
- ^{3}H-BaP was added to the soil prior to weathering so that it was included in the weathering process.
- It was important to verify stability of the ^{3}H-BaP during the 8-week weathering process (i.e., the absence of significant degradation of BaP or loss of the ^{3}H label from the BaP molecule).
- Radiochromatography revealed that most soils (the 15 soils listed above out of 18 total) had little or no degradation of the 3H label after the weathering process.
- For three soils (reduced peat, humus instead of peat, and montmorillonite instead of kaolinite soils, evidence of BaP degradation and/or loss of label was observed and these soils were excluded from analysis.

In vivo Oral Soil Dose Containing 3H-BaP

Figure 1



Relative Bioavailability Based on Food

- Food was used for comparison to these soils because the cancer slope factor for BaP is derived from studies in rats in which the exposure medium was diet.
- 3H-BaP in food was dosed to animals where the [BaP] was either 1, 10 or 100 ppm.
- The ^{3}H blood AUCs showed no significant difference in food with varied BaP doses.
- Because the linear kinetics of food were directly proportional to dose (Figure 2), all soils analyzed had RBAs calculated from an averaged food basis.

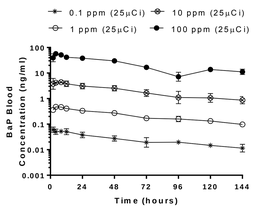


Figure 2. Linear Kinetics of BaP Blood Concentrations Following Administration of ^{3}H-BaP in Food. Rats (n = 3/ treatment; results show mean ± SEM) were administered BaP (0.1, 1, 10 or 100 ppm)/ ^{3}H-BaP(25 µCi) in food. The BaP concentrations in blood were directly proportional to dose over this dose range.

Effect of PAH Source Material on AUCs

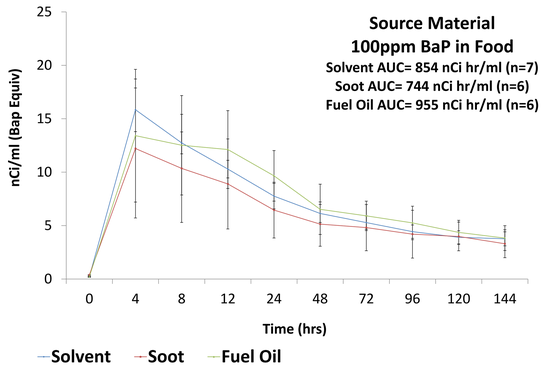


Figure 3. Blood Concentrations of BaP (as ^{3}H) Following Oral Administration of BaP/^{3}H-BaP in food with Solvent, Soot or Fuel oil as the PAH Source Materials. Rats (n = 6 or 7; mean ± SEM) were administered a single dose of BaP (100 ppm)/ ^{3}H-BaP(25 µCi) in food containing either solvent, soot or fuel oil as the source material. The AUCs were calculated from blood levels of ^{3}H.

RBAs of Selected Soils

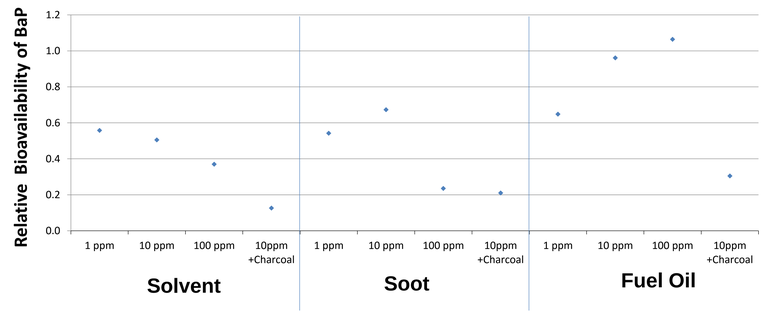
Table 2. BaP Blood Relative Bioavailability

		Source Material: Solvent		Soot		Fuel Oil	
Matrix	BaP Dose (ppm)	AUC (nCi hr/ml)	RBA	AUC (nCi hr/ml)	RBA	AUC (nCi hr/ml)	RBA
Baseline	1	455 ± 30	**0.558**	442 ± 27	**0.542**	529 ± 3	**0.648**
Soil (BSS)	10	413 ± 8	**0.505**	550 ± 26	**0.673**	785 ± 97	**0.961**
	100	302 ± 25	**0.370**	192 ± 13	**0.235**	869 ± 119	**1.064**
BSS + Charcoal	10	103 ± 16	**0.126**	171 ± 4	**0.210**	249 ± 24	**0.305**
BSS - Clay	10	244 ± 16	**0.299**	-	-	698 ± 40	**0.854**
BSS - Peat	10	-	-	-	-	621 ± 70	**0.761**
Food	1,10,100	*817.28*					

Table 3. BaP Adipose Relative Bioavailability

		Source Material: Solvent		Soot		Fuel Oil	
Matrix	BaP Dose (ppm)	Total nCi	RBA	Total nCi	RBA	Total nCi	RBA
Baseline	1	27 ± 6	**0.353**	27 ± 6	**0.336**	75 ± 22	**0.998**
Soil (BSS)	10	29 ± 10	**0.393**	30 ± 4	**0.400**	44 ±10	**0.581**
	100	41 ± 10	**0.549**	33 ±16	**0.435**	50 ± 11	**0.667**
BSS +Charcoal	10	16 ± 7	**0.211**	24 ±8	**0.210**	47 ± 26	**0.631**
BSS - Clay	10	40 ± 11	**0.532**	-	-	57 ± 26	**0.768**
BSS - Peat	10	-	-	-	-	58 ± 14	**0.755**
Food	1,10,100	*74.86*					

Figure 4



Conclusions

- ^{3}H-BaP was detectable and stable in most soils weathered after eight weeks with BaP at environmentally relevant levels of 1, 10 and 100 ppm.
- Soil BaP RBAs are based upon BaP in food. The AUCs for animals dosed with BaP at varying concentrations showed linear kinetics allowing averaging of food data over all soil types and BaP ranges.
- The AUCs from all constructed soils were significantly lower than the AUC from BaP in food with only some fuel oil sourced PAHs in soils approaching food levels. Blood RBAs seem to provide a better indicator than adipose RBAs for predicting PAHs in source materials.
- Food spiked with PAHs from varying sources (control, soot and fuel oil) showed that fuel oil provided higher AUCs than the others.
- These preliminary findings suggest that bioavailability of PAHs from soils are influenced by source, PAH concentration and soil characteristics.
- Ultimately, these soils and associated RBA data will support the development of an *in vitro* method to predict RBA.

Acknowledgments

Special thanks is directed to Georgia Hinkley, Ahmed Alaliewe and Kevin Kircheval (University of Florida), for their assistance with lab and animal procedures.

This work is supported in part by a grant from the Strategic Environmental Research and Development Program (SERDP).

EXPOSURE TO PAHS FROM SOIL: CURRENT RESEARCH EFFORT

Yvette Wieder Lowney, Exponent
Michael Ruby, Integral

November, 2013

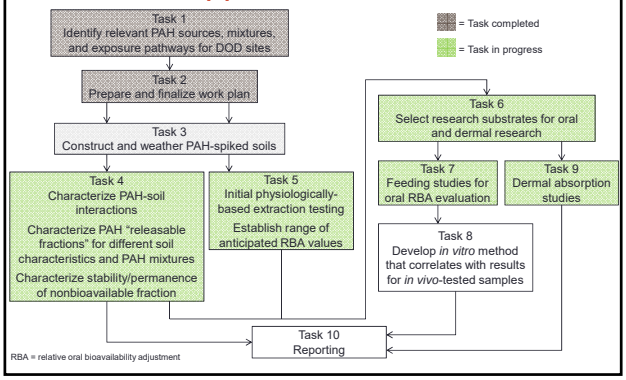
SERDP Project Team

- **Yvette Wieder Lowney**
  - Exponent
  - Bioavailability research and application in risk assessment
- **Michael V. Ruby**
  - Integral Consulting
  - Bioavailability research and *in vitro* method development
- **Upal Ghosh**
  - University of Maryland, Baltimore County
  - Chemical interactions with soils and sediments
- **Stephen Roberts**
  - University of Florida
  - Animal models for bioavailability research
- **Annette Bunge and John Kissel**
  - Colorado School of Mines and University of Washington, respectively
  - Dermal absorption of chemicals from soil
- **Charles Menzie**
  - Exponent
  - Technical Review

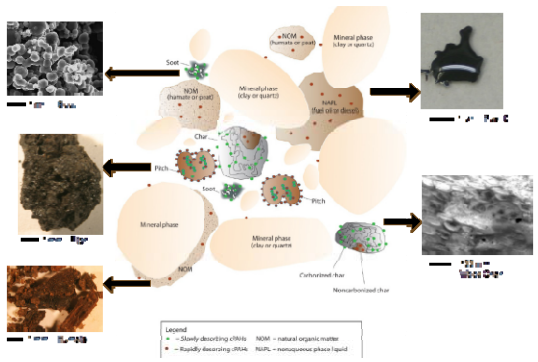
Technical Objectives

1. Confirm which specific PAHs, exposure pathways, and sources drive risks and remedial decisions
2. Develop an understanding of the physical and chemical interactions between soils and PAHs
3. Develop reliable methods for establishing the oral and dermal bioavailability of PAHs from soil
4. Develop a rapid screening tool for estimating the oral bioavailability of PAHs from contaminated soils
5. Develop information to support changes in regulatory policy regarding risk assessment of PAHs from soil

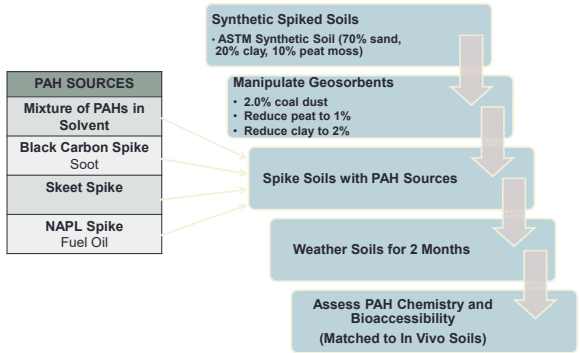
Technical Approach



PAH interactions with soil



Technical Approach: Construct Soils



Technical Approach: Construct Soils

Test Soil Matrix (ASTM synthetic soil; 70% sand, 20% clay, 10% peat)				
PAH Sources	Synthetic soil	Synthetic soil with 2 percent charcoal fines	Synthetic soil with peat content reduced to 1 percent	Synthetic soil with kaolinite content reduced to 2 percent
Mixture of PAHs in Dichloromethane	0.1, 1.0, 10, and 100 mg/kg BaP	0.1, 1.0, and 10 mg/kg BaP	0.1 and 1.0 mg/kg BaP	0.1 and 1.0 mg/kg BaP
Soot	0.1, 1.0, 10, and 100 mg/kg BaP	1.0 mg/kg BaP	—	—
Skeet Particles	0.1, 1.0, 10 mg/kg BaP	1.0 mg/kg BaP	1.0 mg/kg BaP	1.0 mg/kg BaP
Fuel Oil	0.1, 1.0, 10, and 100 mg/kg BaP	0.1, 1.0, and 10 mg/kg BaP	1.0 mg/kg BaP	1.0 mg/kg BaP

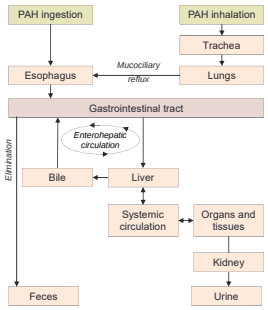
Progress to date

- Chemistry: Equilibrium testing of all test soils has been completed.
- Data evaluation ongoing
- A model of factors controlling solubility of PAHs will be completed by the end of the year.
- *In vitro* extraction: Extractions in simulated gastrointestinal fluid have been completed.
- Presented in poster & platform at SETAC 2013 (Xia et al.)
- Indicate that source materials and soil characteristics control dissolution of PAHs from weathered soils

RBA of PAHs from Soil: *In Vivo* Study

- Need standardized *in vivo* model for site assessment
- Animal studies required for regulatory acceptance in U.S.
- Need data from library of soils with diverse characteristics and contaminant sources
- Animal data needed to assess predictive value of *in vitro* method

Absorption and Distribution in vivo



Source: Adapted from Ramesh et al. 2004.

RBA of PAHs from Soil: *In Vivo* Study

Animal Studies

- Several published studies have measured bioavailability of BaP from soil *in vivo* in animals (1991–2007)
  - Different animal models (mice, rats, hamsters, swine)
  - Different study designs and measurement endpoints
  - No rigorous evaluation of bioavailability measurement methods
  - Most use PAH doses well above environmentally relevant range
  - ***Our focus is to develop methods that can be used to examine bioavailability at environmentally-relevant PAH concentrations (low ppm)***

In Vivo Study: Endpoints Evaluated

- Urinary excretion of BaP metabolites in mice
  - Individual
  - Pooled
- Urinary excretion in additional mouse strains
- Urinary excretion of BaP metabolites in rats
- BaP and metabolites in blood in rats
- Label from ^{14}C-BaP in blood, urine, tissue, and feces of rats
- Label from ^{3}H-BaP in blood, urine, tissue, and feces of rats

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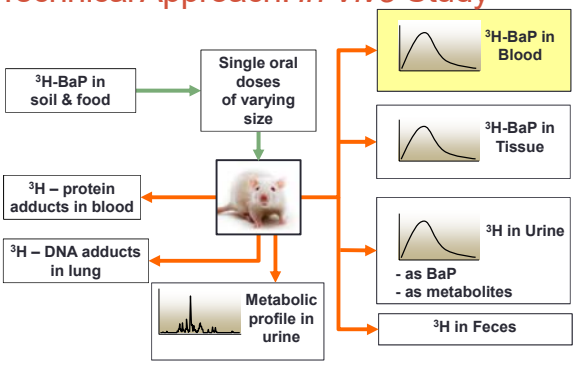
Technical Approach: *In Vivo* Study



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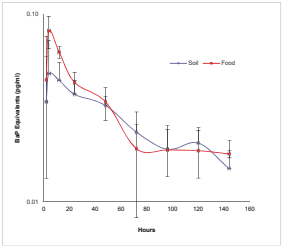
^{3}H-BaP in food versus soil

- Food used for comparison because the cancer slope factor comes from BaP in food.
- Rats given a single dose of BaP in food or soil at 0.2 ppm.
- Blood samples taken over several days
- Counts in blood used to quantify BaP + metabolites based on specific activity of BaP
- This experiment used unweathered soil.
- RBA about 90%.



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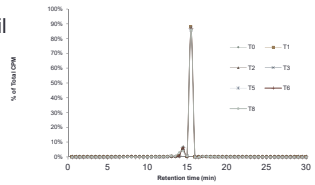
Stability of label during weathering

- Soil spiked with ^{3}H-BaP and weathered for 8 weeks
- Each week an aliquot of soil was extracted and fluorescence and radiochromatograms were obtained.
- Radiochromatograms indicate no degradation of BaP or loss of radiolabel



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The importance of linear pharmacokinetics across dose range

- Interpretation of bioavailability data is straightforward when pharmacokinetics are linear.
- There are at least two potential sources of non-linearity with PAH bioavailability studies
  - Dose-dependent enzyme induction
  - Non-linear absorption, e.g., from capacity-limited uptake
- Confounding effects of induction are minimized by measuring bioavailability from a single dose.
- To determine the potential for other sources of non-linearity, AUCs over a wide dose range were compared.

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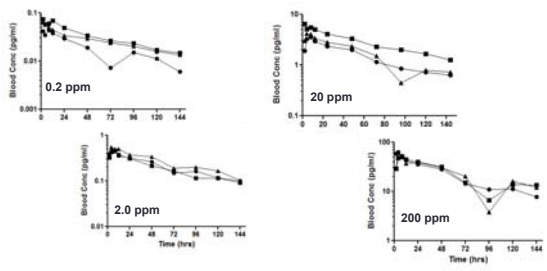
^{3}H-BaP in food administered to rats



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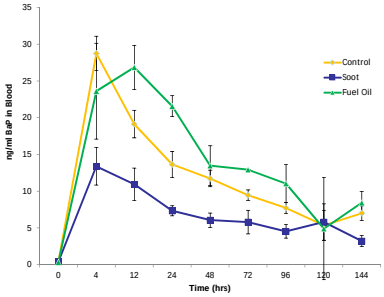
Comparative bioavailability from different source materials



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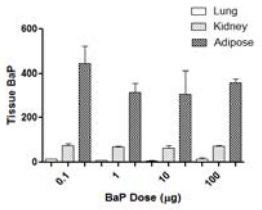
Tissue Levels as Measurement Endpoint

- Rats dosed with ^{3}H-BaP in food or unweathered soil.
- AUCs from blood showed RBA of about 90%. Adipose tissue comparison at 5 days showed RBA of 96%.
- Results from kidney and lung too low to use.



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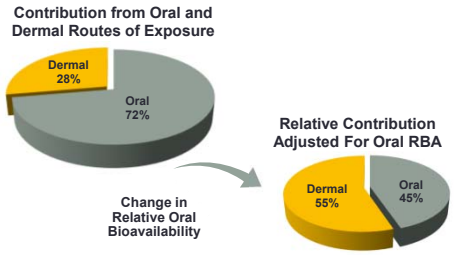
Matrix of Materials for In Vivo Testing

(the concentrations indicated should be confirmed.)

Test Soil Matrix: PAH Sources, Soil Characteristics, and BaP Conc					
PAH Sources	Synthetic Soil (70% sand, 20% clay, 10% peat)	Synthetic Soil + 2 % Charcoal	Synthetic Soil Peat Reduced to 1%	Synthetic Soil Clay Reduced to 2%	Synthetic Soil montmorillonite in place of kaolinite
Mixture of PAHs in Solvent	0.1, 1.0, 10, 100 mg/kg	0.1, 1.0, and 10 mg/kg	0.1 and 1.0 mg/kg	0.1 and 1.0 mg/kg	10 mg/kg
Soot	0.1, 1.0, 10, 100 mg/kg	1.0 mg/kg	—	—	
Fuel oil	0.1, 1.0, 10, 100 mg/kg	0.1, 1.0, and 10 mg/kg	1.0 mg/kg	1.0 mg/kg	

Dermal Study

Effect of Oral Bioavailability on Total Cancer Risk



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Dermal Absorption of PAHs

- Current EPA default assumption is 13% dermal absorption of BaP from soil
- Based on Wester et al. (1990) study in primates
- Study limitations:
  - Soil sieved to 180 to 320 µm (fine to medium sand)
  - No weathering of BaP/soil mixture
  - Dose uncertainty

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Dermal Absorption of PAHs

- Dermal study limitations to address
  - Soil fraction includes fines (<63 µm or <150 µm)
  - Presence of solvent
  - Weathering of PAH in soil
  - Continuous contact ensured
  - Use monolayer soil loading (or more) and report flux
  - Concentration of PAH less than soil saturation
    - ideally at environmentally relevant concentrations)

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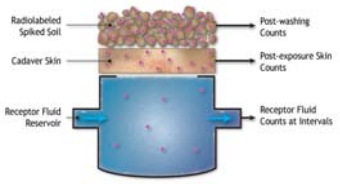
Dermal Absorption Study for BaP in Soil

- Testing four soils with a range of TOC and BC content
- 14C-BaP weathered into soil
- In vitro method using human cadaver skin

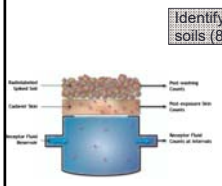


Dermal Study

Soil Code	Soil Source	Total Organic Carbon (%, <63 µm fraction)	Black Carbon (%, <63 µm fraction)
CAMT	California residential soil	1.48	0.36
COSS	Colorado soil from near historical smelter	1.72	0.37
CSU	Colorado agricultural soil	0.99	0.14
ISU	Iowa agricultural soil	3.13	0.23
MTSS	Montana soil from residential area near smelter	3.91	1.23
NYOS	New York orchard soil	6.92	0.37
NYPF2	New York urban soil	7.00	0.47
YOLO	Yolo County soil	0.97	0.09

= Soils selected for dermal absorption study

Dermal Study



Identify candidate soils (8)

TOC & BC analyses (<63 µm fraction)

Select 4 study soils

Pilot testing:
- Assess spiking methods & homogeneity
- Confirm detection limits for ^{14}C-BaP and ^{14}C-fluoranthene

Spike & weather test soils (n=16)
- 4 soils
- 2 chemicals
- 2 concentrations

Dermal absorption testing

Data analysis & reporting

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Progress to date

- Soils selected, sieved, and weathered
- In vitro testing on human cadaver skin complete
- Data reduction in progress
- Anticipate results early 2014
- Will be presented at SOT 2014

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Pathway to Developing In Vitro Method

- Conventional approach
  1. Measure RBA *in vivo* in a suite of soils from different sites
  2. Compare *in vitro* test results with *in vivo* RBA values for the same soils
  3. Adjust conditions of *in vitro* test until results predict *in vivo* results
  4. Use *in vitro* test to measure RBA at contaminated sites
- Challenge with PAHs in Step 1
  - We don't have an animal model that can measure PAH RBA at low concentrations
  - We don't have a library of soils to use in in vitro method validation

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Technical Approach

Task 1
Identify relevant PAH sources, mixtures, and exposure pathways for DOD sites

Task 2
Prepare and finalize work plan

Task 3
Construct and weather PAH-spiked soils

Task 4
Characterize PAH-soil interactions
Characterize PAH "releasable fractions" for different soil characteristics and PAH mixtures
Characterize stability/permanence of nonbioavailable fraction

Task 5
Initial physiologically-based extraction testing
Establish range of anticipated RBA values

Task 6
Select research substrates for oral and dermal research

Task 7
Feeding studies for oral RBA evaluation

Task 8
Develop *in vitro* method that correlates with results for *in vivo*-tested samples

Task 9
Dermal absorption studies

Task 10
Reporting

RBA = relative oral bioavailability adjustment

Questions?

Chemical Controls on Human Exposures to PAHs in Soil

SETAC North America 35th Annual Meeting

Michael Ruby
Yvette Lowney
Upal Ghosh
Steve Roberts
Annette Bunge
John Kissel

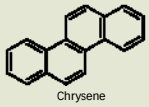
November 10, 2014

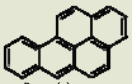
Research Team (SERDP Project)

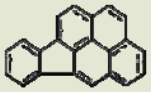
- **Michael Ruby**
  - Integral Consulting
- **Yvette Wieder Lowney**
  - Exponent
  - Bioavailability/ bioaccessibility research and application in risk assessment
- **Upal Ghosh**
  - University of Maryland, Baltimore County
  - Chemical interactions of PAHs with soils
- **Stephen Roberts**
  - University of Florida
  - Animal models for bioavailability research
- **Annette Bunge and John Kissel**
  - Colorado School of Mines and University of Washington, respectively
  - Dermal absorption of chemicals from soil

Chemistry of Carcinogenic PAHs

- Seven PAHs currently considered carcinogenic by EPA
  - 4 rings: benz(a)anthracene, chrysene
  - 5 rings: benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenz(a,h)anthracene
  - 6 rings: indeno(1,2,3-cd)pyrene


Chrysene


Benzo(a)pyrene

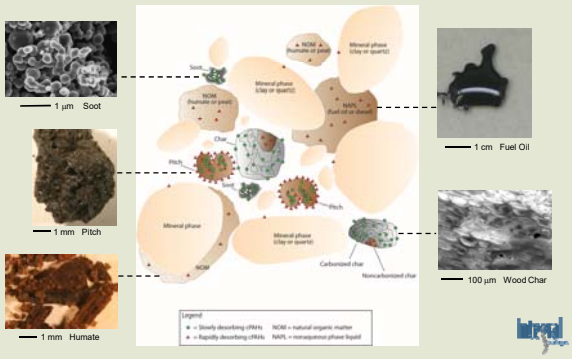

Indeno(1,2,3-cd)pyrene

- Lipophilic, log K_{ow} range from 5.2 to 6.6
- Low water solubility (0.01 to 0.00076 µg/mL)
- Low vapor pressure (6.3×10^{-7} to 9.6×10^{-11} mm Hg)

Sources of PAHs to Soils

Type	PAH Source	Primary PAH-bearing Materials
Natural	Forest fires	Soot, char
	Grass fires	Soot, char
	Volcanic eruptions	Soot, char
	Oil seeps	Weathered crude oil
Industrial	Manufactured gas plants	Coal tar, pitch, coal, char, soot
	Coking operations	Coal tar, coal, coke, soot
	Aluminum production	Coal tar pitch (making and disposing of anodes)
	Foundries	Coal tar pitch, creosote, fuel oil (used in making sand casts), soot
	Wood treating	Creosote
	Refineries	Soot, various NAPLs (crude oil, fuel oil, diesel, etc.)
	Carbon black manufacture	Soot, oil tar
	Fuel spills and/or disposal	Various NAPLs (crude oil, fuel oil, waste oil, diesel)
Non-industrial Sources	Skeet	Coal tar pitch or bitumen (used as binder in targets)
	Asphalt sealants	Coal tar
	Landfills	Creosote (treated wood), soot, char
	Incinerators (municipal, hospital)	Soot
	Open burning	Soot, char
	Fire training	Soot
	Fires	Soot, char
	Auto/truck emissions	Soot

Chemical Controls on PAH Availability

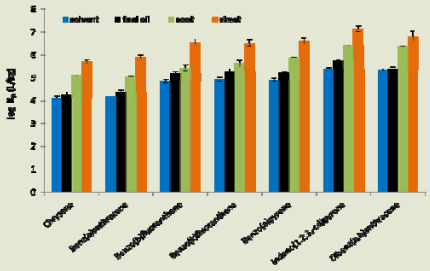


Target BaP Concentrations in Chemistry Study Test Soils

ASTM synthetic soil: 70% sand, 20% kaolinite, 10% peat		
PAH Sources	**Synthetic Soil**	**Synthetic Soil with 2% Charcoal Fines**
Dichloromethane	0.1, 1, 10, 100 mg/kg BaP	0.1, 1, 10 mg/kg BaP
Fuel oil	1, 10, 100 mg/kg BaP	0.1, 1, 10 mg/kg BaP
Soot	0.1, 1, 10, 100 mg/kg BaP	1 mg/kg BaP
Skeet	0.1, 1, 10, 100 mg/kg BaP	1 mg/kg BaP

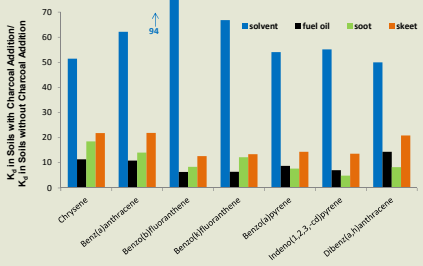
Oral bioavailability study used a subset of these soils (spiked with 3H-BaP); dermal absorption study used four environmental soils spiked with 14C-BaP.

K_d Values for Soils Containing Different PAH Source Materials



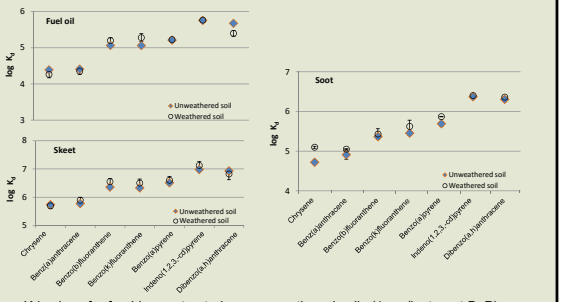
Synthetic soil (sand, clay, peat) spiked with PAHs in solvent (DCM), fuel oil, soot, or skeet at 1 mg/kg BaP

Reduction of K_d after Addition of Charcoal Fines to Test Soils



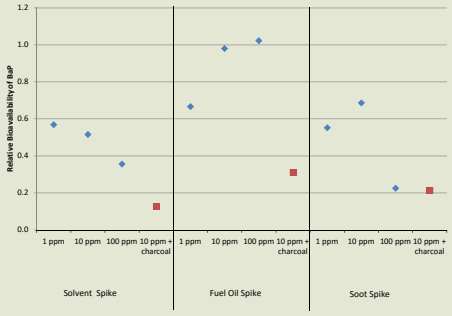
Ratio of K_d values for weathered soils (1 mg/kg target BaP concentration) with and without addition of 2% charcoal fines

Effect of Weathering (2 Months) on K_d Values



Kd values for freshly constructed versus weathered soils (1 mg/kg target BaP)

Effect of PAH Source and Addition of Charcoal Fines on the Oral Bioavailability of BaP



Take Home Messages . . .

- These results are specific to soils (not sediments)
- PAH source materials control oral bioavailability of cPAHs and likely dermal absorption as well
- Presence (or addition) of BC in soil will reduce K_d values and oral bioavailability
- Artificial weathering of spiked soils for 2 months did not decrease K_d values or dermal absorption (effect on RBA under evaluation)

Questions?

Bioavailability of BaP from Soils: Effect of Soil Characteristics, PAH Sources, and PAH Concentrations

Yvette Wieder Lowney, Exponent
Michael Ruby, Integral

SETAC November, 2014

Exponent

SERDP Project Team

Yvette Wieder Lowney	Exponent	Bioavailability/bioaccessibility research and application in risk assessment
Michael V. Ruby	Integral Consulting	Bioavailability research and *in vitro* method development
Upal Ghosh	University of Maryland, Baltimore County	Chemical interactions with soils and sediments
Stephen Roberts	University of Florida	Animal models for bioavailability research
Annette Bunge and John Kissel	Colorado School of Mines and University of Washington, respectively	Dermal absorption of chemicals from soil
Charles Menzie	Exponent	Technical review

Exponent

Technical Approach

Construct and weather PAH-spiked soils

Characterize PAH/soil interactions

Characterize PAH "releasable fractions" for different soil characteristics and PAH mixtures

Initial physiologically based extraction testing

Select research substrates for oral and dermal research

Relative oral bioavailability feeding studies

Dermal absorption flux studies

In vitro method development: Correlate with oral RBA

RBA = relative oral bioavailability

Exponent

Sources of PAHs to Soils

Type	PAH Source	Primary PAH-bearing Materials
Natural	Forest fires	Soot, char
	Grass fires	Soot, char
	Volcanic eruptions	Soot, char
	Oil seeps	Weathered crude oil
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	Coking operations	Coal tar, coal, coke, soot
	Aluminum production	Coal tar pitch (making and disposing of anodes)
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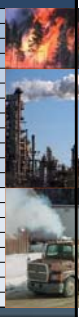
	Wood treating	Creosote
	Refineries	Soot, various NAPLs (crude oil, fuel oil, diesel, etc.)
	Carbon black manufacture	Soot, oil tar
	Fuel spills and/or disposal	Various NAPLs (crude oil, fuel oil, waste oil, diesel)
Non-industrial Sources	Skeet	Coal tar pitch or bitumen (used as binder in targets)
	Asphalt sealants	Coal tar
	Landfills	Creosote (treated wood), soot, char
	Incinerators (municipal, hospital)	Soot
	Open burning	Soot, char
	Fire training	Soot
	Fires	Soot, char
	Auto/truck emissions	Soot



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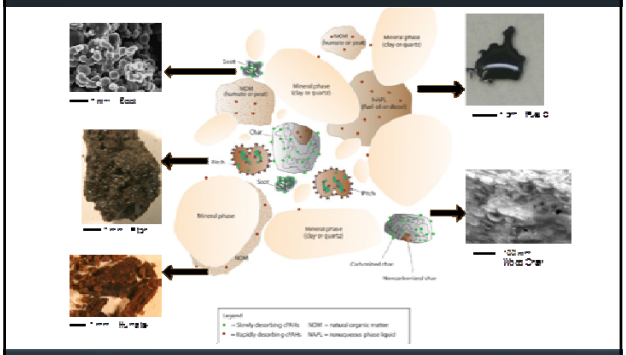
Soil/Chemical Interactions for PAHs at Contaminated Sites



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Technical Objective of *In Vivo* RBA Study

- EPA default is to assume that the RBA of PAHs in soil is 100%
  - Assumes that absorption from soil is equivalent to absorption from spiked food
- Existing research supports site-specific adjustments

- Our research evaluates the effect of potential site-related parameters on the relative oral bioavailability (RBA) of BaP from soils
  - Source material
  - Soil characteristics
  - PAH concentration
  - Studies at environmentally relevant concentrations
    - Soil/chemical interactions
    - Pharmacokinetic interactions

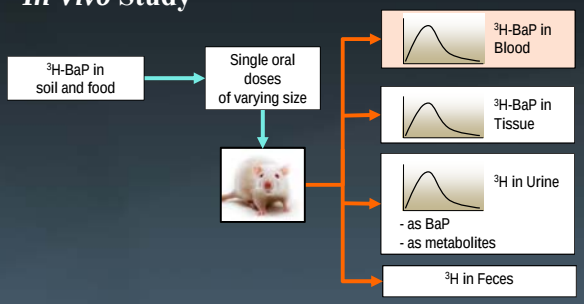
Exponent

Matrix of Materials for *In Vivo* Testing

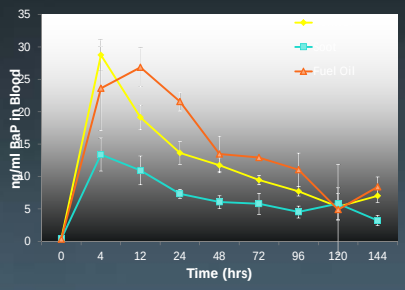
Test Soil Matrix: PAH Sources, Soil Characteristics, and BaP Concentration

PAH Sources	Synthetic Soil 70% Sand 20% Clay 10% Peat	Synthetic Soil + 2% Charcoal	Synthetic Soil Peat Reduced to 1%	Synthetic Soil Clay Reduced to 2%
Mixture of PAHs in:				
Solvent	1.0, 10, 100 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg
Soot	1.0, 10, 100 mg/kg	10 mg/kg		
Fuel Oil	1.0, 10, 100 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg

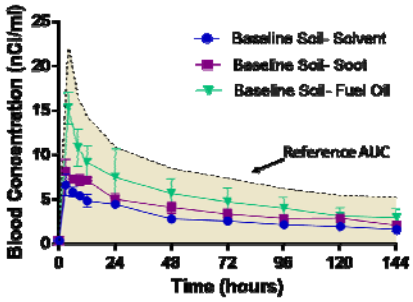
Technical Approach: *In Vivo* Study



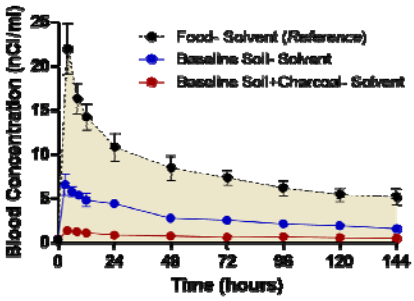
Effect of PAH Source Materials on Bioavailability



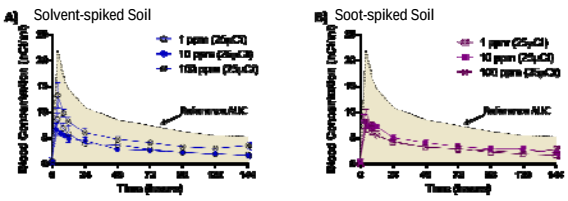
Effect of PAH Source Materials on Bioavailability from Soil



Effect of Soil Characteristics on PAH Bioavailability from Soils



Effect of PAH Concentration on PAH Bioavailability from Soils

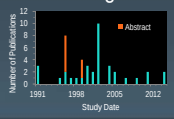


RBA of BaP from Soil: Summary of Data (existing and anticipated)

PAH Source		Solvent		Soot		Fuel Oil	
Soil Matrix	BaP (ppm)	*AUC (nCi hr/ml)*	*RBA*	*AUC (nCi hr/ml)*	*RBA*	*AUC (nCi hr/ml)*	*RBA*
Baseline Soil	1	444 ± 30	0.37	439 ± 30	0.37	—	—
(BSS)	10	411 ± 17	0.35	548 ± 27	0.46	762 ± 99	0.8
	100	664 ± 18	0.56	462 ± 19	0.39	—	—
BSS +Charcoal		102 ± 7	0.09	—	—	—	—
Reduced Clay	10	—	—	—	—	—	—

Conclusions and Implications for Risk Assessment

- Broad research effort undertaken to understand factors that control RBA of BaP in soil
- Default assumption of 100% RBA likely overestimates actual exposures
- Results of RBA testing are similar to existing literature on site-specific investigations



Conclusions and Implications for Risk Assessment *(continued)*

- Preliminary data indicate effects on RBA from
  - PAH source materials
  - Soil characteristics
  - PAH concentration in soil
- Library of soils may support development of *in vitro* methods
- Risk assessment and remedy decisions should incorporate bioavailability considerations
  - Suggest that soil amendments may be effective at affecting RBA (needs further evaluation of efficacy and stability)

Appendix E

In Vitro to *In Vivo* Correlation for PAH RBA from Soils

Materials and Methods

Chemicals

Benzo(a)pyrene and ^{3}H-benzo(a)pyrene (20 Ci/mmol) were purchased from Sigma-Aldrich (St. Louis, MO) and American Radiolabeled Chemicals (St. Louis, MO), respectively. The purity of the radiolabeled benzo(a)pyrene was confirmed to be over 99% by radiochromatography. Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(a)fluoranthene, benzo(k)fluoranthene, indeno(1,2,3-c,d)pyrene, dibenz(a,h)anthracene, and benzo(g,h,i)perylene were obtained from Sigma-Aldrich. Scintillation cocktail (ScintiVerse®), Soluene 350®, hydrogen peroxide (30%), ethanol, sodium sulfate, dichloromethane, methanol, acetone, soot, charcoal, porcine bile, porcine mucin, and bovine serum albumin (BSA) were also obtained from Sigma-Aldrich. Fuel Oil No. 6 was from Chevron (San Ramon, CA). Sand (Sil-CO-Sil®) was purchased from U.S. Silica (Frederick, MD), peat was Miracle-Gro (Marysville, OH), and humus was from Organic Valley (LaFarge, WI).

Soils

Soil samples were constructed using a Baseline Synthetic Soil (BSS) consisting of 70% sand, 20% kaolinite clay and 10% peat. Polycyclic aromatic hydrocarbons (PAHs) from three source materials — solvent, soot, and fuel oil — were added to the baseline soil to achieve target BaP concentrations of 1, 10, or 100 ppm. Composition of the PAH mixtures is shown in Table S-1 (Supplemental Materials). In addition to unlabeled BaP, the PAH mixtures included tritiated BaP (3H-BaP; final concentration 50 µCi/g soil). (Table 1). In addition to source and BaP concentration as variables, addition soils were included to evaluate the effect of organic carbon type (peat vs humus) and clay type (kaolinite vs montmorillonite) on BaP relative bioavailability (Table 1). A test soil was also constructed with 2% charcoal fines, which were added to evaluate the effect of a strong geosorbent on BaP RBA values. PAHs were added to soil using a procedure described elsewhere (Roberts et al.), and the soils were thoroughly mixed, air dried, and sieved to 150 µm.

The constructed soil samples were weathered for 8 weeks with wet-dry cycles following PAH addition (including ^{3}H-BaP). Weathering was accomplished by adding deionized water to the soil samples in an amount equivalent to the water holding capacity of the soil (estimated to be 56% by weight). The soil

sample was then allowed to dry in air at room temperature. This wetting procedure was repeated at weekly intervals over the 8-week weathering period.

Bioaccessibility extraction methods

3550C

A 0.25 g aliquot of weathered soil was weighed (to the nearest 0.001g) into a 20 ml glass vial and 3 ml of the extraction solvent (1:1 Methylene Chloride/Acetone) was added. The vial contents were pulse-sonicated for 3 minutes (50% on and 50% off with 30 seconds interval) with 100% power and then centrifuged at 500 x g for 2 minutes. The supernatant was removed and filtered (Whatman 41 Ashless Quantitative Filter Paper). This extraction was repeated a total of three times and the filtered extracts were combined and mixed thoroughly. A 100 µl aliquot of the filtered extracts was added to 15 ml scintillation fluid in 20 ml scintillation vials, mixed, and allowed to stand for 24 hours before measuring radioactivity content by liquid scintillation counting. Each of the test soils was evaluated in triplicate. A laboratory blank, blank spike, and matrix spike were created for quality control purposes. Blanks were created by following the extraction procedure without the initial addition of soil. Triplicate blank spikes were created by adding 12.5 µCi ^{3}H-BaP to 3 ml of extraction solvent [without soil] and following the extraction procedure. For matrix spikes, 12.5 µCi ^{3}H-BaP was added to BSS soil [without PAHs], and the soil extraction procedure was followed.

n-Butanol Method

This procedure was carried out using a modification of the butanol soil extraction method described in Duan et al., 2014[1]. A 0.25g aliquot of weathered soil was weighed (recorded to the nearest 0.01 mg) into tared 4 ml glass vials. N-Butanol (500 µl of 99+ % purity) was added to each of the vials and the contents were vortexed for 50 seconds. The samples were allowed to settle for 25 minutes and then centrifuged for 5 minutes at 500 x g. A 25 µl aliquot of the supernatant was added to 15 ml of scintillation fluid, mixed, and allowed to stand for 24 hours before counting. Each of the soils was evaluated in triplicate, as well as laboratory blanks, blank spikes, and matrix spikes.

Physiologically-based Extraction Test

[1] Duan, L., Palanisami, T., Yanju, L., Dong, Z., Mallavarapu, M., Kuchel, T., Semple, T., Naidu, R. 2014. Effects of ageing and soil properties on the oral bioavailability of penzoapyrene using a swine model. Environment International 70; 192-202.

To create the physiologically-based extraction fluid, 0.80 g of porcine bile (4 g/L in final extraction fluid), 0.50 g porcine mucin (2.5 g/L in final extraction fluid), and 1.0 g of BSA (bovine serum albumin) (5 g/L in final extraction fluid) were mixed with 140 ml of ASTM type II deionized water in a conical flask, followed by addition of 36.4 ml of 0.2M Na_2HPO_4 and 13.6 ml of 0.1M citrate. After the addition of the components, the solution was brought to 200 ml using ASTM type II deionized water. The extraction fluid was heated in a water bath to 37^0C. The fluid was then vortexed to ensure complete dissolution of solids, and the pH of the fluid was adjusted to 6.6 ± 0.2 by drop-wise addition of dilute HCl or NaOH.

For the extraction procedure, **a** 40 mg aliquot of soil sample was weighed (to the nearest 0.01mg) into amber glass vials and 4 ml of extraction matrix (mucin, bile, and bovine serum albumin) was added. The vials were incubated at 37^0C for 4 hours and then centrifuged at 1500 x g for 10 minutes. The pH of the samples was measured after centrifugation to ensure that all samples were within 0.5 pH units from pH 6.6. A 1.0 ml aliquot of the supernatant was collected and combined with 100 µl of hydrogen peroxide, vortexed for 60 seconds, and allowed to stand for 24 hours before adding 15 ml of scintillation fluid. Samples were then vortexed for 60 seconds and then allowed to sit for 48 hours before counting. As with the other extraction methods, triplicate laboratory blanks, blank spikes, and matrix blanks were prepared.

Instrumental analysis

Beckman LS 6500 Liquid Scintillation Counter (BECKMAN COULTER, Brea, California) was used for the Benzo(a)Pyrene analysis in all the three experiments and beta counting was used to calculate the % ^{3}H-BaP extracted from the soil samples. An auto-DPM program was used to factor out any quench effects. Bioaccessability was determined by reporting the extracted BaP value compared to the actual value in the soil (extracted µCi/g soil / original µCi/g soil).

Table 1.

Soil	Soil type	Source	BaP (PPM)	Original µCi/g soil
1	BSS	Solvent	1	50
2	BSS	Solvent	10	50
3	BSS	Solvent	100	50
7	Char	Solvent	10	50
10	2% Clay	Solvent	10	50
13	BSS	Soot	1	50
14	BSS	Soot	10	50
15	BSS	Soot	100	50

18	Char	Soot	10	50
19	BSS	Fuel oil	1	50
20	BSS	Fuel oil	10	50
21	BSS	Fuel oil	100	50
25	Char	Fuel oil	10	50
26	1% Peat	Fuel oil	10	50
27	2% Clay	Fuel oil	10	50

Table 1 Raw data from in vitro extraction testing and in vivo RBA testing of soils spiked with benzo(a)pyrene at University of Florida, and measurements of Kd for soils at UMBC

																			Relative Oral Bioavailability as Reported in Rats							Data from UMBC Evaluation of Kd for Similarly-Prepared Soi				
UfI Soil ID	Soil type	PAH Source	Concentration of BaP in Soil (mg/kg)	3550 C run 1	3550C run 2	3550C run 3	3550 C mean	SD	% of invivo	PBET run 1	PBET run 2	PBET mean	SD	% of invivo	n-Butanol run 1	n-Butanol run 2	n-Butanol run 3	n-Butanol Mean	SD	% of invivo	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean	SD	UMBC 3550C	log Kd based on measured [BaP]	Kd based on measured [BaP]	Kd based on target spike [BaP]	log Kd based on target spike
1	BSS	Solvent	1	0.293	0.328	0.377	0.333	0.042	60	0.568	0.568	0.568	0.000	102	0.220	0.250	0.220	0.230	0.017	41	0.666	0.455	0.608	0.549	0.510	0.558	0.084	0.12	4.91	81283	6.77E+05	5.83
2	BSS	Solvent	10	0.249	0.295	0.265	0.270	0.023	53	0.433	0.434	0.433	0.001	86	0.173	0.175	0.211	0.186	0.022	37	0.519	0.517	0.468	0.512	0.510	0.505	0.022	2.6	4.82	66069	2.54E+05	5.41
3	BSS	Solvent	100	0.730	0.631	0.584	0.648	0.075	124	0.737	0.737	0.737	0.000	141	0.420	0.408	0.458	0.429	0.026	82	0.473	0.624	0.452	0.471	0.590	0.522	0.079	18.1	5.4	251189	1.39E+06	6.14
13	BSS	Soot	1	0.454	0.465	0.541	0.486	0.047	90	0.536	0.514	0.524	0.015	97	0.278	0.280	0.316	0.291	0.022	54	0.534	0.594	0.621	0.429	0.531	0.542	0.075	0.75	5.87	741310	9.88E+05	5.99
14	BSS	Soot	10	0.731	0.725	0.758	0.738	0.018	110	0.395	0.377	0.386	0.012	57	0.190	0.196	0.208	0.198	0.010	29	0.665	0.674	0.668	0.780	0.577	0.673	0.074	5.1	6.71	5128614	1.01E+07	7.00
15	BSS	Soot	100	0.554	0.639	0.492	0.562	0.074	239	0.133	0.123	0.128	0.007	54	0.039	0.019	0.029	0.029	0.010	12	0.220	0.233	0.214	0.214	0.295	0.235	0.033	58.9	7.78	60255959	1.02E+08	8.01
19	BSS	Fuel oil	1	0.359	0.351	0.401	0.370	0.027	57	0.595	0.61	0.602	0.010	93	0.233	0.237	0.259	0.243	0.014	38	0.652	0.641	0.642	0.644	0.660	0.648	0.008	0.11	5.23	169824	1.54E+06	6.19
20	BSS	Fuel oil	10	0.689	0.734	0.787	0.737	0.049	77	0.729	0.677	0.702	0.037	73	0.573	0.712	0.632	0.639	0.069	66	0.782	0.833	1.431	0.910	0.848	0.961	0.272	3.7	6.02	1047129	2.83E+06	6.45
21	BSS	Fuel oil	100	1.592	1.618	1.332	1.514	0.158	142	0.569	0.5	0.534	0.048	50	1.113	1.218	1.249	1.193	0.071	112	0.553	1.259	1.033	1.059	1.415	1.064	0.313	NA	NA	NA	NA	NA
26	less Peat	Fuel oil	10	1.004	1.094	0.880	0.993	0.107	130	0.657	0.657	0.657	0.000	86	0.873	0.711	0.791	0.792	0.081	104	0.431	0.815	0.921	0.794	0.844	0.761	0.195	NA	NA	NA	NA	NA
27	less Clay	Fuel oil	10	0.752	0.875	0.596	0.741	0.140	87	0.711	0.77	0.74	0.042	87	0.637	0.577	0.569	0.594	0.038	70	0.751	0.737	0.899	0.882	1.002	0.854	0.113	NA	NA	NA	NA	NA
10	less Clay	Solvent	10	0.426	0.478	0.351	0.418	0.064	102	0.625	0.646	0.635	0.015	155	0.165	0.227	0.258	0.217	0.048	53	0.455	0.448	0.415	0.323		0.410	0.060	NA	NA	NA	NA	NA
25	+charc	Fuel oil	10	0.623	0.807	0.644	0.692	0.101	227	0.208	0.197	0.202	0.008	66	0.289	0.218	0.247	0.252	0.036	82	0.420	0.288	0.274	0.254	0.288	0.305	0.067	9.7	5.96	912011	9.40E+05	5.97
18	+charc	Soot	10	0.412	0.539	0.385	0.445	0.082	212	0.117	0.117	0.117	0.000	56	0.025	0.018	0.025	0.023	0.004	11	0.202	0.193	0.209	0.222	0.222	0.210	0.013	NA	NA	NA	NA	NA
7	+charc	Solvent	10	0.403	0.453	0.399	0.418	0.030	332	0.068	0.064	0.066	0.003	52	0.054	0.055	0.053	0.054	0.001	43	0.112	0.143	0.113	0.109	0.150	0.126	0.020	3.5	6.04	1096478	3.13E+06	6.50

Notes:

"BSS" is ASTM Baseline Synthetic Soil

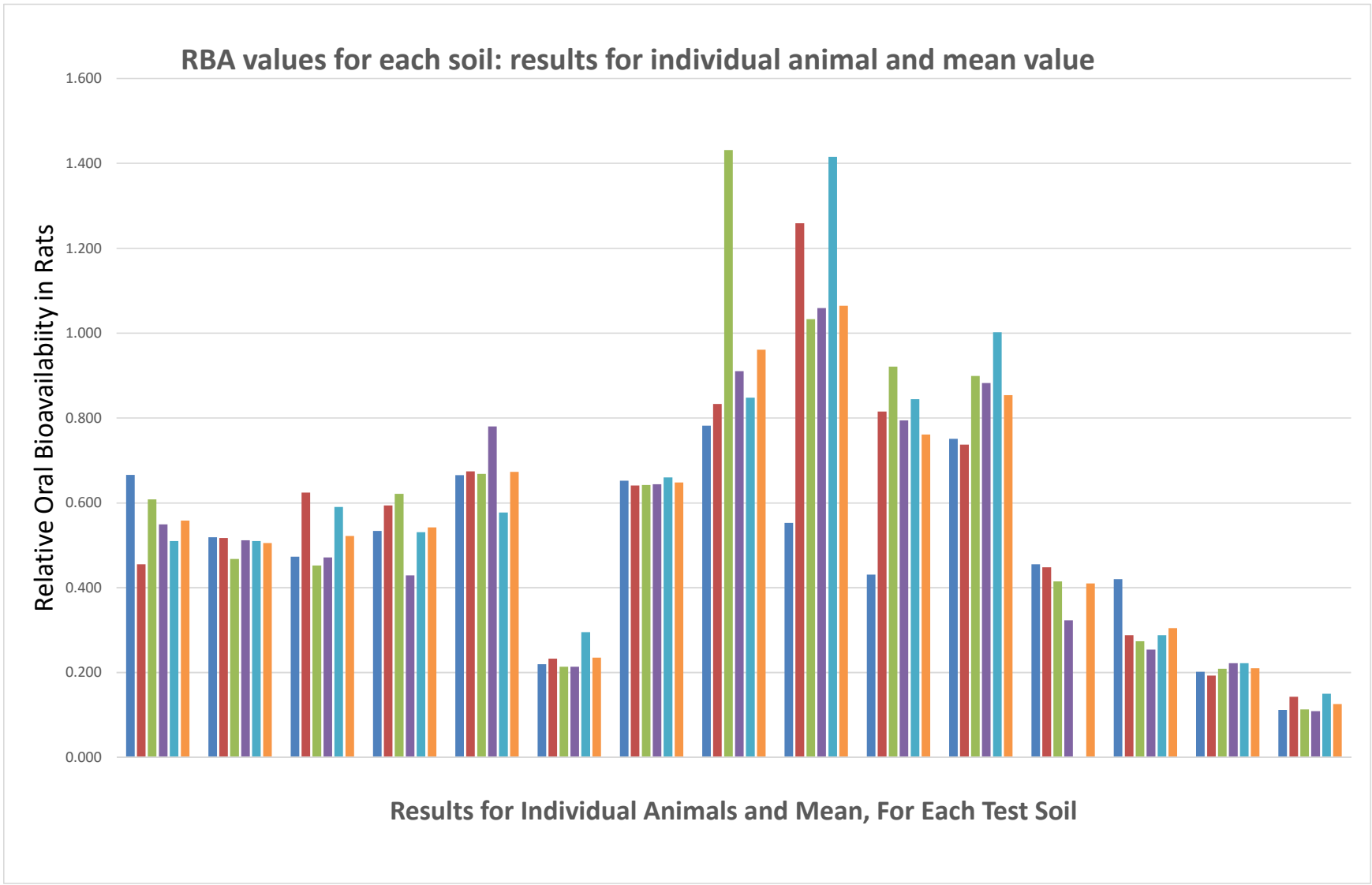
BaP concentrations represent spiked concentrations

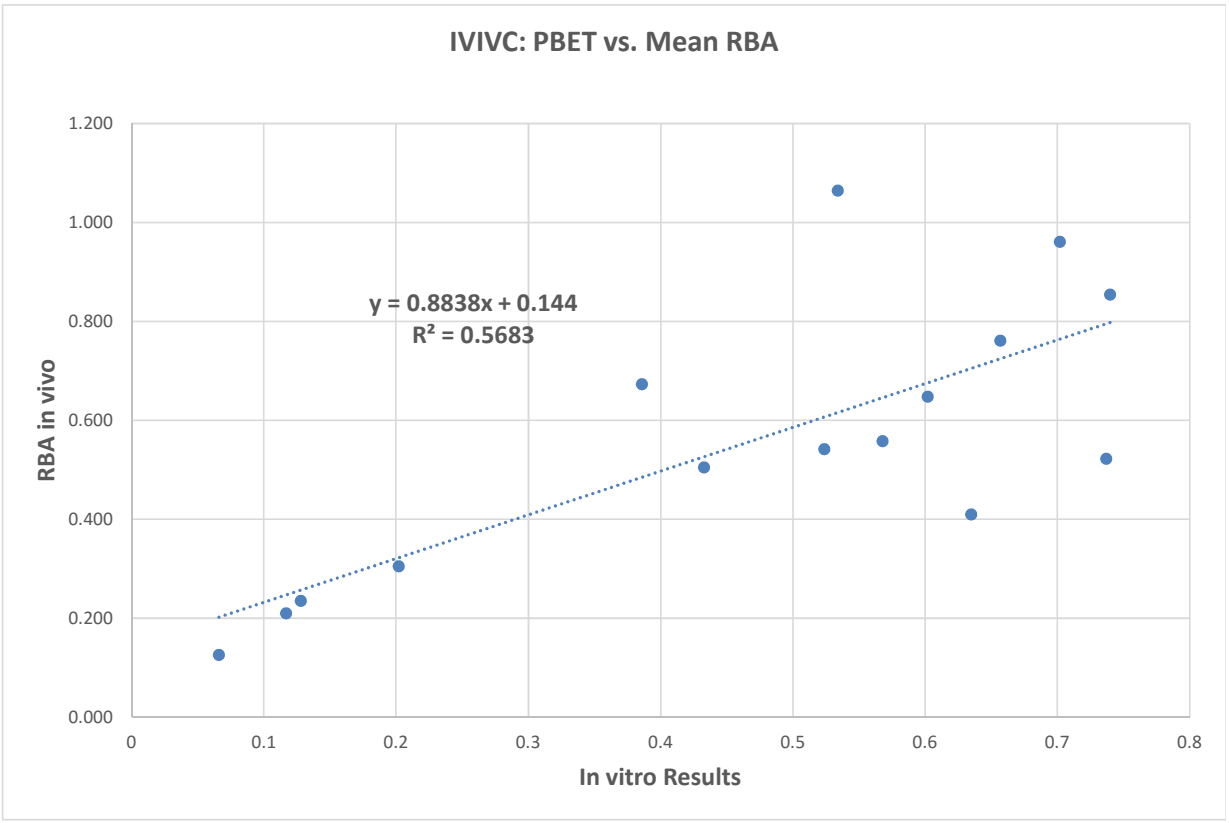
"UMBC 3550C" represents the measured concentration of BaP in soil

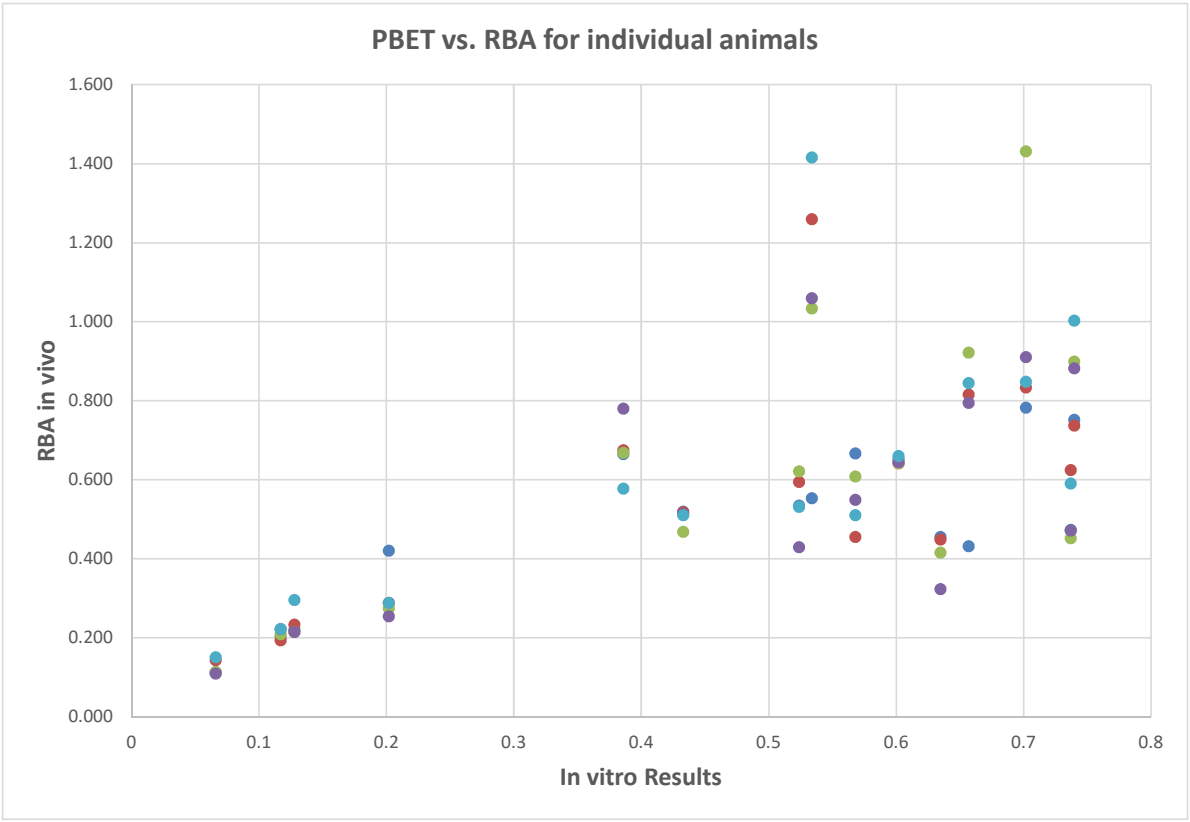
Relative Oral Bioavailability is from studies in rats at UFI as reported in Roberts et al., 2016

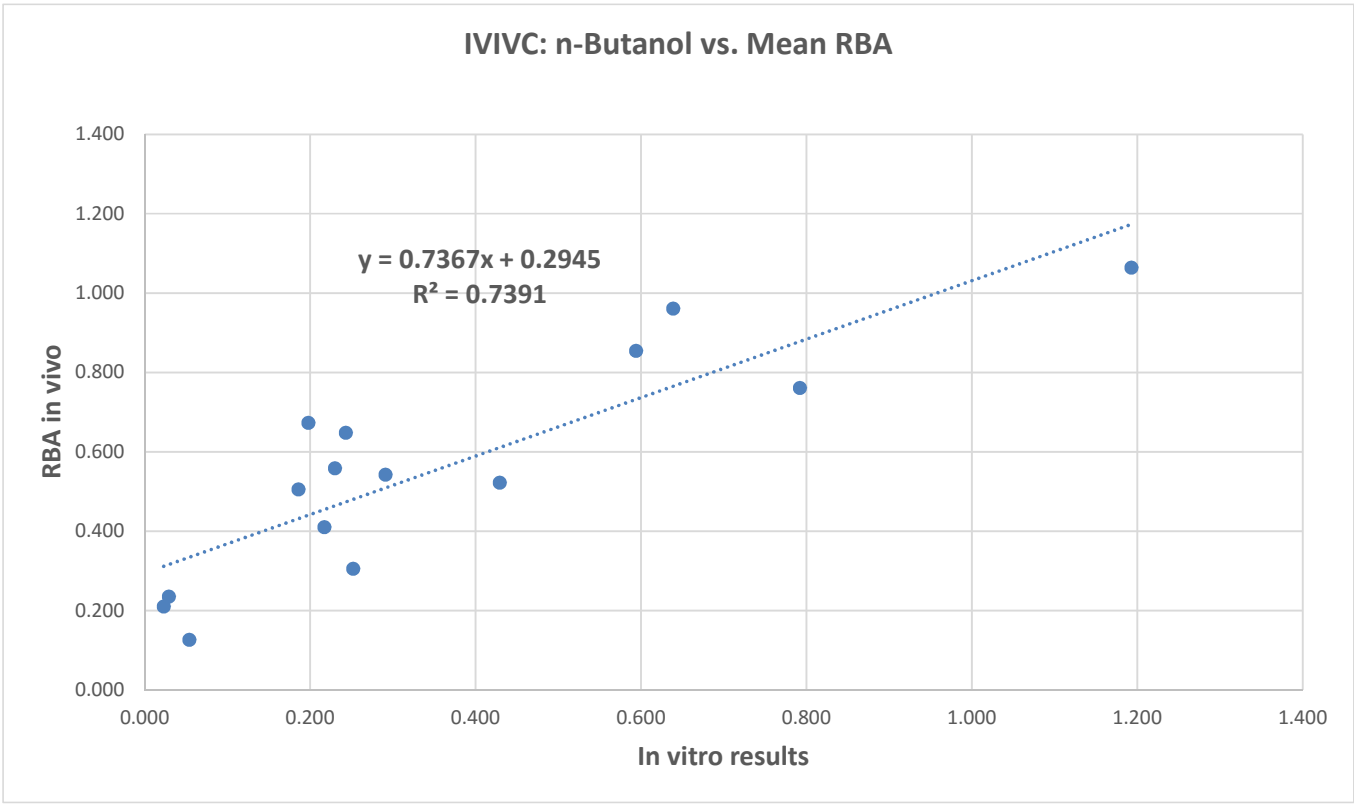
Kd values are for soils constructed at UMBC, but without radiolable. Methods used to generate soils were similar at UFI and UMBC

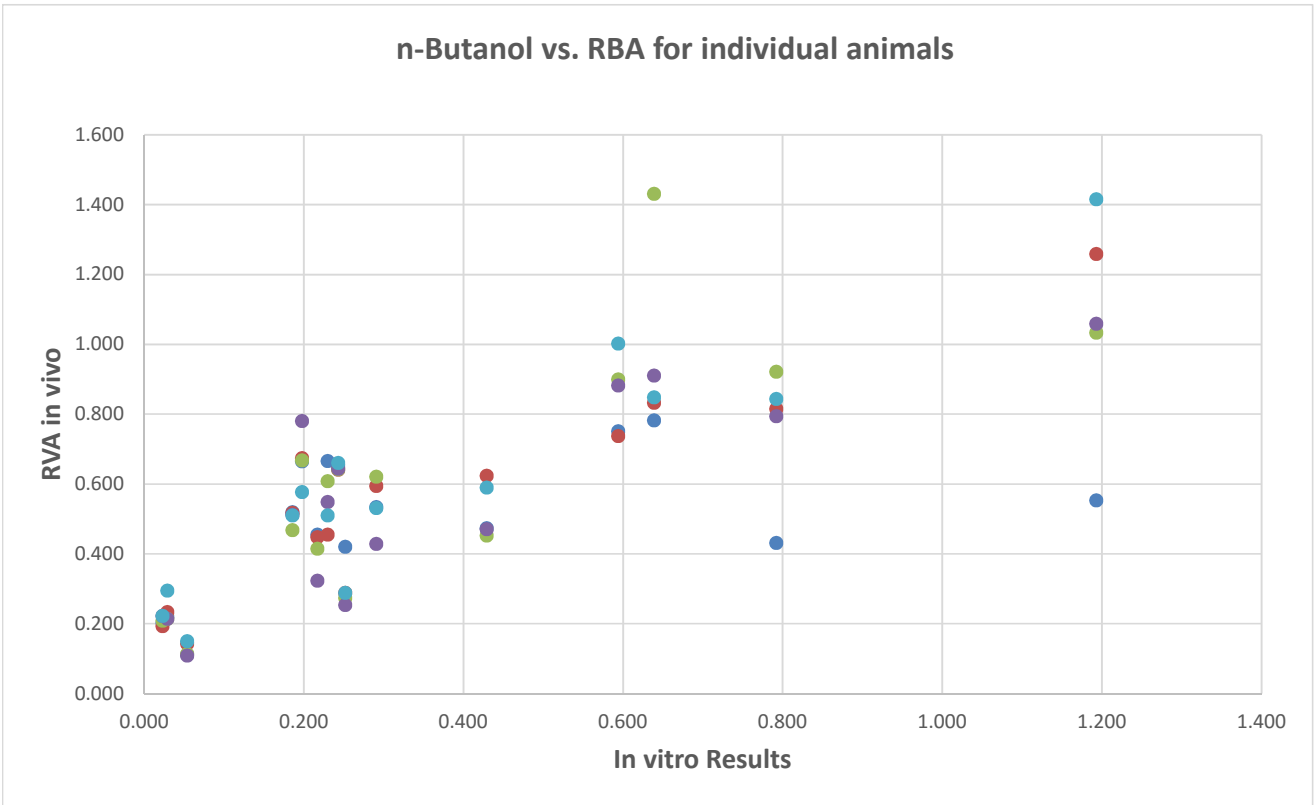
Kd values are reported based on the measured concentrations of BaP in the soil using a modified EPA Method 3550C (as Reported in Xia et al., 2016), and calculated based on the concentration of BaP spiked to soil prior to weathering

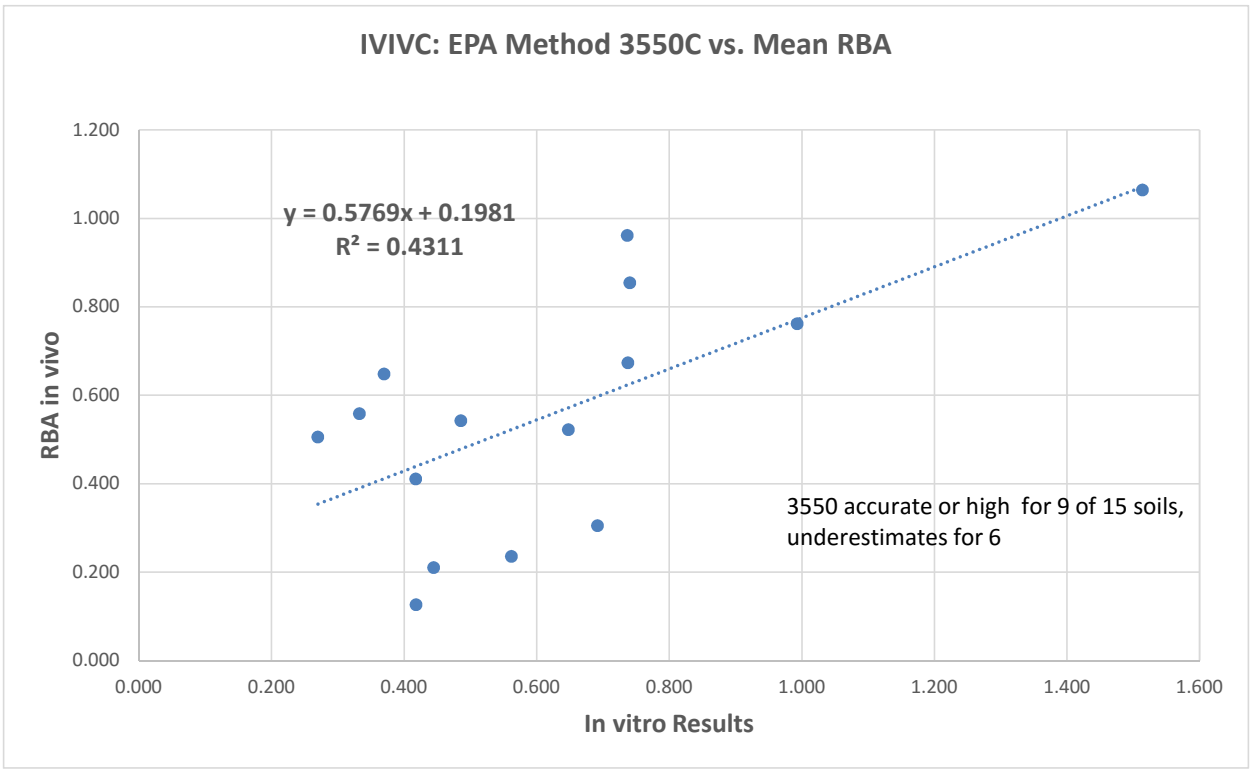

RBA values for each soil: results for individual animal and mean value
Relative Oral Bioavailabiity in Rats
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Results for Individual Animals and Mean, For Each Test Soil

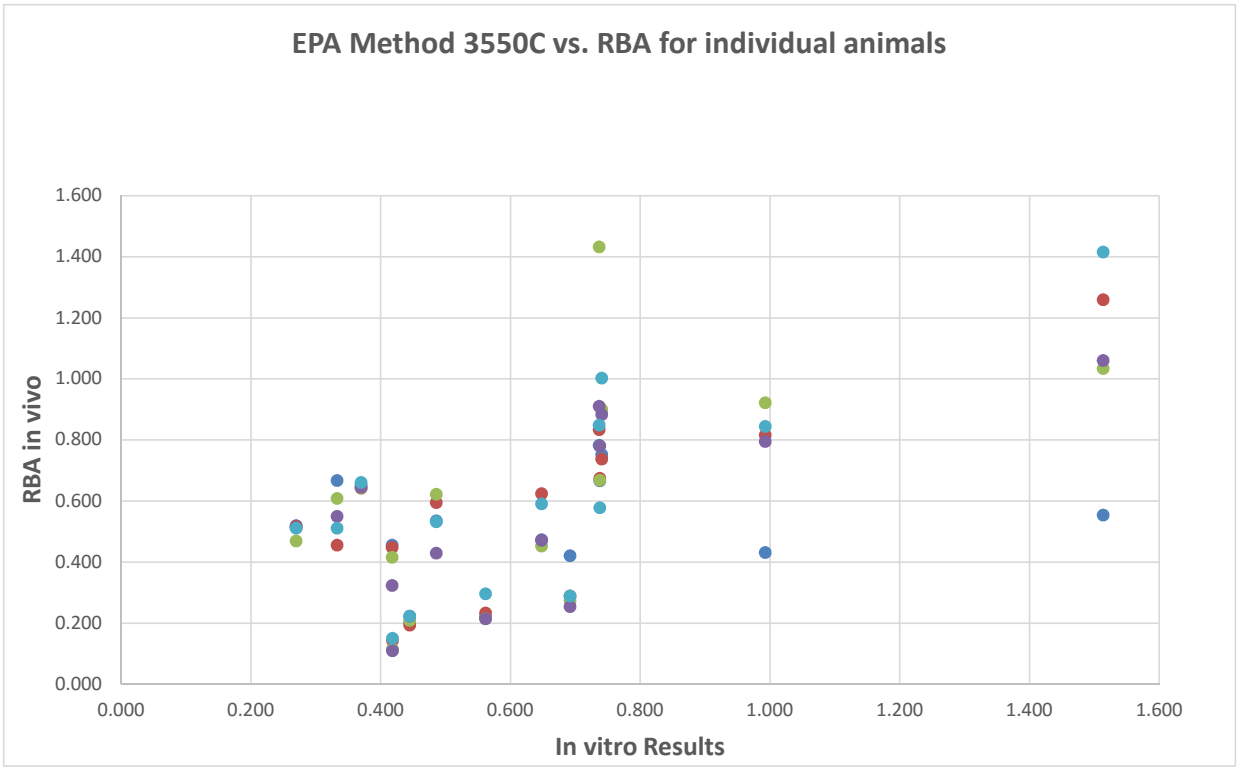

IVIVC: PBET vs. Mean RBA
y = 0.8838x + 0.144
R² = 0.5683
RBA in vivo
In vitro Results
1.200
1.000
0.800
0.600
0.400
0.200
0.000
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8

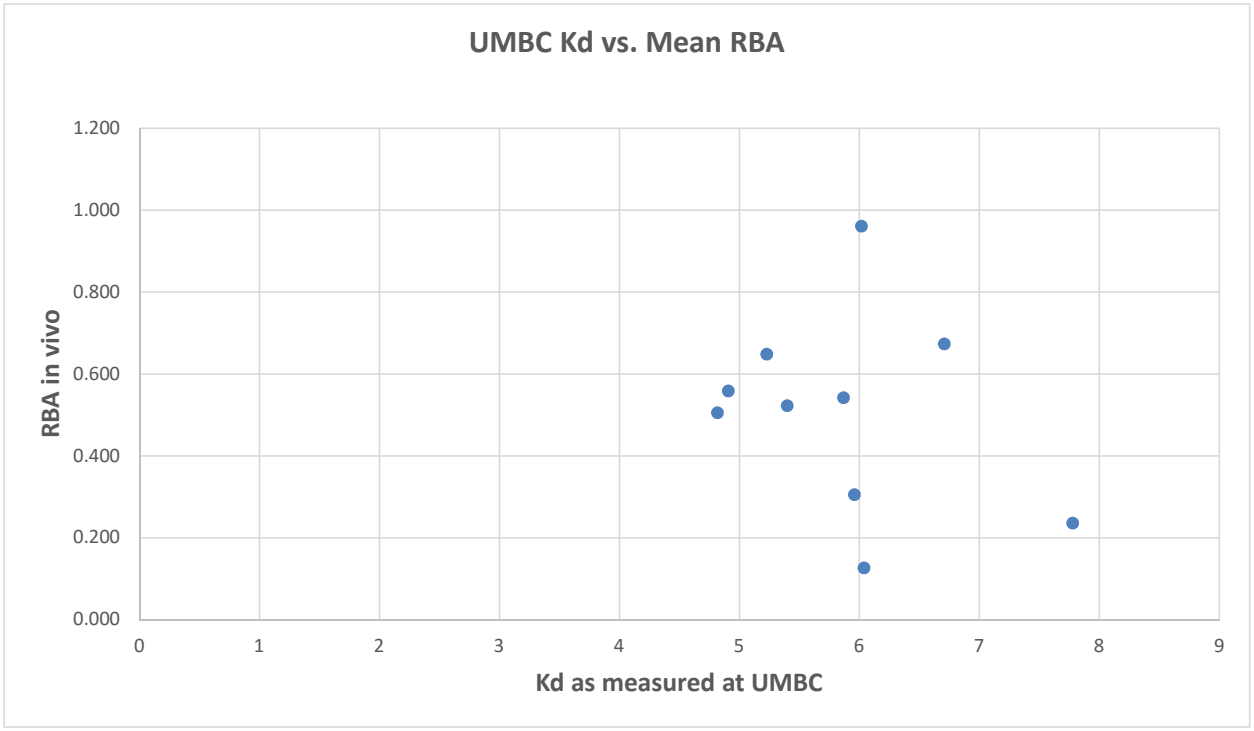

PBET vs. RBA for individual animals
RBA in vivo
In vitro Results
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8

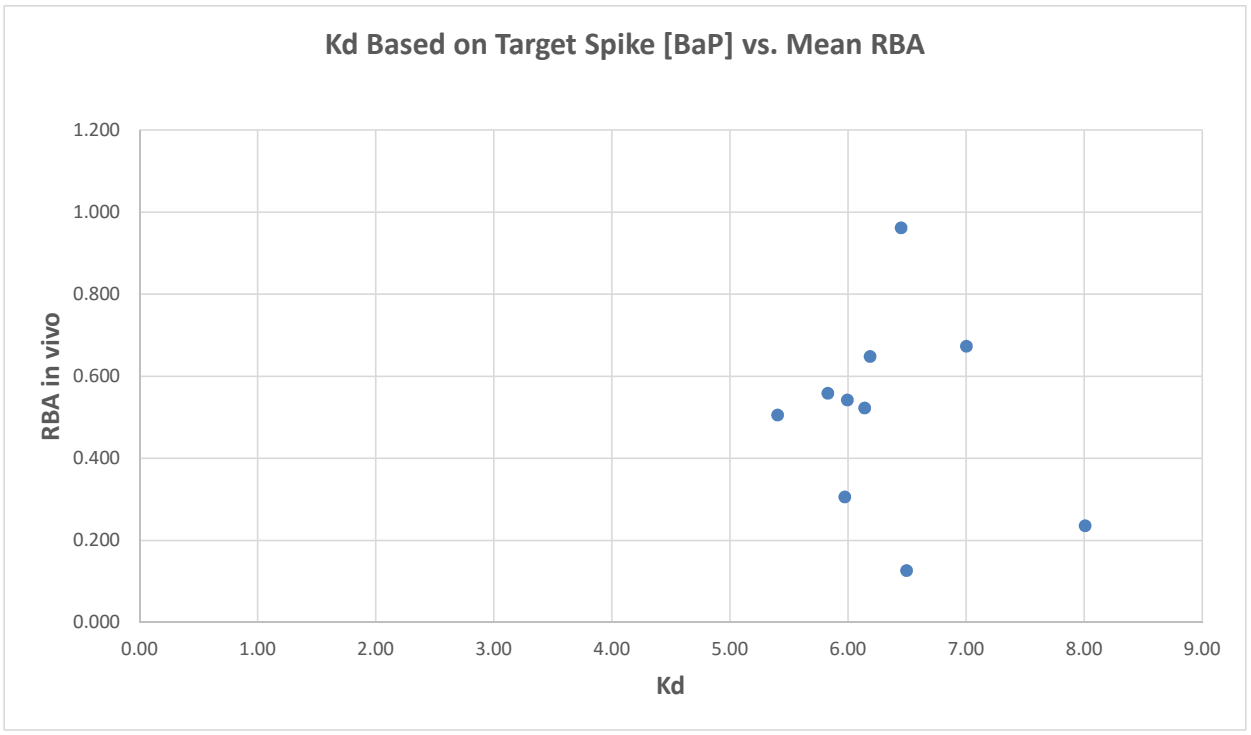

IVIVC: n-Butanol vs. Mean RBA
y = 0.7367x + 0.2945
R² = 0.7391
RBA in vivo
In vitro results
0.000
0.200
0.400
0.600
0.800
1.000
1.200
1.400


n-Butanol vs. RBA for individual animals
RVA in vivo
In vitro Results
0.000
0.200
0.400
0.600
0.800
1.000
1.200
1.400
1.600


IVIVC: EPA Method 3550C vs. Mean RBA
y = 0.5769x + 0.1981
R² = 0.4311
3550 accurate or high for 9 of 15 soils, underestimates for 6
RBA in vivo
In vitro Results
0.000
0.200
0.400
0.600
0.800
1.000
1.200
1.400
1.600


EPA Method 3550C vs. RBA for individual animals
RBA in vivo
In vitro Results
0.000
0.200
0.400
0.600
0.800
1.000
1.200
1.400
1.600


UMBC Kd vs. Mean RBA
RBA in vivo
1.200
1.000
0.800
0.600
0.400
0.200
0.000
0
1
2
3
4
5
6
7
8
9
Kd as measured at UMBC


Kd Based on Target Spike [BaP] vs. Mean RBA
RBA in vivo
1.200
1.000
0.800
0.600
0.400
0.200
0.000
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
Kd

Appendix F

Dermal Absorption of PAHs from Soil

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ORIGINAL ARTICLE

Dermal absorption of benzo[a]pyrene into human skin from soil: Effect of artificial weathering, concentration, and exposure duration

Trevor K. Peckham[1], Jeffry H. Shirai[1], Annette L. Bunge[2], Yvette W. Lowney[3], Michael V. Ruby[4] and John C. Kissel[1]

In vitro assessments of ^{14}C-benzo[a]pyrene (BaP) absorption through human epidermis were conducted with the sub-63-μm fraction of four test soils containing different amounts of organic and black carbon. Soils were artificially weathered for eight weeks and applied to epidermis at nominal BaP concentrations of 3 and 10 mg/kg for 8 or 24 h. Experiments were also conducted at 24 h with unweathered soils and with BaP deposited onto skin from acetone at a comparable chemical load. For the weathered soils, absorption was independent of the amount of organic or black carbon, the mass in the receptor fluid was proportional to exposure duration but independent of concentration, and the mass recovered in the skin after washing was proportional to concentration and independent of exposure time. Results from the weathered and unweathered soils were similar except for the mass recovered in the washed skin, which was lower for the weathered soil only at the higher concentration. We hypothesize that chemical concentrations exceeded the BaP sorption capacity accessible within the artificial weathering timeframe for all soils tested, and that BaP mass in the washed skin was dominated by particles that were not removed by washing. Fluxes into and through skin from soils were lower by an order of magnitude than from acetone-deposited BaP.

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Keywords: dermal exposure; exposure modeling; polycyclic aromatic hydrocarbons

INTRODUCTION

Soil cleanup standards and assessment of human health risks at contaminated sites are based in part on predicted human exposure to soil contaminants, including from direct skin contact. Percutaneous absorption of soil-bound chemicals requires transfer from soil particles to the skin surface and then diffusion through the protective epidermis into the underlying dermis. Characterization of the rate of skin uptake is therefore important in predicting the absorbed dose.

Polycyclic aromatic hydrocarbons (PAHs) are commonly present in soil at or near hazardous waste sites and often drive risk and remedial decision making. Benzo[a]pyrene (BaP) is the index chemical under the current regulatory paradigm for PAH toxicity. It was ranked #8 on the Agency for Toxic Substances and Disease Registry (ATSDR) Priority List of Hazardous Substances in 2013, based on a combination frequency of appearance, toxicity and potential for human exposure at National Priority List (NPL) sites.[1] Several studies have attempted to characterize the dermal absorption of BaP from contaminated soil or sediment, including *in vivo* studies performed on rats[2] and rhesus monkeys,[3] and *in vitro* experiments using skin of rats,[2,4,5] pigs,[6] guinea pigs,[7] and humans.[3,4,7–11]

Theoretical and empirical evidence suggests that fractional absorption is dependent on the mass of soil on the skin (the soil load) when the soil load covers the exposed area completely (i.e., the fraction absorbed decreases as the soil load increases).[5,9,12–14] Therefore, dermal absorption is best described in terms of gradient-driven flux, not percent absorption. Although fractional absorption has normally been reported by previous investigators, a recent review of dermal absorption studies of contaminated soils found that average BaP uptake reported as flux from six of the studies listed above[2–4,6,8,9] spanned a range of six orders of magnitude (0.19–420 000 pg/cm^2/h).[14] A thorough examination of this literature is warranted, but will not be attempted here. A recent abbreviated review[15] noted that important gaps in the existing literature include quantification of the effects of chemical concentration in the soil and of soil characteristics on uptake.

To improve the general understanding of the potential for dermal absorption of PAHs from contaminated soil, *in vitro* assessments of absorption through human cadaver skin were conducted with four test soils spiked with radiolabeled BaP. For comparison with the soil measurements, absorption from BaP applied to skin in solvent was also evaluated. The present study was developed and performed with attention to important methodological criteria, including soil layering effects, appropriateness of particle size distribution employed, degree of chemical saturation of soil, and soil-chemical contact (i.e., "aging") time. Prior experiments have generally failed to account for one or more of these criteria in either reporting or execution.[14,15] Further considerations were implemented in the present study to produce conditions that represent realistic exposure scenarios; these included employing soil with BaP concentrations in a range that

[1]Department of Environmental and Occupational Health Services, School of Public Health, University of Washington, Seattle, WA, USA; [2]Chemical and Biological Engineering Department, Colorado School of Mines, Golden, CO, USA; [3]Exponent, Boulder, CO, USA and [4]Integral Consulting, Louisville, CO, USA. Correspondence: John C Kissel, Department of Environmental and Occupational Health Services, School of Public Health, University of Washington, Box 354695, 4225 Roosevelt Way NE, Seattle 98195, WA, USA.
Tel: +1 202 543-5111.
E-mail: jkissel@uw.edu
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might reasonably be found at a contaminated site, and artificially weathering and aging spiked soils prior to experimental application.

MATERIALS AND METHODS

Chemicals

^{14}C-BaP (specific activity = 26.6 μCi/μmol; 98.6% purity; 7-^{14}C-labeled) in toluene was obtained from American Radiolabeled Chemicals (St. Louis, MO, USA). BaP-toluene stock solutions used to spike soils were prepared with anhydrous toluene (99.8% purity; Sigma-Aldrich, St Louis, MO, USA). For acetone-delivered experiments, toluene was removed by evaporation and BaP dissolved into acetone.

Study Soils

Soil experiments were conducted using the sub-63 μm fraction of four soils with varying total organic content (TOC) and black carbon (BC) as listed in Table 1. TOC was measured by combustion at 900 °C after removal of inorganic carbon with hydrochloric acid, and BC content was measured using a chemo-thermal oxidation method (CTO-375).[16] The CSU and ISU soils were collected from Colorado State University (Fort Collins, CO, USA) and Iowa State University Agricultural Station (Ames, IA, USA), respectively, and prepared following procedures described by Choate et al.[17] The Yolo soil, collected from the University of California (UC), Davis student farm[18] was acquired from the UC Davis soils laboratory, which was also the source of the Yolo County soil used by Wester et al.[3] (personal communication, R Wester, 1994); see Supporting Information for additional details. The MTSS soil is a composite of soils collected from nine residences near the smelter in Anaconda, MT that has been used in oral bioavailability studies.[19–21]

Study Design

The study design is summarized in Table 1. Experiments were performed using weathered samples of all four soils and unweathered samples of the MTSS and Yolo soils. All soils were applied to skin from the same three donors. The soil load of ~30 mg/cm^2 was sufficient to cover the skin with multiple layers of particles. In the acetone-delivered experiments, ~80 ng/cm^2 of ^{14}C-BaP, a load similar to the mass applied in soils at the 3 mg/kg concentration, was deposited onto the skin surface in 50 μl of acetone. The experiments with BaP in weathered soil and acetone were randomized within two subsets (trials lasting 24 h and those lasting 4 or 8 h) that were performed in alternating weeks. The testing of unweathered soils was completed in a single experimental run performed 2 days after the soil was prepared.

Soil Preparation

Test soil concentrations were chosen within the constraints of adequate detection of BaP (i.e., the specific activity of the available radiolabel), which limited the lower soil concentration to 3 mg/kg, and the estimated capacity of the test soils to sorb BaP. The higher concentration was limited to 10 mg/kg to reduce the likelihood of soil saturation. The selected levels are similar to those found in urban and nonindustrial soils in the United States; several studies characterizing PAHs in soils have reported BaP concentrations as high as 17 mg/kg with arithmetic means primarily in the 0.5 to 1.3 mg/kg range.[22–27]

Soil concentrations of 3 and 10 mg/kg of BaP on 1.5–2 g of soil were achieved by adding 9 and 30 μl, respectively, of stock solution (325 μg/ml) per gram of the soils that were then subjected to weathering, and about 30 and 100 μl/g of stock solution at a concentration of 100 μg/ml to the soils tested unweathered. After spiking, the vials were placed onto a Labquake rotator (Barnstead Thermolyne, Dubuque, IA, USA) for 1 h, uncapped and placed in fume hood for 0.5 or 1 h, for low and high concentrations, respectively, to allow for volatilization of toluene vehicle, and then recapped and placed back on the rotator for a total of 72 h of mixing to ensure sufficient homogenization of the radiolabeled BaP in the soils.

Artificial weathering of soils was conducted by adding 0.5 ml of deionized (DI) water (ACS reagent grade, Ricca Chemical Co, Arlington, TX, USA) per gram of weathered soil once a week for 8 weeks to vials containing the eight test soils. The vials were then capped tightly for 3 days, after which the caps were removed and the samples were air dried for 2 days, followed by capping and mixing on the rotator for 2 more days. Experiments began 3 weeks after weathering was completed and were performed over a period of 14 weeks. During this time, soils were stored at 3 °C to limit any microbial activity. To test for homogenization and sample stability, 5 mg aliquots of each test soil were taken in triplicate during each experimental run and analyzed. Measured soil concentrations compared across the experimental period were not significantly different (see Supporting Information, Supplementary Figure S1).

Q14

Table 1. Experimental matrix and carbon content of sub-63 μm fraction of the test soils

Vehicle	*Soils studied*	*Nominal C mg/kg*	*Duration h*	*Replicates*[a]
Weathered soil	CSU, ISU, MTSS, Yolo	3, 10	8, 24	2
Unweathered soil	MTSS, Yolo	3, 10	24	1
Acetone	n/a	n/a	4, 8, 24	2
Soil	*CSU*	*ISU*	*MTSS*	*Yolo*
TOC[b] (%)	0.99	3.1	3.9	0.97
BC[b] (%)	0.14	0.23	1.2	0.09

[a]All studies were performed on the same three donors. [b]Determinations of total organic carbon (TOC) and black carbon (BC) are from Ghosh (University of Maryland, Baltimore County).

Skin Source and Preparation

Frozen split-thickness human cadaver abdominal skin (~400 μm thick) acquired from the abdomen within 24 h postmortem from three subjects (Caucasian; two females and one male, ages 59, 79, and 48 years, respectively) was purchased from the National Disease Research Interchange (NDRI, Philadelphia, PA) and stored at −20 °C until used. For highly lipophilic chemicals like BaP, the dermis can present a significant additional barrier to dermal absorption that is not present *in vivo*, because the dermis is vascularized.[28] Therefore, dermal absorption was measured through only the epidermis, which was prepared by placing skin samples, cut into usable sections while still partially frozen, into water at 60 °C for 1 min.[29] The epidermis was peeled carefully from the dermis and placed in DI water until it was positioned on the diffusion cells.

Diffusion Cell Experiments

Dermal absorption was measured using vertical flow-through Teflon diffusion cells (9 mm, Series 1, in-line) from PermeGear (Bethlehem, PA, USA), with a diffusion area of 0.64 cm^2 and a receptor volume of ~0.25 ml. The receptor fluid was 10 mM phosphate-buffered saline (PBS; 0.138 M NaCl; 0.0027 M KCl) with 4% bovine serum albumin (BSA) added to increase BaP solubility after degassing by vacuum filtration (0.45 μm pore size cellulose acetate membrane, Corning, Tewksbury, MA, USA) to prevent bubbles in the system.

Twelve cells were used per experimental run. The epidermal membranes were mounted between the receptor chamber and donor chamber with the stratum corneum facing up. The 12 diffusion cells used in each experiment and sample collection system were housed within an environmental chamber in which temperature and relative humidity were controlled at 32 °C and 40%, respectively. Once loaded with skin, the diffusion cells were equilibrated for ~12 h, with the receptor fluid flow rate delivered to each cell set to 0.6 ml/h. After equilibration, 20 mg of the test soils or 50 μl of the acetone-BaP solution were applied to the skin surface. Addition of sweat simulant to skin before addition of labeled soil or acetone was deliberately avoided as unrepresentative of chronic exposure. BaP is a carcinogen, and chronic rather than acute toxicity is the concern.

The receptor fluid flow rate to each cell from the multichannel Ismatec peristaltic pump (IDEX Health & Science, Oak Harbor, WA, USA) was set to 1.5 ml/h and collected into borosilicate scintillation vials (VWR, Radnor, PA, USA). The actual volume of receptor solution delivered to each cell was determined gravimetrically. The receptor fluid solution was collected at specified intervals throughout the 8 or 24 h exposure period (either 2, 4, and 8 h or 3, 6, 12, and 24 h) and then mixed with 12 ml of scintillation cocktail.

Table 2. Summary results of BaP absorption into and through skin from soil or deposited onto the skin from acetone[a]

# of soils	n	Weathered? Y/N	t_{exp} h	Nominal C mg/kg	Measured C mg/kg	BaP load ng/cm^2	Mass balance %	J_{in} ng/cm^2-h	J_{out} ng/cm^2-h	M_{sk}/A ng/cm^2	M_{rf}/A ng/cm^2
4	23	Y	8	3	2.7 (0.07)	82.7 (1.6)	103 (3.2)	0.083 (0.016)	0.013 (0.002)	0.56 (0.13)	0.11 (0.014)
4	24	Y	24	3	2.8 (0.05)	84.1 (1.6)	99.2 (2.6)	0.033 (0.008)	0.012 (0.001)	0.50 (0.17)	0.28 (0.025)
4	24	Y	8	10	8.8 (0.21)	271 (5.1)	103 (2.8)	0.21 (0.062)	0.012 (0.001)	1.6 (0.49)	0.10 (0.012)
4	23	Y	24	10	9.1 (0.17)	279 (5.0)	98.7 (2.4)	0.075 (0.014)	0.012 (0.001)	1.5 (0.32)	0.28 (0.027)
2[b,c]	12	Y	24	3	2.9 (0.05)	87.7 (1.7)	98.6 (4.3)	0.037 (0.015)	0.012 (0.001)	0.60 (0.35)	0.29 (0.032)
2[b,c]	12	Y	24	10	9.4 (0.16)	293 (5.9)	98.1 (1.7)	0.078 (0.023)	0.012 (0.002)	1.6 (0.54)	0.30 (0.046)
2[b]	6	N	24	3	3.4 (0.12)	104 (4.4)	89.7 (3.0)	0.052 (0.017)	0.014 (0.002)	0.90 (0.42)	0.34 (0.051)
2[b]	6	N	24	10	12.9 (1.0)	396 (24)	89.2 (2.0)	0.16 (0.062)	0.014 (0.002)	3.6 (1.5)	0.33 (0.052)
n/a[d]	5	—	4	—	—	83.2 (5.4)	78.0 (19)	6.9 (2.8)	0.012 (0.008)	27.4 (11.3)	0.048 (0.034)
n/a[d]	5	—	8	—	—	80.4 (5.2)	83.7 (20)	3.3 (1.1)	0.019 (0.02)	26.1 (8.4)	0.15 (0.16)
n/a[d]	6	—	24	—	—	76.7 (4.0)	77.2 (11)	1.2 (0.24)	0.11 (0.11)	26.1 (4.4)	2.7 (2.6)

[a]Experimental results presented as mean (maximum error of the mean at 95% confidence level); n = total number of experimental measurements; C, soil concentration; t_{exp}, time of exposure; J_{in}, average flux over exposure period into skin; J_{out}, average flux over exposure period through skin and into receptor fluid; M_{sk}/A, mass of BaP per skin surface area recovered from washed skin; and M_{rf}/A, mass of BaP per skin surface area recovered from receptor fluid. [b]MTSS and Yolo soils only. [c]Data also included in results of weathered trials with all four soils; presented for comparison with unweathered soil experiments. [d]BaP delivered to skin surface via acetone-vehicle.

At the end of each trial, 150 μl of DI water was pipetted into the donor chamber, and the skin surface was wiped with two dry cotton applicator tips (Puritan Medical, Guilford, ME, USA) to collect the moistened soil or the acetone-deposited BaP. The tips were clipped into scintillation vials ($\leq$2 per vial) and extracted with cocktail. This wetting/wiping cycle was repeated twice per cell. The skin sample was then carefully removed from the receptor chamber with tweezers and rinsed by swirling the sample in DI water as a final step. The donor chamber was rinsed with Hionic-Fluor scintillation cocktail (Perkin Elmer, Waltham, MA, USA), and the receptor chamber was wiped with a DI water-soaked cotton tip. All materials used in quantitating and loading the soils into the diffusion cells were rinsed with scintillation cocktail. Skin samples were solubilized in 2 ml of Soluene 350 (Perkin Elmer, Waltham, MA, USA) with sonication (Branson Ultrasonics, Danbury, CT, USA) for 2 h at 65 °C and mixed with 10 ml of scintillation cocktail. Hionic-Fluor was used in all samples and rinses, except the receptor fluid and aqueous skin rinse solutions, which utilized Ultima Gold XR (also from Perkin Elmer).

Radiolabel Counting

Radioactivity of each sample vial was counted in a Beckman LS 6000SC liquid scintillation analyzer (Beckman Coulter, Brea, CA, USA) five times over the course of 11 weeks. Except for the vials containing high amounts of soils (i.e., skin-washing materials and run-specific soil aliquots), counts were stable over time and averages of the five counts were used after adjusting for background from cocktail blanks (29.5 ± 0.46 disintegrations per minute (d.p.m.), mean ± 95% confidence interval, corresponding to a detection limit of ~0.13 ng BaP). Interferences from high solids content and slow partitioning to the scintillation cocktail were evident during counting of vials containing more than ~5 mg of soil. These issues were resolved by diluting 1-ml aliquots of well-mixed cocktail-soil solution with an additional 10 ml of cocktail and recounting.

Data Analysis

Radioactivity counts in d.p.m. were converted to BaP mass at 2.3×10^5 d.p.m./μg as derived from the molecular weight of BaP (252.3 μg/μmol) and specific activity of the ^{14}C-BaP (26.6 μCi/μmol). Mass balances were calculated for each experimental trial. Experiments reported here were deliberately conducted at high soil loads to avoid issues with uneven distribution of soil on the skin surface, rendering direct reporting of fractional absorption inappropriate.[14,30] The primary results normalized by skin surface area (A) were cumulative BaP mass in the receptor fluid (M_{rf}) and BaP mass recovered from the washed skin (including BaP that was in and/or on the washed skin) at the end of the experiment (M_{sk}), from which the average flux of BaP over the exposure duration into skin (J_{in}) and through skin and into the receptor fluid (J_{out}) were calculated. For risk assessment purposes, J_{in}, which includes both BaP found in and/or on skin after washing and BaP collected in the receptor fluid over the exposure period, is most relevant.

Statistical analyses were completed using Stata 12 (StataCorp, College Station, TX, USA). One-way ANOVA and two-sample t-tests were employed to assess differences. In cases in which equal variance could not be assumed, a Kruskal–Wallis test (considered a non-parametric analog to the one-way ANOVA) or an unequal variance t-test was also condiucted. In all cases the result, whether a finding of significant difference or lack thereof, was consistent with the prior ANOVA (data not shown). Dixon's Q test was used to identify outliers at the 99% confidence level in BaP determinations of the receptor fluid and solubilized skin. Results are reported as mean with corresponding 95% confidence intervals (shown as error bars in figures, and as maximum errors on the mean in text and tables) calculated for all measurements.

RESULTS

The study results are summarized in Table 2 and in the Supporting Information (Supplementary Tables S1 and S2). A total of 126 trials were attempted. Of these, four were excluded from the final analysis. Results (unusually high levels of BaP in the receptor fluid) from two weathered soil trials failed the Dixon's Q test at the 99% level, reflecting probable membrane failures. Two acetone-vehicle trials were excluded due to total radioactivity recoveries $<$ 50%, (one each in the 4 and 8 h experiments). These poor recoveries may have been attributable to inadequate collection of residues from the donor chamber, which were typically higher in the acetone trials than in the soil trials (see Supplementary Table S1). Exclusion of these two acetone trials had no significant effect on measured absorption metrics. The average total radioactivity recovered was 101% (83–117% range) for weathered-soil, 89% (85–93%) for unweathered soil and 80% (61–98%) for acetone. Lower recovery from acetone deposition than from soil application experiments is consistent with prior results reported by Wester et al.[3]

Weathered Soils

Results for M_{rf}/A, J_{out} and M_{sk}/A are presented in Figure 1. No significant differences were seen among the four soil types for any of the end points measured. Accordingly, results for J_{in}, J_{out}, M_{rf}/A, and M_{sk}/A presented in Table 2 are averages across the test soils. Data for individual soil types are presented in Supplementary Tables S1 and S2. No significant differences in M_{rf}/A were seen between the 3 and 10 mg/kg trials of the same duration (8 h: $P = 0.42$; 24 h: $P = 0.79$). At 24 h, M_{rf}/A was greater than at 8 h by

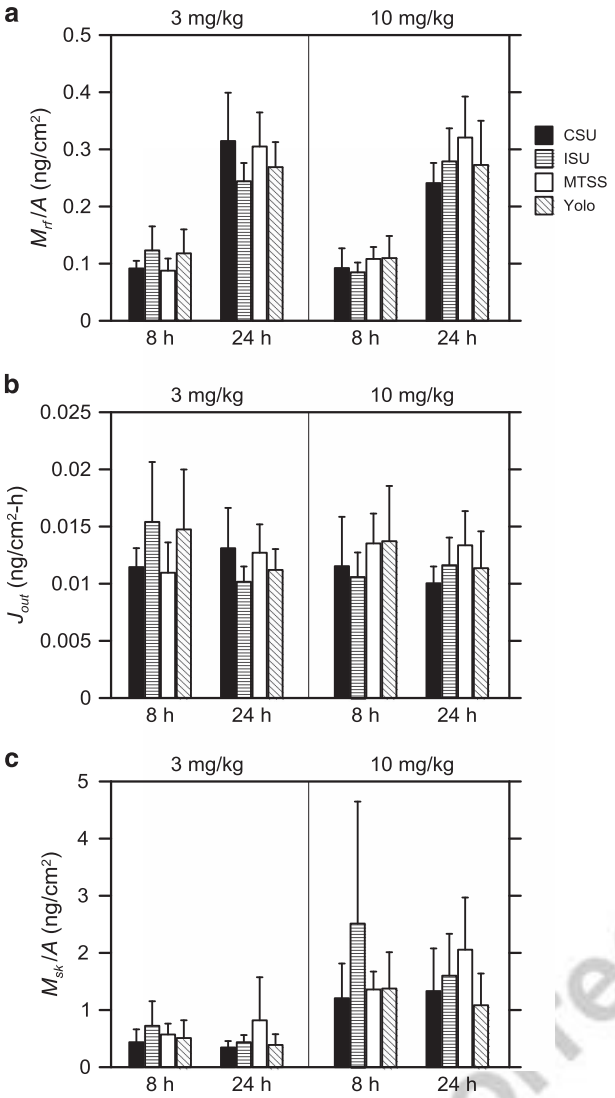

Figure 1. Results for four weathered soils at 3 and 10 mg/kg BaP concentration after 8 and 24 h exposures: (**a**) M_{rf}/A; (**b**) J_{out}; and (**c**) M_{sk}/A. Error bars represent 95% confidence intervals. BaP, ^{14}C-benzo [a]pyrene.

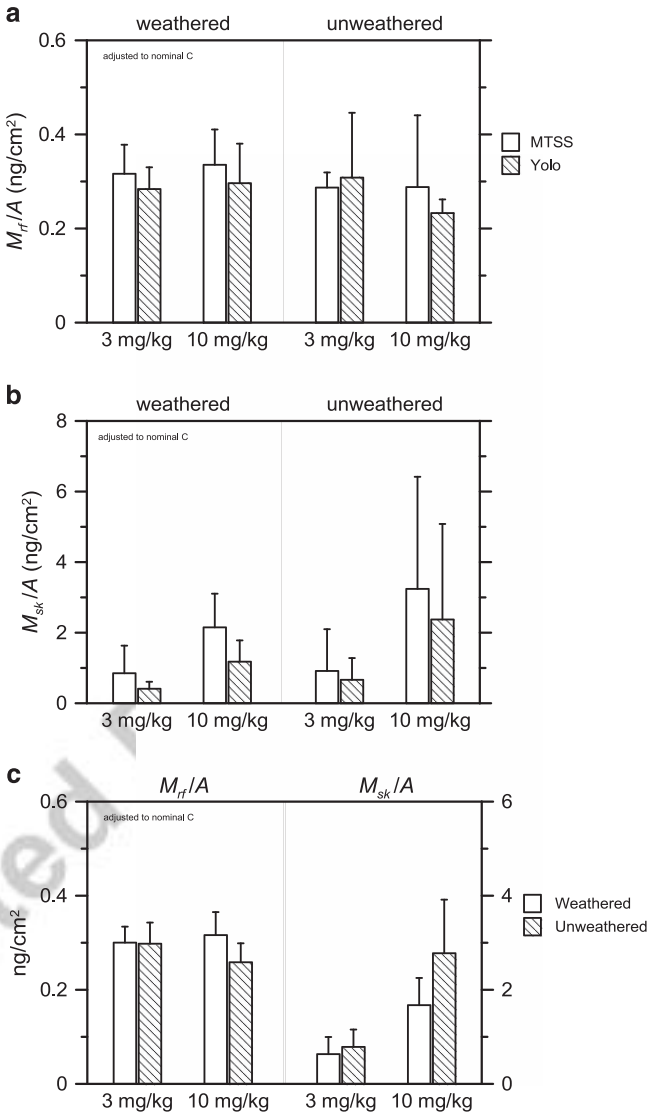

Figure 2. Results for weathered and unweathered Yolo and MTSS soils adjusted to the nominal BaP concentrations of 3 and 10 mg/kg after a 24 h exposure: (**a**) M_{rf}/A for each soil; (**b**) M_{sk}/A for each soil; and (**c**) average of Yolo and MTSS soils combined for M_{rf}/A (left axis) and M_{sk}/A (right axis); $n = 3$ and 6 for each unweathered and weathered soil, respectively. Error bars represent 95% confidence intervals.

an amount that was proportional to the exposure duration (Figure 1a). As a result, J_{out} was approximately constant across both exposure duration and soil concentration (Figure 1b). Within each soil concentration examined, no difference was seen in M_{sk}/A between 8 and 24 h (3 mg/kg: $P = 0.55$; 10 mg/kg: $P = 0.73$). Values of M_{sk}/A in the 3 and 10 mg/kg trials were statistically different ($P \leq 0.0001$) by an amount that was approximately proportional to concentration.

Unweathered Soils

Results from trials with unweathered MTSS and Yolo soils are compared in Figure 2 with results from the same two soils after weathering. As with the weathered soils, no significant differences were seen between the two unweathered soils for any of the end points measured. After adjusting for the differences in the actual compared with nominal soil concentrations (multiplying by ratio of nominal to actual concentration), differences between the Yolo-MTSS average weathered and unweathered soils were not statistically significantly different for M_{rf}/A at either soil concentration or for M_{sk}/A in the 3 mg/kg trials ($P > 0.13$). For soils at 10 mg/kg of BaP, M_{sk}/A was larger from the unweathered soils by a statistically significant difference (2.8 ± 1.2 vs 1.7 ± 0.9 ng/cm^2, $P = 0.03$). Driven by this greater recovery of BaP from skin, J_{in} was larger from unweathered soils in the 10 mg/kg trials after adjusting for actual concentration (0.13 ± 0.05 vs. 0.08 ± 0.04 ng/cm^2/h, $P = 0.047$).

Acetone Compared with Soils

Distribution of radioactivity observed in the acetone-delivered trials differed from the soil experiments: less mass was recovered in the skin surface wash, while a relatively larger mass was collected from the donor chamber (see Supplementary Table S1 for details). Compared with the weathered and unweathered soil experiments at similar BaP load (3 mg/kg concentration), M_{sk}/A for BaP delivered in acetone was between one and two orders of magnitude greater (Table 2; $P < 0.0001$) and did not vary with the length of the exposure for exposure times as short as 4 h. The appearance of BaP in the receptor

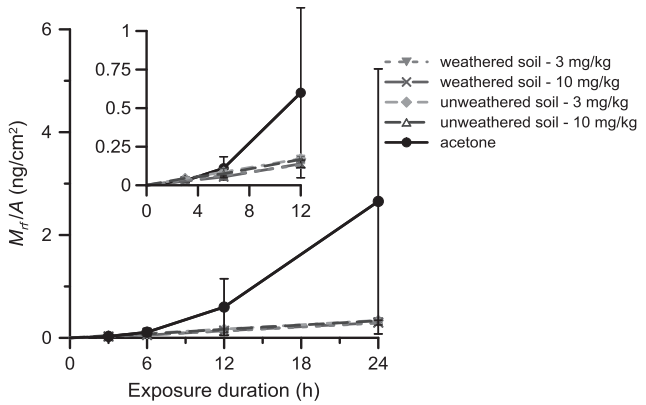

Figure 3. M_{rf}/A from BaP deposited in acetone and from BaP in the weathered and unweathered MTSS and Yolo soils at 3 and 10 mg/kg BaP concentrations. Lines connecting the results are drawn to guide the eye. Inset is an enlargement of the data to 12 h. Error bars represent 95% confidence intervals.

fluid was similar from soil or acetone up to the 6 h measurement (Figure 3). After 24 h, M_{rf}/A was greater from the acetone-delivery experiments by approximately one order of magnitude compared with soil experiments at both concentrations. For BaP delivered in acetone, J_{out} increased with time and was significantly greater than J_{out} in the soil experiments at 24 h (Tables 2, $P = 0.002$), which remained nearly constant for exposure periods of 6 h or greater.

DISCUSSION

Results from this study are discussed and then compared with prior studies and related to risk assessments.

Q6 Observations of this Study

Six significant outcomes have been identified, which are described individually and then considered together.

Absence of an effect of soil concentration on transfer to receptor fluid. Chemical penetration through skin is driven by thermodynamic activity, which, for a given vehicle, usually varies with concentration. The observation that M_{rf}/A did not vary with BaP concentration in either the weathered (Figure 1) or unweathered (Figure 2) soil experiments was therefore unexpected. A plausible explanation for this finding is that the soil saturation limit (within the constraints of the weathering protocol) for BaP was less than 3 mg/kg for all test soils, which caused the thermodynamic activity of BaP to be independent of soil concentration and soil type. Absence of concentration dependence of dermal absorption at concentrations above the experimentally determined soil saturation limit has been observed in experiments with methyl paraben on the 38–63 μm fraction of the same ISU and CSU soils tested in this study.[31]

To evaluate this possibility, the soil saturation limit ($C_{soil,sat}$) was estimated using equation (1), which has been proposed as suitable for non-ionizable lipophilic chemicals:[14]

$$C_{soil,sat} = TOC \times K_{oc} \times C_{w,sat} \quad (1)$$

In this equation $C_{w,sat}$ is the chemical saturation limit in water, and K_{oc} is the organic carbon water partition coefficient. For BaP, experimental values for $C_{w,sat}$ are reported to be 0.0016 mg/l [ref. 32] and 0.0038 mg/l,[33] and logK_{oc} (for K_{oc} in units of L/kg) is estimated to be 5.3–5.8.[34] Combining these numbers into equation (1) with the 1–4% TOC values of the test soils in this study, $C_{soil,sat}$ for BaP is estimated as 3–96 mg/kg, which is not too different from the soil concentrations in this study. Given the considerable uncertainty in the estimate of K_{oc} for BaP[35,36] and in the suitability of equation (1) for calculating $C_{soil,sat}$, soil saturation is a plausible explanation of the observed results, especially as it is consistent with other observations described below.

Absence of an effect of soil characteristics on uptake of BaP. These experiments were conducted using soils with a fourfold range of TOC and 13-fold range of BC with the expectation that these characteristics would affect the sorbent capacity for BaP and hence, the thermodynamic activity and driving force for transfer from soil to and through the skin. No consistent effects of TOC or BC were observed for either the weathered or unweathered soils for any of the end points measured (Figures 1 and 2). This is consistent with the hypothesis that even the soils with the greatest expected sorbent capacity were effectively saturated with BaP. Had the concentrations been sub-saturated, differences among soils might have been observed. A further consideration is that all soils were pre-sieved to the sub-63 μm fraction. An alternative hypothesis is that sorption on the increased surface areas associated with fine particles might have diminished the influence of carbon content.[37]

Proportionality of mass in washed skin to soil concentration. In both weathered and unweathered soils, M_{sk}/A varied directly with BaP concentration in the applied soil (Figures 1c and 2b). This observation could be explained by concentration-dependent transfer into the skin from soil, or by the amount of BaP found in the skin being primarily attributable to residual soil that was not removed by washing. The former explanation is not consistent with the observation that M_{rf}/A was not affected by differences in soil concentration or type as described above. The latter explanation is further supported by a lack of time dependence of skin residues, which is necessary but not sufficient evidence (see next paragraph). If post-wash skin residues are attributable primarily to unrecovered soil, the amount of soil that would have to remain on the skin to yield these results would be on the order of 0.1 mg/cm^2, which is certainly plausible given initial soil loads of ~30 mg/cm^2 and an estimated monolayer load of about 1 mg/cm^2.[17]

Absence of time dependence on BaP in skin residues following soil exposure. If M_{sk}/A is dominated by unremoved soil, relevant processes should be relatively rapid in an *in vitro* system, that is, particles that cannot be cleaned from the skin surface probably adhere soon after the soil is applied. Therefore, M_{sk}/A should be insensitive to experimental duration. This is consistent with the observation of no difference in the 8 and 24 h trials (Figure 1c and Table 2), although a more rigorous test would have been measurement M_{sk}/A after less than an hour of exposure.

Absence of a clear effect of weathering. No statistically significant difference was observed in M_{rf}/A from the weathered and unweathered soils (Figure 2a). This can be explained by saturation of the soils, which could have caused some readily available BaP to remain at the surface of each soil after weathering. Alternatively, the artificial weathering process applied here may have been insufficiently rigorous or carried out over too little time to see an effect. In a previous study of BaP on aged (but not weathered) soils, Roy and Singh[9] observed no effect after 45 days and only a twofold effect on M_{rf}/A after 110 days. However, bioaccessibility studies (not involving skin) have shown that soil wetting/drying cycles increase sequestration and decrease extractability of PAHs[38–41] and several pesticides.[42,43] Weathering did appear to effect M_{sk}/A at the 10 mg/kg concentration (Figure 2b and c). This could occur if more neat BaP is held less tightly on the outer surfaces of a saturated soil before weathering compared

Q15

Table 3. Average BaP flux into and through skin over 24 h from Yolo soil and deposited onto the skin with acetone measured in this study compared with results from Wester et al.[3]

Study/vehicle	*Type*	*Species*	*n*	*Soil Weathered?*	t_{exp} *h*	*Nominal Soil C mg/kg*	*Nominal BaP load ng/cm²*	J_{in} *ng/cm² h*	J_{out} *ng/cm² h*[a]
Soil									
Wester et al.[3]	*In vivo*	Monkey	4	N	24	10	400	2.2 (0.89)	n/a[b]
	In vitro	Human	6	N	24	10	400	0.24 (0.18)	0.0019 (0.0007)
This study	*In vitro*	Human	6	Y	24	10	300	0.057 (0.026)	0.011 (0.003)
	In vitro	Human	3	N	24	10	300	0.15 (0.16)	0.013 (0.002)
Acetone									
Wester et al.[3]	*In vivo*	Monkey	4	—	24	—	500	11 (7.3)	n/a[b]
	In vitro	Human	6	—	24	—	500	5.0 (2.1)	0.019 (0.013)
This study	*In vitro*	Human	6	—	24	—	80	1.2 (0.25)	0.11 (0.11)

[a]Experimental results presented as mean (maximum error of the mean at 95% confidence level); n = total number of experimental measurements; C = concentration; t_{exp} = time of exposure; J_{in} = average flux over exposure period into skin; and J_{out} = average flux over exposure period through skin and into receptor fluid. [b]Not available; experimental protocol prevented determination of this number.

with after weathering. In this scenario, transfer of loosely held particles of neat BaP could increase the total amount of BaP in skin above that from soil particles left after washing. This effect would be expected to be greater for the soils contaminated with more BaP, which could explain the observation that M_{sk}/A was statistically significantly different in 10 mg/kg trials but not 3 mg/kg trials.

Vehicle-dependent time course of BaP penetration to receptor fluid. In 24 h experiments, the cumulative mass of BaP in the receptor fluid increased by a significantly larger rate from acetone than from soil beyond about 6 h (Figure 3) leading to a nearly 10-fold larger J_{out} (Table 2). This likely reflects differences in the amount of BaP that directly contacts skin in the soil *versus* acetone experiments. All BaP delivered by acetone would be at the skin surface, whereas a large fraction of the BaP applied in multiple layers of soil would be some distance from the skin surface. The observation that the rate of BaP transfer into the receptor from soil remained approximately constant up to 24 h (Figure 3) suggests that BaP transfer from the soil to the skin was not rate limiting and that BaP at the skin–soil interface was not depleted during an initial 24 h exposure.

Summarizing. Taken together the significant observations of this study suggest that BaP contamination levels, although low, may have exceeded the sorbent capacity of the soils used in this study and that the quantity of BaP measured in the skin was primarily attributable to residual soil not removed by the washing step. Although alternative hypotheses can explain individual observations (e.g., mass of BaP in the skin is proportional to the soil concentrations below saturation), we were unable to identify other explanations that were consistent with all six observations. For example, BaP soil concentrations less than saturation can explain the observed concentration dependence of the mass of BaP in the skin but not the absence of a concentration effect in the mass of BaP in the receptor fluid.

Soil saturation can be assessed experimentally. If vapor pressure of a soil contaminant is adequate, then thermodynamic activity can be assessed by measuring contaminant concentration in the head space in equilibrium with the contaminated soil compared with the pure contaminant (similar to the assessment by of 4-chloro-3-methylphenol in liquid solutions[44]). For less volatile compounds such as BaP, contaminant uptake into a sorbent material from soil compared with the neat contaminant or differential scanning calorimetry can be used.[37] Because exceeding the soil sorption capacity was not anticipated, the scope of the present study did not include such measurements. It would be useful to include soil saturation measurements in future studies of contaminated soils.

Comparison with Prior Results

Prior investigations of dermal absorption of BaP from soil have been reviewed elsewhere.[14,15] The US Environmental Protection Agency (USEPA) currently recommends that risk assessments assume the dermal absorption of PAHs from soils to be 13% of the total applied dose without consideration of differences in soils, exposure period, soil load or which PAH species.[45] This guidance relies on a subset of experiments reported by Wester et al.[3] that were conducted *in vivo* using rhesus monkeys and BaP. The experiments by Wester et al. were similar to experiments reported in this study with some important differences. Wester et al. used one of the four soils used here (Yolo) but sieved to 180–300 μm rather than to sub-63 μm. At this particle size fraction, their nominal soil load of 40 mg/cm^2, although larger than in this study, is estimated to cover the skin with only a single layer of particles.[12,14] The initial BaP concentration of 10 mg/kg in Wester et al. matched the higher concentration in this study. Wester et al. also conducted *in vitro* experiments with human skin, and applied BaP in acetone as well as soil. Soil and acetone results from Wester et al., which were all 24 h exposures, are compared with this study in Table 3 in terms of J_{in} and J_{out}.

Wester et al. estimated absorption in the rhesus monkeys by dividing the amount of radiotracer collected in excreta over 7 days by 6.6%, which was the fraction collected over 7 days following intravenous administration. The result should represent BaP that penetrated through the skin in 24 h, plus residual BaP in the skin after washing at the end of the 24 h exposure period and subsequently subject to systemic uptake. This corresponds to J_{in} equal to 2.2 ng/cm^2 h, or a cumulative uptake into and penetration through the skin of 52.8 ng/cm^2. The study's *in vitro* results using human skin were roughly one order of magnitude lower. Results obtained *in vitro* here are similar to the Wester et al. *in vitro* results with respect to J_{in} (i.e., substantially lower than the Wester et al. *in vivo* rhesus monkey results). Higher J_{out} in the current study compared with the *in vitro* experiments from Wester et al. for both soil and acetone experiments is consistent with the different skin sample preparations used in the two studies (i.e., heat-separated *versus* dermatomed skin). The hydrophilic dermis layer in dermatomed skin has been shown to present an additional barrier to highly lipophilic chemicals like BaP that is

missing entirely in heat-separated skin.[28] Use of heat-separated skin may therefore be more appropriate than use of split thickness skin in *in vitro* trials involving lipophilic agents.

Questions have been raised previously regarding the Wester et al. *in vivo* trials.[14] Dermal soil experiments conducted *in vivo* in surrogate animals are limited by inherent difficulties in controlling animal behaviors. Soil was applied to the monkeys in the Wester et al. trials while they were lightly anesthetized and lying on their backs.[15] They were then positioned upright in metabolic chairs with restraints. Given the large particle sizes used (180–300 μm; fine to coarse sand) and the volume of the eye-guard used to cover the application site,[3] it is unclear that soil-skin contact could reasonably be expected to be maintained for 24 h. However, if the soil was supersaturated and/or if the solvent used to deliver the BaP to the soil had not completely evaporated, then the initial transfer from soil surfaces or solvent could have left sufficient chemical on the skin surface to cause measurable absorption even if longer term soil contact did not occur. Also, mass balances were not reported making it impossible to assess uncertainties in the estimated absorbed dose, which is sensitive to the intravenous correction factor (1/0.066 = 15.2) by which the mass of BaP collected in urine was multiplied. This correction is based on unproven assumptions that the disposition, metabolism and elimination pathways are identical for dermal and intravenous routes of dose administration.

In vivo and *in vitro* J_{in} values observed by Wester et al. subsequent to acetone-delivery of BaP are less disparate than the corresponding *in vivo/vitro* soil results, suggesting that much of the *in vivo/vitro* soil differential is attributable to something other than interspecies skin differences. Acetone deposition experiments reported here were conducted at lower initial chemical load (80 ng/cm^2 compared with 500 ng/cm^2 in Wester et al.), which, as expected due to lower surface coverage, produced a proportionally lower J_{in}. When multiplied by the ratio of the chemical load in the two studies (i.e., 500/80), the extrapolation of J_{in} from this study to the chemical load used by Wester et al. study is 7.5 ng/cm^2 h, which is between the *in vivo* and *in vitro* J_{in} (11 and 5, respectively) from Wester et al. Average J_{in} from acetone reported here is similar to the Wester et al. *in vivo* soil result, which further suggests that the latter may represent transfer from residual solvent rather than from soil.

In a small *in vitro* study ($n = 3$), Wester et al. observed smaller amounts of BaP in skin exposed to soil for 25 min compared with 24 h, suggesting that residual soil left by washing was not significant. This is not surprising given that particles smaller than 180 μm had been removed by sieving. Skin-washing effectiveness is likely to be better for a soil with particles >180 μm than for soils in this study containing a significant fraction of particles <25 μm.

Application to Risk Assessment

BaP is the index chemical for PAH-risk assessment. In addition, in 2013, USEPA proposed, for the first time, a dermal carcinogenic slope factor for BaP.[46] That value is undergoing further review, but could potentially increase the importance of dermal exposures in evaluation of risks from contaminated sites. A careful review of the cancer bioassay studies upon which the proposed dermal slope factor was derived suggests that estimates of cancer risk should be based on absorbed rather than exposed dose, making the results of the experiments reported here directly relevant to the assessment of cancer risks from exposure to PAHs. The presumed primacy of the Wester et al.[3] *in vivo* soil experiment results is called into question by their similarity to *in vitro* acetone deposition results reported here. Evidence is also presented for rapid adherence of a portion of soil-borne contaminant. Some of that rapidly adhering mass may be on fine particles that are not easily removed. A health-protective assumption would be that non-removable particle-bound material is functionally equivalent to the same mass of neat compound in the outer layers of skin. Results presented here also highlight the importance of soil concentration relative to sorption capacity. Soils may be weak sorbents, with low mg/kg levels of BaP representing soil saturation. BaP is routinely found in soils at or above concentrations used in this study,[27] suggesting soils in the environment might exist at supersaturated conditions with obvious implications for transfer to skin. However, the apparent saturation limit of soils might be influenced by both duration of weathering and source of the BaP. Sorption capacity might increase if soil is amended with partitioning phases in the form of soot or other carbonaceous material. Soils in experiments reported here were spiked with pure chemical using a volatile solvent, a procedure that might have contributed to saturation exceedance. Nevertheless, results reported here do suggest that uptake from saturated soil is slower than uptake from a similar amount of BaP deposited from acetone.

CONFLICT OF INTEREST

Funding for this research was provided by the Strategic Environmental Research and Development Program (SERDP). Y.W.L. works and M.V.R. worked for scientific consulting firms specializing in risk assessment services to private and public sector clients. With SERDP's knowledge, they coordinate an industry-agency advisory group that receives updates on the project work, but does not control the direction of the research effort. The other authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Journal of Exposure Science and Environmental Epidemiology website (http://www.nature.com/jes)

SUPPLEMENTARY INFORMATION

Dermal absorption of benzo[a]pyrene into human skin from soil: Effect of artificial weathering, concentration and exposure duration

Trevor K. Peckham[1], Jeffry H. Shirai[1], Annette L. Bunge[2], Yvette W. Lowney[3], Michael V. Ruby[4], John C. Kissel[1*]

[1] Department of Environmental and Occupational Health Services, University of Washington, Seattle, WA 98195, USA

[2] Chemical and Biological Engineering Department, Colorado School of Mines, Golden, CO 80401, USA

[3]Exponent, Boulder, CO 80302, USA

[4]Integral Consulting, Louisville, CO 80027, USA (Deceased)

*Correspondence: University of Washington School of Public Health, Department of Environmental and Occupational Health Sciences, Box 354695, 4225 Roosevelt Way NE, Seattle, WA 98195, United States. Tel: (206) 543-5111; Fax: (206) 543-8123; E-mail: jkissel@uw.edu.

File contents:

Yolo soil (additional information). The Yolo soil, collected from the University of California (UC), Davis, student farm[1] was provided by William Reifenrath (Stratacor, Richmond, CA), who acquired it from the UC Davis soils lab. This was the source of the Yolo County soil used by Wester et al.[2] (personal communication, R. Wester, 1994), although there are differences in the reported percentages of clay and sand (classified by size): 26% sand and clay reported by Wester et al., and 34% sand and 20% clay reported by Reifenrath et al. It appears that the numbers in both studies are for the original samples and not the sieve fractions that were actually used in the studies.

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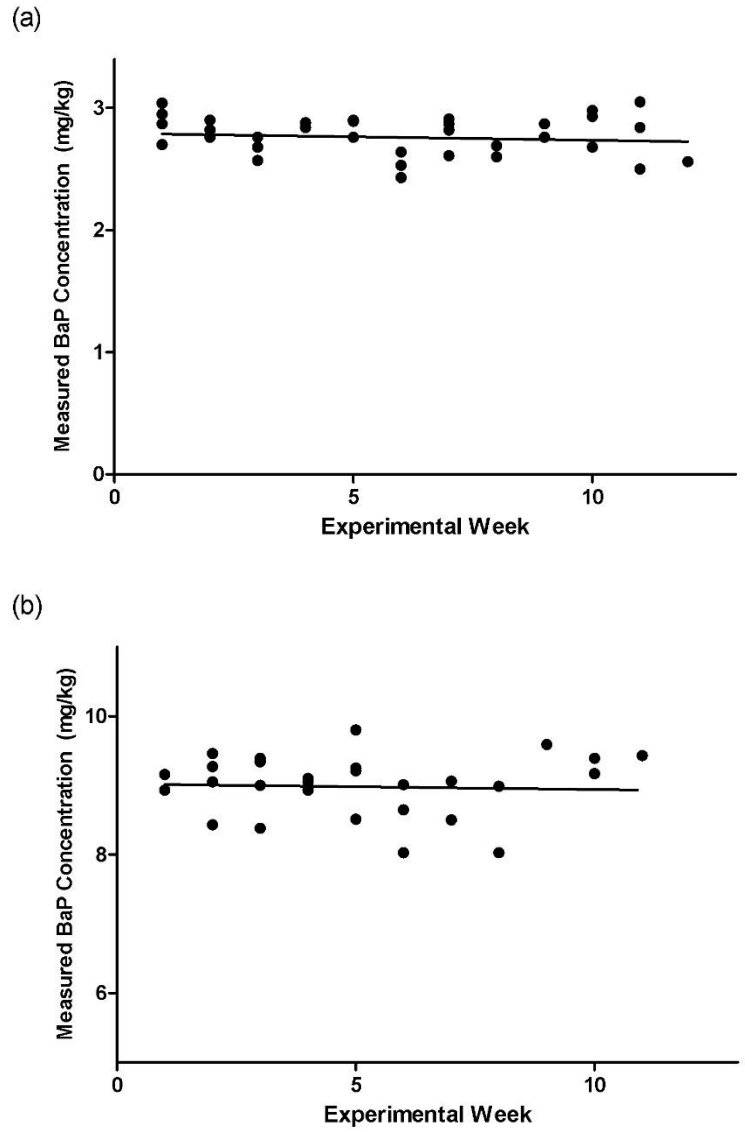


Figure S1. Average measured BaP soil concentrations from three 5-mg weathered soil aliquots taken each week across the experimental period and linear regression for (a) nominal 3 mg/kg soils (slope: -0.007; 95% Confidence Interval: -0.06 to 0.04) and (b) nominal 10 mg/kg soils (slope: -0.005; 95% Confidence Interval: -0.02 to 0.008).

Table S1. Average BaP mass recovered from each experimental compartment and mass balance for all study conditions, including by individual soil type[a]

Vehicle	Weathered?	t_{exp}	nominal		BaP mass loaded			mass recovered from: receptor fluid			washed skin			donor chamber rinse			skin washing[b]			cell wipe			% of applied dose recovered		
			C																						
	Y/N	hr	mg/kg	n	ng/cm^2	± SD	± E	ng	± SD	± E	ng	± SD	± E	ng	± SD	± E	ng	± SD	± E	ng	± SD	± E	%	± SD	± E
Weathered Trials																									
CSU	yes	8	3	6	83.0	2.1	2.2	0.058	0.008	0.008	0.28	0.14	0.14	0.23	0.24	0.25	53.3	1.5	1.5	0.28	0.64	0.68	103	6.2	6.5
ISU	yes	8	3	6	76.8	3.3	3.5	0.078	0.025	0.027	0.46	0.26	0.27	0.25	0.09	0.09	51.7	3.0	3.2	0.28	0.50	0.52	108	8.9	9.3
MTSS[c]	yes	8	3	5	85.0	3.7	4.7	0.056	0.011	0.013	0.36	0.10	0.12	0.36	0.43	0.53	53.2	3.8	4.7	1.04	2.27	2.82	102	5.8	7.2
Yolo	yes	8	3	6	86.1	2.7	2.8	0.075	0.025	0.027	0.32	0.19	0.20	0.20	0.09	0.09	54.4	1.7	1.8	0.062	0.08	0.082	101	7.4	7.8
			Group:	**23**	**82.7**	**3.7**	**1.6**	**0.067**	**0.021**	**0.009**	**0.36**	**0.19**	**0.08**	**0.26**	**0.23**	**0.10**	**53.1**	**2.6**	**1.1**	**0.42**	**1.11**	**0.48**	**103**	**7.4**	**3.2**
CSU	yes	24	3	6	82.5	3.7	3.9	0.20	0.051	0.054	0.22	0.07	0.07	0.28	0.17	0.17	50.5	2.8	2.9	0.185	0.25	0.26	98	6.1	6.4
ISU	yes	24	3	6	78.3	2.5	2.6	0.16	0.017	0.018	0.28	0.08	0.08	0.13	0.05	0.06	49.9	3.9	4.1	0.098	0.13	0.14	102	5.0	5.3
MTSS	yes	24	3	6	87.9	3.1	3.2	0.19	0.036	0.038	0.52	0.46	0.48	0.19	0.09	0.09	55.6	2.7	2.8	0.008	0.00	0.003	101	7.1	7.4
Yolo	yes	24	3	6	87.6	2.6	2.7	0.17	0.027	0.028	0.25	0.11	0.12	0.19	0.15	0.15	52.8	4.1	4.3	0.032	0.03	0.033	96	5.9	6.1
			Group:	**24**	**84.1**	**3.8**	**1.6**	**0.18**	**0.037**	**0.015**	**0.32**	**0.26**	**0.11**	**0.20**	**0.13**	**0.05**	**52.2**	**3.9**	**1.7**	**0.081**	**0.15**	**0.063**	**99**	**6.1**	**2.6**
CSU	yes	8	10	6	278	8.5	8.9	0.061	0.020	0.021	0.77	0.37	0.39	0.84	0.53	0.55	174	7.0	7.3	0.49	1.15	1.21	100	3.9	4.1
ISU	yes	8	10	6	243	2.4	2.5	0.054	0.010	0.011	1.6	1.3	1.4	1.3	1.3	1.4	168	8.1	8.5	1.2	1.8	1.9	112	3.4	3.6
MTSS	yes	8	10	6	277	5.9	6.2	0.069	0.013	0.013	0.87	0.19	0.20	0.64	0.52	0.54	179	3.3	3.5	0.40	0.95	0.99	103	3.7	3.9
Yolo	yes	8	10	6	285	5.1	5.3	0.072	0.021	0.022	0.88	0.39	0.40	0.38	0.21	0.22	175	7.1	7.5	0.084	0.15	0.16	97	4.8	5.0
			Group:	**24**	**271**	**12.0**	**5.1**	**0.064**	**0.017**	**0.007**	**1.0**	**0.74**	**0.31**	**0.79**	**0.78**	**0.33**	**174**	**7.3**	**3.1**	**0.55**	**1.2**	**0.50**	**103**	**6.7**	**2.8**
CSU	yes	24	10	6	275	3.8	4.0	0.16	0.013	0.013	0.85	0.45	0.47	0.71	0.50	0.52	171	11.8	12.4	0.28	0.65	0.68	99	4.8	5.0
ISU[c]	yes	24	10	5	257	2.9	3.5	0.18	0.030	0.037	1.0	0.38	0.47	0.57	0.30	0.37	161	14.8	18.3	0.19	0.31	0.38	100	10.9	13.5
MTSS	yes	24	10	6	295	11.7	12.2	0.20	0.044	0.046	1.3	0.55	0.58	0.78	0.40	0.42	180	12.2	12.8	0.51	1.22	1.28	97	3.2	3.3
Yolo	yes	24	10	6	291	7.0	7.4	0.17	0.047	0.049	0.69	0.34	0.35	0.56	0.42	0.44	181	9.7	10.2	0.025	0.03	0.030	99	2.0	2.0
			Group:	**23**	**279**	**11.6**	**5.0**	**0.18**	**0.038**	**0.016**	**0.97**	**0.47**	**0.20**	**0.65**	**0.40**	**0.17**	**173**	**13.8**	**6.0**	**0.25**	**0.70**	**0.30**	**99**	**5.6**	**2.4**
Unweathered Trials																									
MTSS	no	24	3	3	98.7	1.5	3.8	0.20	0.009	0.023	0.64	0.34	0.83	0.41	0.28	0.69	56.1	1.9	4.8	0.075	0.105	0.260	91	1.7	4.2
Yolo	no	24	3	3	110	1.8	4.5	0.23	0.042	0.103	0.50	0.19	0.46	0.42	0.09	0.21	60.4	2.0	5.1	0.050	0.057	0.14	88	3.0	7.5
			Group:	**6**	**104**	**4.2**	**4.4**	**0.22**	**0.031**	**0.033**	**0.57**	**0.26**	**0.27**	**0.41**	**0.18**	**0.19**	**58.2**	**3.0**	**3.1**	**0.062**	**0.077**	**0.080**	**90**	2.8	**3.0**
MTSS	no	24	10	3	366	11.3	28.1	0.22	0.047	0.116	2.5	0.98	2.42	0.68	0.21	0.52	200	6.0	14.8	4.1	7.0	17	89	2.4	5.9
Yolo	no	24	10	3	427	2.9	7.2	0.20	0.010	0.025	2.1	0.96	2.37	0.81	0.068	0.17	239	7.5	18.7	0.024	0.019	0.048	89	1.9	4.8
			Group:	**6**	**396**	**22.5**	**23.7**	**0.21**	**0.031**	**0.033**	**2.3**	**0.89**	**0.93**	**0.74**	**0.15**	**0.16**	**219**	**21.9**	**22.9**	**2.1**	**5.0**	**5.2**	**89**	**1.9**	**2.0**
Acetone-vehicle Trials																									
BaP-acetone[d]	--	4	--	**5**	**83.2**	**4.4**	**5.4**	**0.03**	**0.017**	**0.021**	**17.4**	**5.8**	**7.2**	**15.4**	**5.7**	**7.1**	**7.6**	**8.9**	**11**	**0.52**	**0.50**	**0.62**	**78**	**16**	**19**
BaP-acetone[d]	--	8	--	**5**	**80.4**	**4.2**	**5.2**	**0.10**	**0.083**	**0.10**	**16.6**	**4.3**	**5.3**	**12.3**	**5.4**	**6.7**	**13**	**8.8**	**11**	**0.60**	**0.97**	**1.2**	**84**	**16**	**20**
BaP-acetone	--	24	--	**6**	**76.7**	**3.8**	**4.0**	**1.7**	**1.6**	**1.6**	**16.6**	**2.6**	**2.8**	**10.6**	**3.3**	**3.5**	**7.9**	**6.5**	**6.8**	**0.81**	**1.0**	**1.1**	**77**	**10**	**11**
Subset of Weathered Trials for Comparison with Unweathered Trials																									
MTSS	yes	24	3	6	87.9	3.1	3.2	0.19	0.036	0.038	0.52	0.46	0.48	0.19	0.09	0.09	55.6	2.7	2.8	0.008	0.003	0.003	101	7.1	7.4
Yolo	yes	24	3	6	87.6	2.6	2.7	0.17	0.027	0.028	0.25	0.11	0.12	0.19	0.15	0.15	52.8	4.1	4.3	0.032	0.031	0.033	96	5.9	6.1
			Group:	**12**	**87.7**	**2.7**	**1.7**	**0.18**	**0.033**	**0.021**	**0.38**	**0.35**	**0.22**	**0.19**	**0.11**	**0.07**	**54.2**	**3.6**	**2.3**	**0.020**	**0.024**	**0.016**	**99**	**6.8**	**4.3**
MTSS	yes	24	10	6	295	11.7	12.2	0.20	0.044	0.046	1.31	0.55	0.58	0.78	0.40	0.42	180	12.2	12.8	0.51	1.22	1.28	97	3.2	3.3
Yolo	yes	24	10	6	291	7.0	7.4	0.17	0.047	0.049	0.69	0.34	0.35	0.56	0.42	0.44	181	9.7	10.2	0.03	0.03	0.03	99	2.0	2.0
			Group:	**12**	**293**	**9.3**	**5.9**	**0.19**	**0.046**	**0.029**	**1.0**	**0.54**	**0.35**	**0.67**	**0.41**	**0.26**	**181**	**10.5**	**6.7**	**0.27**	**0.86**	**0.55**	**98**	**2.6**	**1.7**

[a] n = total number of experimental measurements; C = concentration; texp = time of exposure; SD = standard deviation; E = maximum error of the mean at a 95% confidence level. [b] Due to high amounts of soil in these vials, 1 mL aliquots of well-mixed cocktail-soil solution were added to an additional 10 mL of cocktail and re-counted. [c] One cell had receptor fluid values that failed outlier analysis (Dixon's Q test at 99% level). [d] One trial was removed from analysis due to total radioactivity recoveries of <50%.

Table S2. Average flux into and through skin for all experimental conditions, including by individual soil type[a]

Vehicle	Weath-ered?	t_{exp}	nominal C		measured C[b]			average flux: J_{out}			J_{in}			M_{rf}/A			M_{sk}/A		
	Y/N	hr	mg/kg	n	mg/kg	± SD	± E	ng/cm2-hr	± SD	± E	ng/cm2-hr	± SD	± E	ng/cm2	± SD	± E	ng/cm2	± SD	± E
Weathered Trials																			
CSU	yes	8	3	6	2.7	0.13	0.14	0.011	0.002	0.002	0.066	0.026	0.028	0.092	0.013	0.013	0.44	0.21	0.23
ISU	yes	8	3	6	2.6	0.16	0.16	0.015	0.005	0.005	0.106	0.054	0.056	0.12	0.040	0.042	0.72	0.41	0.43
MTSS[c]	yes	8	3	5	2.8	0.16	0.20	0.011	0.002	0.003	0.082	0.020	0.025	0.088	0.017	0.021	0.57	0.15	0.19
Yolo	yes	8	3	6	2.8	0.14	0.15	0.015	0.005	0.005	0.078	0.036	0.038	0.12	0.005	0.005	0.51	0.30	0.31
			Group:	**23**	**2.7**	**0.17**	**0.07**	**0.013**	**0.004**	**0.002**	**0.083**	**0.038**	**0.016**	**0.11**	**0.033**	**0.014**	**0.56**	**0.29**	**0.13**
CSU	yes	24	3	6	2.8	0.13	0.13	0.013	0.003	0.004	0.027	0.007	0.007	0.31	0.081	0.085	0.35	0.11	0.11
ISU	yes	24	3	6	2.7	0.07	0.08	0.010	0.001	0.001	0.028	0.005	0.005	0.24	0.031	0.032	0.44	0.12	0.12
MTSS	yes	24	3	6	2.9	0.09	0.09	0.013	0.002	0.002	0.047	0.031	0.033	0.31	0.057	0.060	0.82	0.72	0.75
Yolo	yes	24	3	6	2.8	0.08	0.08	0.011	0.002	0.002	0.027	0.009	0.010	0.27	0.042	0.044	0.39	0.18	0.19
			Group:	**24**	**2.8**	**0.12**	**0.05**	**0.012**	**0.002**	**0.001**	**0.033**	**0.018**	**0.008**	**0.28**	**0.059**	**0.025**	**0.50**	**0.40**	**0.17**
CSU	yes	8	10	6	9.0	0.09	0.10	0.012	0.004	0.004	0.16	0.075	0.079	0.09	0.033	0.035	1.2	0.58	0.61
ISU	yes	8	10	6	8.1	0.16	0.17	0.011	0.002	0.002	0.32	0.26	0.27	0.08	0.016	0.017	2.5	2.0	2.14
MTSS	yes	8	10	6	9.0	0.31	0.32	0.014	0.002	0.003	0.18	0.039	0.041	0.11	0.020	0.021	1.4	0.30	0.31
Yolo	yes	8	10	6	9.3	0.19	0.19	0.014	0.005	0.005	0.19	0.080	0.084	0.11	0.037	0.039	1.4	0.61	0.64
			Group:	**24**	**8.8**	**0.49**	**0.21**	**0.012**	**0.004**	**0.001**	**0.21**	**0.15**	**0.062**	**0.10**	**0.028**	**0.012**	**1.6**	**1.2**	**0.49**
CSU	yes	24	10	6	9.1	0.08	0.08	0.010	0.001	0.001	0.066	0.030	0.031	0.24	0.034	0.035	1.3	0.71	0.75
ISU[c]	yes	24	10	5	8.6	0.26	0.32	0.012	0.002	0.002	0.078	0.025	0.032	0.28	0.047	0.058	1.6	0.59	0.73
MTSS	yes	24	10	6	9.6	0.20	0.21	0.013	0.003	0.003	0.099	0.036	0.038	0.32	0.068	0.072	2.1	0.87	0.91
Yolo	yes	24	10	6	9.2	0.11	0.12	0.011	0.003	0.003	0.057	0.024	0.026	0.27	0.074	0.077	1.1	0.53	0.55
			Group:	**23**	**9.1**	**0.39**	**0.17**	**0.012**	**0.003**	**0.001**	**0.075**	**0.032**	**0.014**	**0.28**	**0.062**	**0.027**	**1.5**	**0.75**	**0.32**
Unweathered Trials																			
MTSS	no	24	3	3	3.3	0[e]	0[e]	0.013	0.001	0.001	0.06	0.021	0.053	0.32	0.014	0.036	1.0	0.53	1.3
Yolo	no	24	3	3	3.5	0[e]	0[e]	0.015	0.003	0.007	0.05	0.014	0.034	0.36	0.065	0.162	0.78	0.29	0.73
			Group:	**6**	**3.4**	**0.11**	**0.12**	**0.014**	**0.002**	**0.002**	**0.05**	**0.017**	**0.017**	**0.34**	**0.049**	**0.051**	**0.90**	**0.40**	**0.42**
MTSS	no	24	10	3	12.0	0[e]	0[e]	0.014	0.003	0.008	0.18	0.065	0.160	0.35	0.074	0.18	3.9	1.5	3.8
Yolo	no	24	10	3	13.8	0[e]	0[e]	0.013	0.001	0.002	0.15	0.063	0.156	0.32	0.016	0.040	3.3	1.5	3.7
			Group:	**6**	**12.9**	**0.99**	**1.03**	**0.014**	**0.002**	**0.002**	**0.16**	**0.059**	**0.062**	**0.33**	**0.050**	**0.052**	**3.6**	**1.4**	**1.5**
Acetone-vehicle Trials																			
BaP-acetone[d]	--	4	--	**5**	--	--	--	**0.012**	**0.007**	**0.008**	**6.9**	**2.3**	**2.8**	**0.048**	**0.027**	**0.034**	**27.4**	**9.1**	**11.3**
BaP-acetone[d]	--	8	--	**5**	--	--	--	**0.019**	**0.016**	**0.020**	**3.3**	**0.85**	**1.06**	**0.15**	**0.13**	**0.16**	**26.1**	**6.7**	**8.4**
BaP-acetone	--	24	--	**6**	--	--	--	**0.11**	**0.10**	**0.11**	**1.2**	**0.23**	**0.24**	**2.7**	**2.5**	**2.6**	**26.1**	**4.2**	**4.4**
Subset of Weathered Trials for Comparison with Unweathered Trials																			
MTSS	yes	24	3	6	2.9	0.09	0.09	0.013	0.002	0.002	0.047	0.031	0.033	0.31	0.057	0.060	0.82	0.72	0.75
Yolo	yes	24	3	6	2.8	0.08	0.08	0.011	0.002	0.002	0.027	0.009	0.010	0.27	0.042	0.044	0.39	0.18	0.19
			Group:	**12**	**2.9**	**0.08**	**0.05**	**0.012**	**0.002**	**0.001**	**0.037**	**0.024**	**0.015**	**0.29**	**0.051**	**0.032**	**0.60**	**0.55**	**0.35**
MTSS	yes	24	10	6	9.6	0.20	0.21	0.013	0.003	0.003	0.099	0.036	0.038	0.32	0.068	0.072	2.1	0.87	0.91
Yolo	yes	24	10	6	9.2	0.11	0.12	0.011	0.003	0.003	0.057	0.024	0.026	0.27	0.074	0.077	1.1	0.53	0.55
			Group:	**12**	**9.4**	**0.24**	**0.16**	**0.012**	**0.003**	**0.002**	**0.078**	**0.037**	**0.023**	**0.30**	**0.072**	**0.046**	**1.6**	**0.85**	**0.54**

[a] n = total number of experimental measurements; C = concentration; texp = time of exposure; Jin = average flux into skin; Jout = average flux into receptor fluid; Msk/A = mass of BaP per area in skin; Mrf/A = mass of BaP per area in receptor fluid; SD = standard deviation; E = maximum error of the mean at a 95% confidence level. [b] Due to high amounts of soil in these vials, 1 mL aliquots of well-mixed cocktail-soil solution were added to an additional 10 mL of cocktail and re-counted. [c] One cell had receptor fluid values that failed outlier analysis (Dixon's Q test at 99% level).[d] One trial was removed from analysis due to total radioactivity recoveries of <50%. [e] All experiments took place in same week.

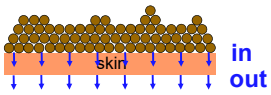
Dermal absorption of benzo[a]pyrene:

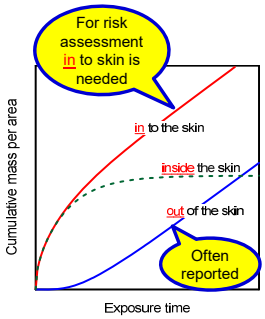
Assessment of flux from weathered soil and application to risk assessment of contaminated sites

Annette L. Bunge[1], Trevor K. Peckham[2], John C. Kissel[2], Jeffry H. Shirai[2], Yvette W. Lowney[3], Michael V. Ruby[4]

[1]Colorado School of Mines, Golden, CO, [2]University of Washington, Seattle, WA, [3]Exponent, Boulder, CO, [4]Integral Consulting, Louisville, CO

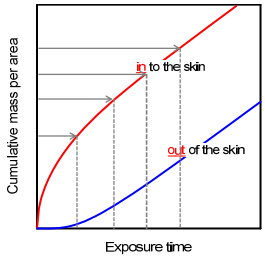
Skin absorption from contaminated soil

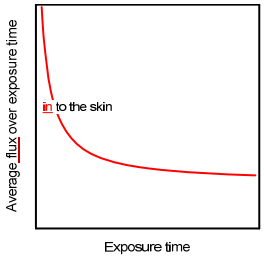




in to the skin = inside the skin + out of the skin

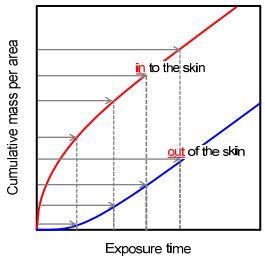
Average flux in to the skin

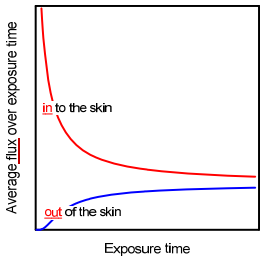




in to the skin = inside the skin + out of the skin

Average flux out of the skin





Study Approach

Experimental methods

- Epidermis layer of human cadaver skin
  - Prepared by heat separation
  - Duplicates of 3 subjects (n = 6) in most cases
- ^{14}C-labeled BaP added to soil using toluene
  - After mixing, toluene was evaporated, then rotated for 72 h, and applied to skin before or after weathering
  - Soil load applied to skin ~30 mg/cm^2 (multiple soil layers insured skin was completely covered with soil)
  - Two concentrations: 3 and 10 mg/kg
- Weathered with 8 weekly hydration-drying cycles
- Weathered and unweathered soils compared with acetone deposited onto skin

Studied 4 soils

Similar higher TOC soils but different BC

Soil	Soil Source	TOC (%) §	Black Carbon (%) §
CSU	Colorado agricultural soil	0.99	0.14
ISU	Iowa agricultural soil	3.13	0.23
MTSS	Montana soil near smelter	3.91	1.23
Yolo	Yolo County, California soil	0.97	0.09

- Varying total organic carbon (TOC) content (1 to 4%)
- Varying black carbon (BC) content (0.1 to 1.2%)
- Yolo soil chosen because EPA default absorption for BaP was measured using this soil
- Small particles that adhere to skin (< [illegible] sieve fraction)

13%

§Values provided by U Ghosh, U MD, Baltimore Co

Selection of BaP soil concentrations

- Selection criteria for two concentrations (C)
  - Small enough to be environmentally relevant
  - Large enough to measure absorption ($\geq$ 3 mg/kg)
  - Small enough to be less than the BaP saturation for the selected soils ($C < C_{sat}$)
- Soil saturation (C_{sat})?
  - Analogous to C_{sat} in a solvent
  - If $C \leq C_{sat}$, all of the chemical is dissolved; dermal absorption is proportional to C
  - If $C > C_{sat}$, some chemical is not dissolved; dermal absorption is proportional to C_{sat}

low C = 3 mg/kg

Example of C_{sat} in skin permeation

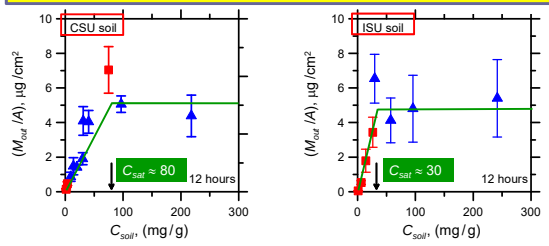
Methyl paraben permeation through split-thickness human skin in 12 hours
(Mean ± standard deviation)

There is a concentration limit for increasing skin permeation

Example of C_{sat} in skin permeation

Methyl paraben permeation through split-thickness human skin in 12 hours
(Mean ± standard deviation)

There is a concentration limit to increasing skin permeation



Estimating soil s

A soil's capacity to "hold" chemical may be affected by factors other than TOC (e.g., soil surface area)

- Assume soil saturatio saturation limit of BaP in soil organic carbon, estimated as:
  - C_{sat} = (TOC/100) K_{oc} $C_{sat,w}$
    - TOC = total organic carbon content (%) = 1 – 4
    - K_{oc} = organic carbon-water partition coefficient (L/kg) = 2.5 x 10^5 §
    - $C_{sat,w}$ = saturation limit in water (mg/L) = 0.013 §
  - C_{sat} = (TOC/100) x (3250 mg/kg) = 30 – 130 mg/kg
  - Estimates for K_{oc} can vary by at least 10-fold
- Chose 10 mg/kg as the "high" C

§Estimates from EpiSuite

Dermal absorption from soils is sometimes reported as percent of applied dose

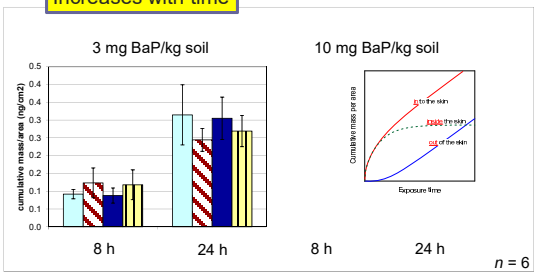
Percent absorbed is affected by soil load applied when soil covers the skin

Better to report results as mass absorbed/area or flux

Weathered soil results

Cumulative mass per area out of skin

Increases with time



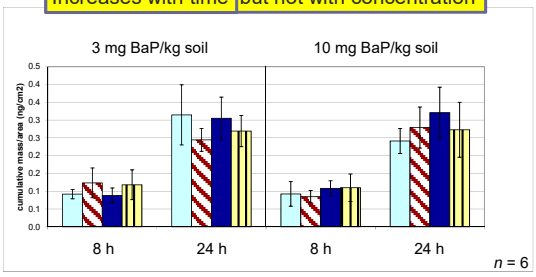
No change with soil

	CSU	ISU	MTSS	Yolo
TOC(%)	0.99	3.13	3.91	0.97
BC(%)	0.14	0.23	1.23	0.09

Mean ± 95% CI

Cumulative mass per area out of skin

Increases with time but not with concentration



No change with soil

	CSU	ISU	MTSS	Yolo
TOC(%)	0.99	3.13	3.91	0.97
BC(%)	0.14	0.23	1.23	0.09

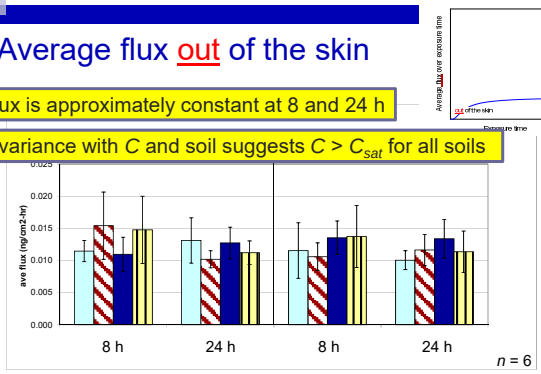
Mean ± 95% CI

Average flux out of the skin

Average flux out of the skin

Flux is approximately constant at 8 and 24 h

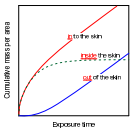
Invariance with C and soil suggests $C > C_{sat}$ for all soils



	CSU	ISU	MTSS	Yolo
TOC(%)	0.99	3.13	3.91	0.97
BC(%)	0.14	0.23	1.23	0.09

Mean ± 95% CI

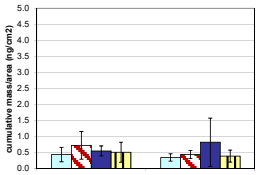
Mass per area inside skin

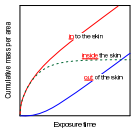


Mass per area inside skin

No change with time

3 mg BaP/kg soil





n = 6

No change with soil

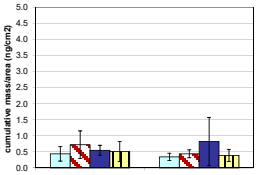
	CSU	ISU	MTSS	Yolo
TOC(%)	0.99	3.13	3.91	0.97
BC(%)	0.14	0.23	1.23	0.09

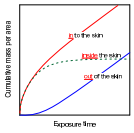
Mean ± 95% CI

Mass per area inside skin

No change with time

3 mg BaP/kg soil





n = 6

No change with soil

	CSU	ISU	MTSS	Yolo
TOC(%)	0.99	3.13	3.91	0.97
BC(%)	0.14	0.23	1.23	0.09

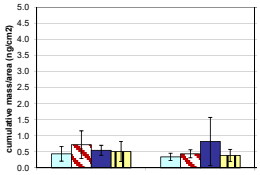
Mean ± 95% CI

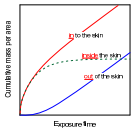
Mass per area inside skin

No change with time

Increases with *C*

3 mg BaP/kg soil





n = 6

No change with soil

	CSU	ISU	MTSS	Yolo
TOC(%)	0.99	3.13	3.91	0.97
BC(%)	0.14	0.23	1.23	0.09

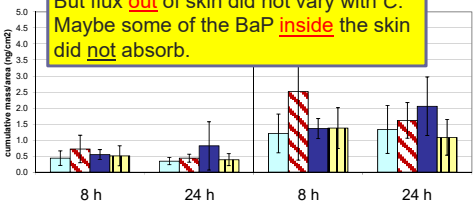
Mean ± 95% CI

Mass per area inside skin

No change with time

Increases with *C*

But flux out of skin did not vary with *C*. Maybe some of the BaP inside the skin did not absorb.



n = 6

No change with soil

	CSU	ISU	MTSS	Yolo
TOC(%)	0.99	3.13	3.91	0.97
BC(%)	0.14	0.23	1.23	0.09

Mean ± 95% CI

Total mass inside the skin = mass on particles stuck to the skin + absorbed mass inside the skin

IF inside the skin

BaP mass from **particles** > BaP mass from **absorption**

And IF

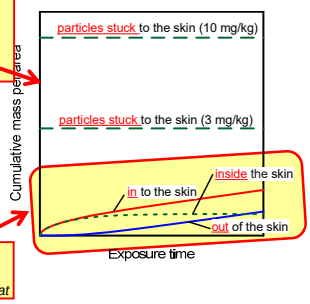
$C > C_{sat}$

THEN

BaP mass inside the skin is proportional to *C*

AND

BaP mass out of the skin is independent of *C*



Is it plausible that BaP mass inside the skin is from particles left "on" the washed skin?

Mass per area inside skin

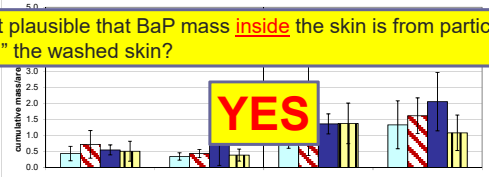
No change with time

Increases with *C*

3 mg BaP/kg soil

10 mg BaP/kg soil

Is it plausible that BaP mass inside the skin is from particles left "on" the washed skin?



YES

Soil load responsible for this BaP mass inside skin ~ 0.1 mg/cm^2

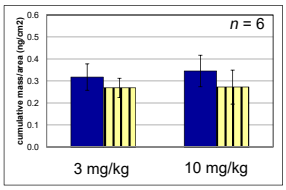
Compare with:
Applied soil load ~ 30 mg/cm^2
Monolayer load ~ 1-2 mg/cm^2

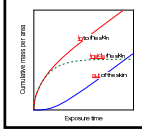
Mean ± 95% CI

Compare weathered & unweathered soil results

Mass per area out of the skin in 24 h

weathered soil





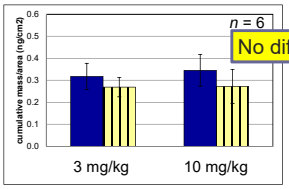
	MTSS	Yolo
TOC(%)	3.91	0.97
BC(%)	1.23	0.09

Mean ± 95% CI

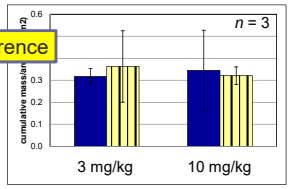
Mass per area out of the skin in 24 h

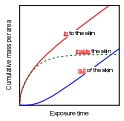
weathered soil

Unweathered soil



No difference



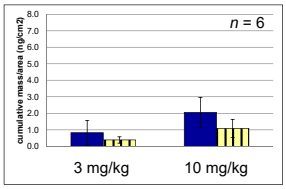


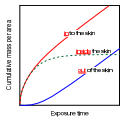
	MTSS	Yolo
TOC(%)	3.91	0.97
BC(%)	1.23	0.09

Mean ± 95% CI

Mass per area inside skin at 24 h

weathered soil





	MTSS	Yolo
TOC(%)	3.91	0.97
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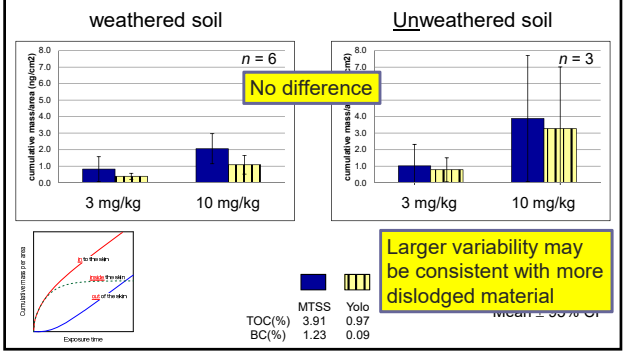
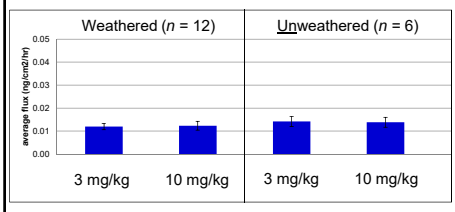
Mean ± 95% CI

Mass per area inside skin at 24 h



Compare soil & acetone deposited onto skin results

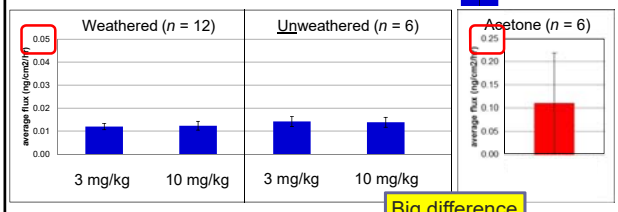
Average flux out of the skin in 24 h



MTSS & Yolo Soils combined

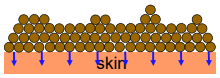
Mean ± 95% CI

Average flux out of the skin in 24 h



MTSS & Yolo Soils combined

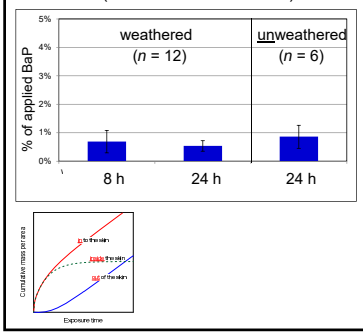
~ same BaP as soil at 3 mg/kg



Mean ± 95% CI

Mass per area inside skin

Soil at *C* = 3 mg/kg
(MTSS & Yolo combined)

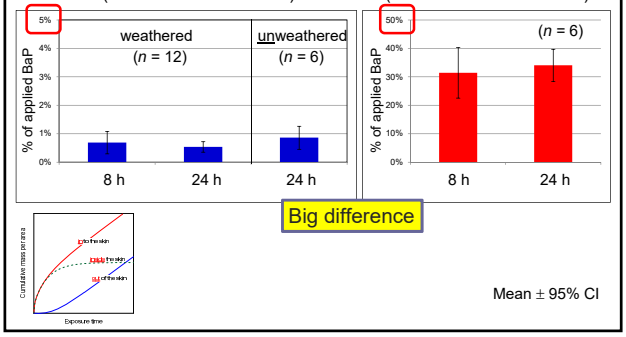


Mean ± 95% CI

Mass per area inside skin

Soil at *C* = 3 mg/kg
(MTSS & Yolo combined)

Acetone deposited
(~ same BaP mass as soil)



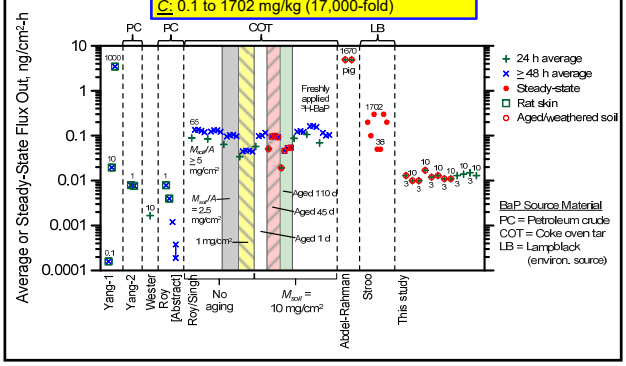
Mean ± 95% CI

Compare this study to prior studies

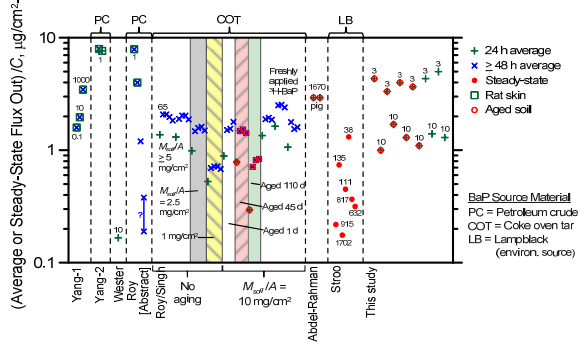
From review of BaP absorption from soils to be submitted soon

Average flux out of skin ≥ 24 h

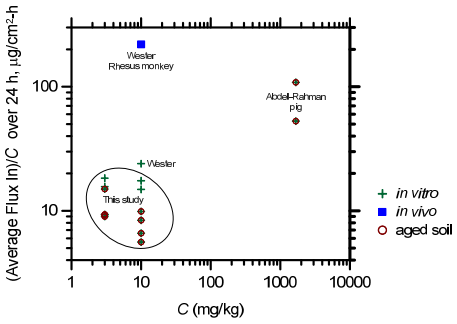
Flux: 1.6 x 10^{-4} to 9.8 ng/cm^2-h (61,000-fold)
C: 0.1 to 1702 mg/kg (17,000-fold)



Average flux out of skin ≥ 24 h / *C*



Average flux in to the skin in 24 h / *C*



Average flux in to the skin in 24 h

Average flux in to the skin from 10 mg/kg over 24 h (pg/cm^2-h)			
This study	*In vitro*	Weathered*	7.6
	In vitro	Unweathered†	16
Wester	*In vitro*	Human	24
Wester	*In vivo*	Monkey	220

* Mean of 4 soils including Yolo County soil used by Wester et al. 1990
† Mean of 2 soils

Summary of study results (I)

- BaP absorption into skin is less from soil than when applied directly to skin in solvent
- For BaP applied to skin in soil:
  - No difference between soils
  - Flux out of skin was independent of *C* = 3 & 10 mg/kg
  - Mass inside the skin was proportional to *C*
- These results are consistent with the hypothesis:
  - $C > C_{sat}$ ("absorbed" mass of BaP is independent of *C*)
  - Total mass of BaP inside the skin is dominated by particles (of soil and/or dislodged neat BaP) that are not removed by washing

Summary of study results (II)

- BaP absorption from weathered soil was not significantly different from unweathered soil
- New results are generally consistent with previous studies of BaP contaminated soils except for the *in vivo* Rhesus monkey study of mass in to the skin from Wester (source of EPA 13% default value)
- Only 2 of the 7 previous BaP studies (Wester and Abdel-Rahman) have reported BaP in to the skin, which is needed for making risk assessments

Acknowledgments

- Funding to the University of Washington and the Colorado School of Mines was from Exponent, Inc. as part of a large project funded by SERDP
- Results have not been reviewed by DOD, DOE or EPA and no endorsement should be inferred
- Experimental assistance from Carley Heinen, Marie Robertson and Anna Fretheim at University of Washington
- Upal Ghosh, University of Maryland, Baltimore County for soil characterization (TOC and BC)

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The End

Questions?

Summary of results from other studies (I)

- Seven prior studies of BaP absorption into skin from soil
  - In 4 studies, BaP was in a matrix (crude oil, coal tar, lampblack)
  - In 3 studies, BaP was applied directly to the soil using a solvent
    - Only 1 in human (1 in pig and 1 in rat)
    - Only aged soil in 1 study (the un-aged samples in this study were excluded because solvent was present)
- Results from most studies can be reported as "flux out" but not "flux in" (which is preferred for risk assessment)

Summary of results from other studies (II)

- Effect of *C* was investigated in only 1 other study (in rat skin)
- Effects of BaP source material are difficult to assess
  - Lampblack study appears to have lower flux at same concentrations, but the effect is less than 10 fold
  - Flux from soils with BaP in matrix may be proportional to *C*

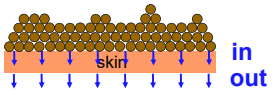
Dermal absorption of benzo[a]pyrene:

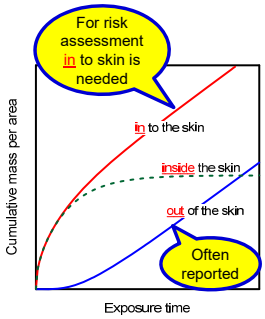
Assessment of flux from weathered soil and application to risk assessment of contaminated sites

Annette L. Bunge[1], Trevor K. Peckham[2], John C. Kissel[2], Jeffry H. Shirai[2], Yvette W. Lowney[3], Michael V. Ruby[4]

[1]Colorado School of Mines, Golden, CO, [2]University of Washington, Seattle, WA, [3]Exponent, Boulder, CO, [4]Integral Consulting, Louisville, CO

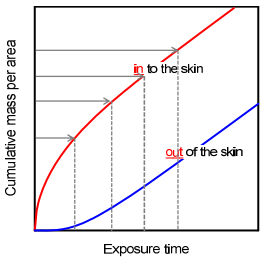
Skin absorption from contaminated soil

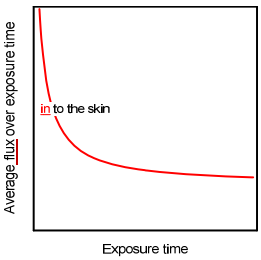




in to the skin = inside the skin + out of the skin

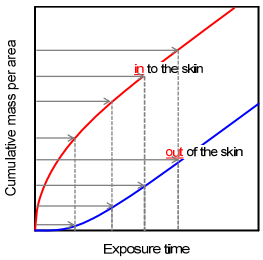
Average flux in to the skin

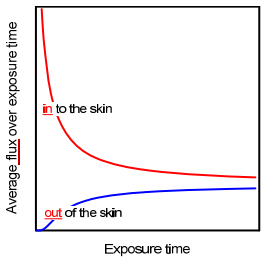




in to the skin = inside the skin + out of the skin

Average flux out of the skin





Study Approach

Experimental methods

- Epidermis layer of human cadaver skin
  - Prepared by heat separation
  - Duplicates of 3 subjects (n = 6) in most cases
- ^{14}C-labeled BaP added to soil using toluene
  - After mixing, toluene was evaporated, then rotated for 72 h, and applied to skin before or after weathering
  - Soil load applied to skin ~30 mg/cm^2 (multiple soil layers insured skin was completely covered with soil)
  - Two concentrations: 3 and 10 mg/kg
- Weathered with 8 weekly hydration-drying cycles
- Weathered and unweathered soils compared with acetone deposited onto skin

Studied 4 soils

Similar higher TOC soils but different BC

Soil	Soil Source	TOC (%) §	Black Carbon (%) §
CSU	Colorado agricultural soil	0.99	0.14
ISU	Iowa agricultural soil	3.13	0.23
MTSS	Montana soil near smelter	3.91	1.23
Yolo	Yolo County, California soil	0.97	0.09

- Small particles that adhere to skin (< 63 μm sieve fraction)
- Varying total organic carbon (TOC) content (1 to 4%)
- Varying black carbon (BC) content (0.1 to 1.2%)
- Yolo soil chosen because EPA default absorption for BaP was measured using this soil

13%

§Values provided by U Ghosh, U MD, Baltimore Co

Selection of BaP soil concentrations

low C = 3 mg/kg

- Selection criteria for two concentrations (C)
  - Large enough to measure absorption (≥ 3 mg/kg)
  - Small enough to be environmentally relevant
  - Small enough to be less than the BaP saturation for the selected soils ($C < C_{sat}$)
- Soil saturation (C_{sat})?
  - Analogous to C_{sat} in a solvent
  - If $C \leq C_{sat}$, all of the chemical is dissolved; dermal absorption is proportional to C
  - If $C > C_{sat}$, some chemical is not dissolved; dermal absorption is proportional to C_{sat}

Example of C_{sat} in skin permeation

Methyl paraben permeation through split-thickness human skin in 12 hours (Mean ± standard deviation)

There is a concentration limit to increasing skin permeation

Estimating soil s

A soil's capacity to "hold" chemical may be affected by factors other than TOC (e.g., soil surface area)

- Assume soil saturatio saturation limit of BaP in soil organic carbon, estimated as:
  - C_{sat} = (TOC/100) K_{oc} $C_{sat,w}$
    - TOC = total organic carbon content (%) = 1 – 4
    - K_{oc} § = organic carbon-water partition coefficient (L/kg) = 2.5 x 10^5
    - $C_{sat,w}$ = saturation limit in water (mg/L) = 0.013 §
  - C_{sat} = (TOC/100) x (3250 mg/kg) = 30 – 130 mg/kg
  - Estimates for K_{oc} can vary by at least 10-fold
- Chose 10 mg/kg as the "high" C

§Estimates from EpiSuite

Dermal absorption from soils is sometimes reported as percent of applied dose

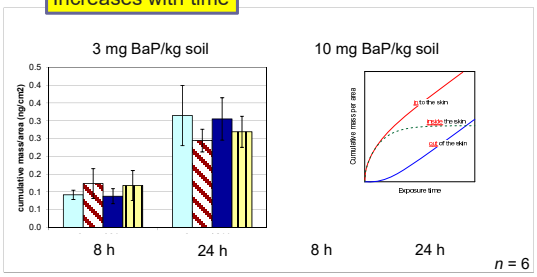
Percent absorbed is affected by soil load applied

Better to report results as mass absorbed/area or flux

Weathered soil results

Cumulative mass per area out of skin

Increases with time



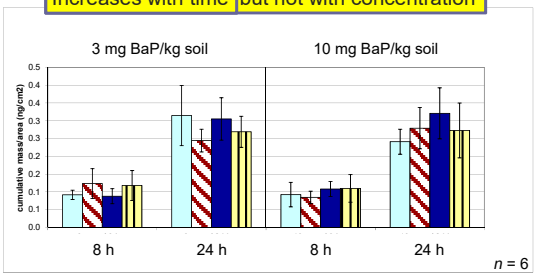
No change with soil

	CSU	ISU	MTSS	Yolo
TOC(%)	0.99	3.13	3.91	0.97
BC(%)	0.14	0.23	1.23	0.09

Mean ± 95% CI

Cumulative mass per area out of skin

Increases with time but not with concentration



No change with soil

	CSU	ISU	MTSS	Yolo
TOC(%)	0.99	3.13	3.91	0.97
BC(%)	0.14	0.23	1.23	0.09

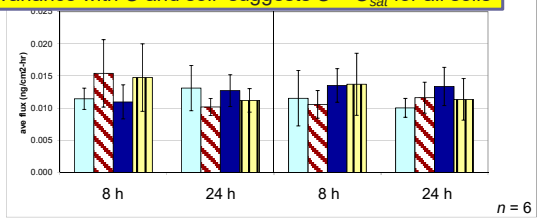
Mean ± 95% CI

Average flux out of the skin

Average flux out of the skin

Flux is approximately constant at 8 and 24 h

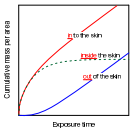
Invariance with C and soil suggests $C > C_{sat}$ for all soils



	CSU	ISU	MTSS	Yolo
TOC(%)	0.99	3.13	3.91	0.97
BC(%)	0.14	0.23	1.23	0.09

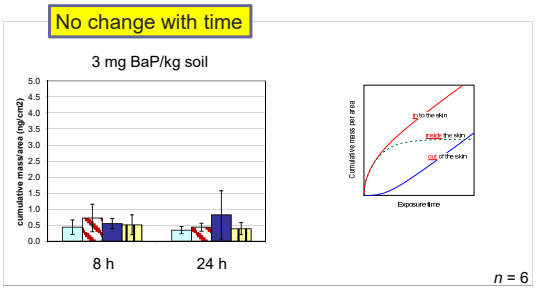
Mean ± 95% CI

Mass per area inside skin



Mass per area inside skin

No change with time



No change with soil

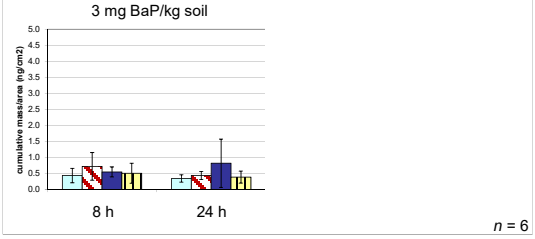
	CSU	ISU	MTSS	Yolo
TOC(%)	0.99	3.13	3.91	0.97
BC(%)	0.14	0.23	1.23	0.09

Mean ± 95% CI

Mass per area inside skin

No change with time

Increases with *C*



No change with soil

	CSU	ISU	MTSS	Yolo
TOC(%)	0.99	3.13	3.91	0.97
BC(%)	0.14	0.23	1.23	0.09

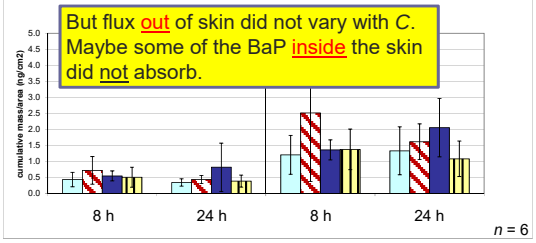
Mean ± 95% CI

Mass per area inside skin

No change with time

Increases with *C*

But flux out of skin did not vary with *C*. Maybe some of the BaP inside the skin did not absorb.



No change with soil

	CSU	ISU	MTSS	Yolo
TOC(%)	0.99	3.13	3.91	0.97
BC(%)	0.14	0.23	1.23	0.09

Mean ± 95% CI

Total mass inside the skin = mass on particles stuck to the skin + absorbed mass inside the skin

IF inside the skin

BaP mass from **particles** > BaP mass from **absorption**

And IF

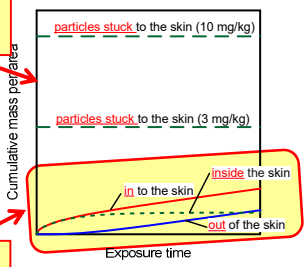
$C > C_{sat}$

THEN

BaP mass inside the skin is proportional to *C*

AND

BaP mass out of the skin is independent of *C*



Is it plausible that BaP mass inside the skin is from particles left "on" the skin by washing?

Mass per area inside skin

No change with time

Increases with *C*

3 mg BaP/kg soil

10 mg BaP/kg soil

Is it plausible that BaP mass inside the skin is from particles left "on" the skin by washing?

Soil load responsible for this BaP mass inside skin ~ 0.1 mg/cm^2

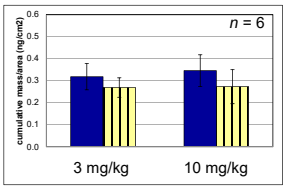
Compare with:
Applied soil load ~ 30 mg/cm^2
Monolayer load ~ 1-2 mg/cm^2

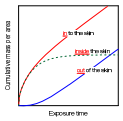
Mean ± 95% CI

Compare weathered & unweathered soil results

Mass per area out of the skin in 24 h

weathered soil



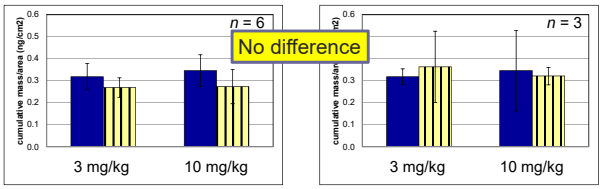


	MTSS	Yolo
TOC(%)	3.91	0.97
BC(%)	1.23	0.09

Mean ± 95% CI

Mass per area out of the skin in 24 h

weathered soil | Unweathered soil

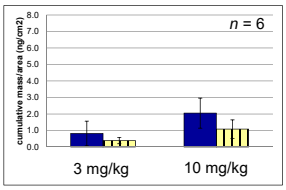


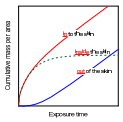
	MTSS	Yolo
TOC(%)	3.91	0.97
BC(%)	1.23	0.09

Mean ± 95% CI

Mass per area inside skin at 24 h

weathered soil



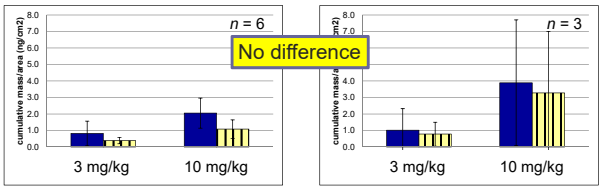


	MTSS	Yolo
TOC(%)	3.91	0.97
BC(%)	1.23	0.09

Mean ± 95% CI

Mass per area inside skin at 24 h

weathered soil | Unweathered soil

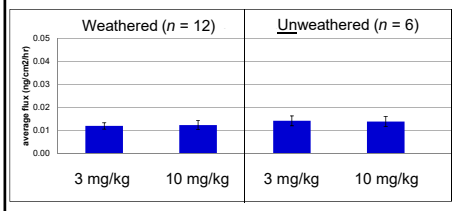


Larger variability may be consistent with more dislodged material

	MTSS	Yolo
TOC(%)	3.91	0.97
BC(%)	1.23	0.09

Compare soil & acetone deposited onto skin results

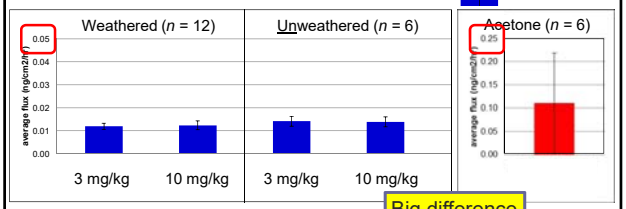
Average flux out of the skin in 24 h



MTSS & Yolo Soils combined

Mean ± 95% CI

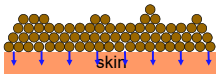
Average flux out of the skin in 24 h



Big difference

MTSS & Yolo Soils combined

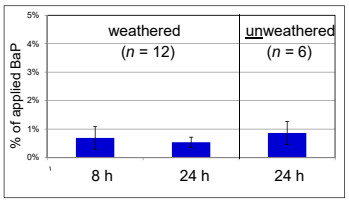
~ same BaP as soil at 3 mg/kg

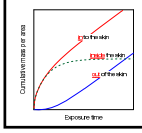


Mean ± 95% CI

Mass per area inside skin

Soil at *C* = 3 mg/kg
(MTSS & Yolo combined)



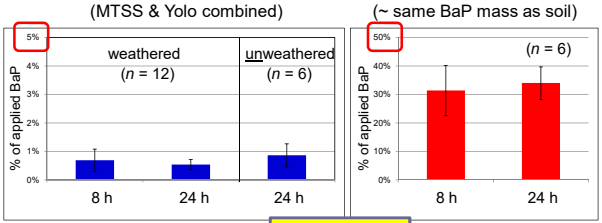


Mean ± 95% CI

Mass per area inside skin

Soil at *C* = 3 mg/kg
(MTSS & Yolo combined)

Acetone deposited
(~ same BaP mass as soil)



Big difference

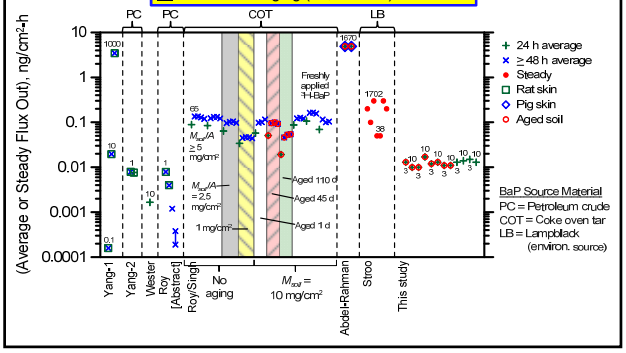
Mean ± 95% CI

Compare this study to prior studies

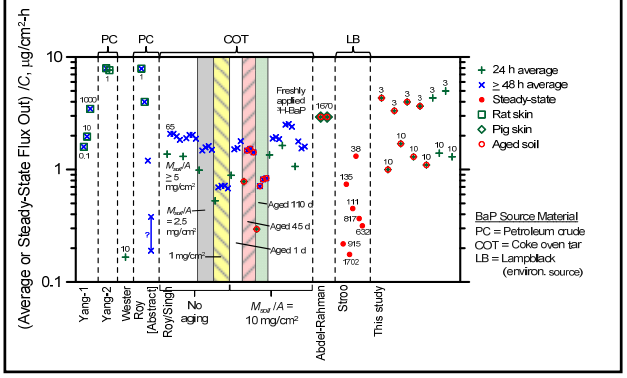
From review of BaP absorption from soils to be submitted soon

Average flux out of skin ≥ 24 h

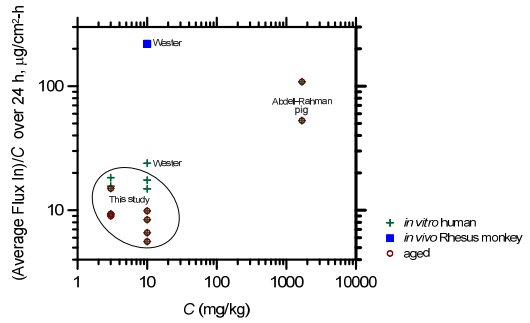
Flux: 1.6 x 10^{-4} to 9.8 ng/cm²-h (61,000-fold)
C: 0.1 to 1702 mg/kg (17,000-fold)



Average flux out of skin ≥ 24 h / *C*



Average flux in to the skin in 24 h / *C*



Average flux in to the skin in 24 h

Average flux in to the skin from 10 mg/kg over 24 h (pg/cm²-h)			
This study	*In vitro*	Weathered*	7.6
	In vitro	Unweathered†	16
Wester	*In vitro*	Human	24
Wester	*In vivo*	Monkey	220

* Mean of 4 soils including Yolo County soil used by Wester et al. 1990
† Mean of 2 soils

Summary of study results (I)

- BaP absorption into skin is less from soil than when applied directly to skin in solvent
- For BaP applied to skin in soil:
  - No difference between soils
  - Flux out of skin was independent of C = 3 & 10 mg/kg
  - Mass inside the skin was proportional to C
- These results are consistent with the hypothesis:
  - $C > C_{sat}$ ("absorbed" mass of BaP is independent of C)
  - Total mass of BaP inside the skin is dominated by particles (of soil and/or dislodged neat BaP) that are not removed by washing

Summary of study results (II)

- BaP absorption from weathered soil was not significantly different from unweathered soil
- New results are generally consistent with previous studies of BaP contaminated soils except for the *in vivo* Rhesus monkey study of mass in to the skin from Wester (source of EPA 13% default value)
- Only 2 of the 7 previous BaP studies (Wester and Abdel-Rahman) have reported BaP in to the skin, which is needed for making risk assessments

Acknowledgments

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- Results have not been reviewed by DOD, DOE or EPA and no endorsement should be inferred
- Experimental assistance from Carley Heinen, Marie Robertson and Anna Fretheim at University of Washington
- Upal Ghosh, University of Maryland, Baltimore County for soil characterization (TOC and BC)

The End

Questions?

Summary of results from other studies (I)

- Seven prior studies of BaP absorption into skin from soil
  - In 4 studies, BaP was in a matrix (crude oil, coal tar, lampblack)
  - In 3 studies, BaP was applied directly to the soil using a solvent
    - Only 1 in human (1 in pig and 1 in rat)
    - Only aged soil in 1 study (the un-aged samples in this study were excluded because solvent was present)
- Results from most studies can be reported as "flux out" but not "flux in" (which is preferred for risk assessment)

Summary of results from other studies (II)

- Effect of *C* was investigated in only 1 other study (in rat skin)
- Effects of BaP source material are difficult to assess
  - Lampblack study appears to have lower flux at same concentrations, but the effect is less than 10 fold
  - Flux from soils with BaP in matrix may be proportional to *C*

Dermal absorption of benzo[a]pyrene from soil: Assessment of flux and application to risk assessment of contaminated sites

Trevor Peckham

A thesis
submitted in partial fulfillment of the
requirements for the degree of

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Committee:
John Kissel (Chair)
Alison Cullen
Rob Duff
Jeff Shirai

Program Authorized to Offer Degree:
School of Public Health-
Department of Environmental and Occupational Health Sciences

University of Washington

ABSTRACT

Dermal absorption of benzo[a]pyrene from soil: Assessment of flux and application to risk assessment of contaminated sites

Trevor Peckham

Chair of the Supervisory Committee:
Professor John C. Kissel
Department of Environmental and Occupational Health Science

Soil cleanup standards and assessment of human health risks at contaminated sites are based in part on predicted human exposure to soil contaminants, including from direct skin contact. Available investigations of dermal absorption from soil are relatively sparse and have been conducted with a variety of different methods, many of which fail to account for important physical and chemical drivers of skin permeation. To improve understanding of the soil-dermal exposure pathway, i*n vitro* assessments of radiolabeled benzo[a]pyrene (BaP) absorption through human epidermis were conducted. Experiments employed four test soils, which were artificially weathered and applied to epidermis at multiple BaP concentrations and exposure durations. Experiments were also conducted with unweathered soils and BaP deposited onto skin from acetone. For weathered soils, absorption was independent of soil type, the mass in the receptor fluid was proportional to exposure duration but independent of concentration, and the mass recovered in the skin after washing was proportional to concentration and independent of exposure time. Results from the weathered and unweathered soils were essentially similar. The findings are consistent with concentrations that exceeded the BaP sorption capacity of all soils

tested, and with BaP mass in the wash skin dominated by particles that were not removed by washing. Flux into and through skin from soils were lower by an order of magnitude from acetone-deposited BaP. Potential barriers and opportunities for improving guidance for the assessment of dermal exposure from contaminated soils were also examined, as the current method is relatively simplistic and based on an experimentally-determined parameter that is susceptible to distortion by common methodological pitfalls. A practical recommendation is described that is easily implemented, empirically and theoretically supported, and represents a more health protective approach until further methodological improvements are feasible.

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First and foremost, I'd like to thank John Kissel and Jeff Shirai for their incredible dedication to my project and graduate education. They spent more face time with me than can be reasonably expected from a faculty or staff. I've acquired many fond memories in my time in the Kissel Lab, including laughing as much as working with Jeff and many hours of solving the world's problems with John in his office.

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Annette Bunge was integral in the study design, interpretation of results, and development of a manuscript describing results from my primary experiments. Thanks as well to my other co-authors, Yvette Lowney and Mike Ruby.

A general thanks is in order for the excellent faculty of the Department of Environmental & Occupational Health Sciences. I feel lucky to have been able to spend my graduate education among such prestigious researchers and amazing people. Similar sentiment can be said about the greater School of Public Health and the Evans School of Public Affairs. From the DEOHS, I have to especially thank Scott Meschke for the opportunity to work for him on several interesting projects (and securing me funding when I needed it!).

I benefited greatly from the support and tireless work of staff at all levels of the DEOHS, especially Julie Tran, Rory Murphy, and Grace Wong. I worked with so many great people in the SPH Dean's Office during my extracurricular activities, including Anna Frazer, Brit Exworthy, Deb Hinchey, Howie Frumkin, and JeShawna Schmidt.

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Last but not least, I thank Desiree, Phil, and Jon Peckham, and the rest of my incredible family, for a lifetime of support that has allowed me to develop my interests in environmental public health.

PREFACE

This thesis is organized into three chapters: (1) an introduction; (2) a manuscript; and (3) a policy recommendation. The introduction intends provide background information regarding dermal exposure to chemicals from soil and the primary chemical of concern, benzo[a]pyrene (BaP). This includes key methodological considerations when performing experiments to characterize this exposure pathway, toxicity and environmental information about BaP, and a brief review of prior investigations that have looked at dermal uptake of BaP from soil. The manuscript summarizes results of *in vitro* experiments conducted using human cadaver skin and artificially spiked soils. This manuscript is intended for publication, and aims to further understanding of the physical and chemical mechanisms driving dermal absorption of BaP. The policy recommendation evaluates dermal-soil exposure and risk assessment guidance emanating from the Environmental Protection Agency. This chapter describes some barriers and opportunities for improving these protocols, and ultimately makes one recommendation that is easily implemented and supported by current scientific knowledge.

Chapter 1. Introduction

Assessment of the potential human health risks associated with contaminated sites subject to cleanup under provisions of the Comprehensive Environmental Response, Compensation, and Liability Act (also known as CERCLA or Superfund) often requires characterization of exposure to contaminated soils, including skin contact. However, available investigations of dermal absorption from soil are relatively sparse and have been conducted with a variety of different methods, making systematic evaluation of results difficult. While dermal contact with contaminated soils is often considered a minor exposure pathway, Johnson and Kissel (1996) found that this pathway accounted for a predicted lifetime excess cancer risk of greater than 1 in 10,000 in nearly 20% of 200 Superfund sites evaluated. In addition, dermal absorption was the dominant exposure route at approximately 5% of sites, supporting the notion that dermal exposure requires assessment (Johnson and Kissel, 1996).

The process of transepidermal uptake of soil-bound chemicals through skin is complex, requiring transfer from soil particles to the skin surface and then through the protective epidermis into the underlying dermis. Total dose from direct skin contact with contaminated soil will depend on several chemical and physical factors, including chemical concentration in the soil, adherence factor of soil to skin (or contact rate), soil particle size distribution, soil-chemical contact time (*i.e.*, "aging"), degree of saturation of soil, sorption capacity of soil particles, duration of exposure, and skin surface area available for contact. Current guidance for assessment of dermal exposures to contaminants in soil from the U.S. Environmental Protection Agency (EPA), however, is relatively simplistic. As defined in Part E of the Risk Assessment Guidance for Superfund (RAGS), calculation of chemical uptake from soil is essentially based on multiplying total chemical loading on skin from soil by an experimentally determined

fractional absorption (ABS) value (USEPA, 2004). This method does not account for physical mechanisms that drive diffusion of chemicals through skin. Further, although the ABS parameter is considered fixed (traditionally calculated as the gross percent of initial contaminant load absorbed in a fixed time frame) determination of this value is heavily influenced by experimental conditions.

Key Methodological Considerations for Investigations of Dermal Absorption from Soil. Designing experiments to investigate dermal uptake of chemical from soil requires understanding of relevant phenomenological concepts at play so that appropriate interpretation of results is possible. In the most comprehensive review of literature on soil-based dermal absorption studies to date, Spalt et al. (2009) document several key phenomenological concepts, methodological considerations, and recommendations for good practices.

Layering effects- Measured fractional absorption is dependent on the configuration of soil loading. The simplest loading to conceptualize is monolayer coverage, which is defined as completed coverage of a given skin surface area by a single layer of soil particles. The mass of soil required to achieve monolayer coverage for a given area will depend on the relative size of soil particles (Duff and Kissel, 1996). In this condition, the interfacial area is maximized and flux of chemical into skin will no longer increase with increased mass loading. It is possible to then infer that increased soil loading above monolayer coverage will correspond with a reduction of apparent gross percent absorbed. Therefore it is inappropriate to report fractional absorption without accounting for soil loading conditions. Fractional absorption measurement from uniform monolayer coverage or less is not susceptible to this artificial suppression. However, because uniform distribution of soil particles across skin is difficult in practice, it is likely preferable to employ supramonolayer experimental soil loadings and report chemical uptake in terms of flux.

Soil saturation – The capacity of a soil to sorb chemicals is limited by its inherent properties. If saturation of chemical in soil is exceeded, free chemical unable to sorb to soil will be available for absorption. Experimental ABS values determined under these conditions would not represent absorption from soil, but rather from neat compound. Experiments should not be conducted with soils that have soil-chemical concentrations approaching this saturation limit.

Particle size distribution – For dry soils, smaller particles tend to adhere to human skin. Excluding particles greater than 150 µm in diameter through sieving is common in environmental health studies involving soil. Research on soil adherence to human skin suggests that a cutoff size of <65 µm might be more appropriate (Kissel et al., 1996). Soil particle size can also influence the mass of soil required for complete monolayer coverage, the soil surface area in contact with skin, and soil porosity—all of which can potentially affect fractional absorption efficiency. Because actual exposures to soil typically involve small particle size fractions, absorption experiments should avoid exclusion of fine particles.

Soil-agent contact time - The process of chemical sorption to soil is not instantaneous. Certain compounds may need long periods of time to reach equilibrium with soil. Dermal absorption experiments should allow for adequate mixing of compound and soil, such that the chemical is well distributed. Additionally, adequate time for solvent evaporation is needed in soil spiking procedures that involve a solvent vehicle.

Other considerations included complete reporting of methodological parameters, assuring continuous soil-skin contact (most relevant to *in vivo* experiments), and use of only the top layer of the epidermis of human skin (*i.e.*, heat-separated skin) for *in vitro* experiments.

Polycyclic Aromatic Hydrocarbons in Soil. Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals formed from incomplete combustion and commonly found in the soil at

or near hazardous waste sites, especially where coal, wood, gasoline, or other products have been burned. PAHs, characterized by two or more benzene rings bonded together with only carbon and hydrogen atoms, generally occur as complex mixtures rather than single compounds. Studies, regulations, and data reporting have focused on a limited number of these compounds. Seven PAHs have been identified as probable human carcinogens, including benzo[a]pyrene (BaP), which is considered the primary risk driver under the current regulatory paradigm for PAH toxicity. The Agency for Toxic Substances and Disease Registry (ATSDR) reported that PAHs ranked #9 on the agency's Priority List of Hazardous Substances in 2013, which is based on a combination of a contaminant's frequency, toxicity, and potential for human exposure at National Priority List (NPL) sites. BaP, also listed as a separate hazardous substance, was found at 545 NPL sites and ranked #8 (ATSDR, 2014).

BaP is also found in urban and nonindustrial environments, likely due to atmospheric deposition after local or long-range transport. Several studies have attempted to characterize background levels of PAHs in soils in the United States. Bradley et al. (1994) report soil BaP concentrations from 62 total samples taken in three New England cities ranging from 40 to 13,000 µg/kg, with an arithmetic mean of 1,323 µg/kg and upper 95% confidence interval on the mean of 1,816 µg/kg (Bradley et al., 1994). A survey by the U.S. Geologic Survey (USGS) of 57 surface soil samples in Chicago, Illinois reported BaP concentrations between 39 and 17,000 µg/kg (excluding one sample that was an outlier at 460,000 µg/kg). The authors note that the distribution of BaP was not uniform, due to their finding of elevated concentrations (>4,000 µg/kg) in certain large regions of the study area and comparatively low concentrations (<400 µg/kg) in others (Kay et al., 2003). Several studies conducted by the Electric Power Research Institute (EPRI) analyzed soil samples in western New York, eastern Pennsylvania, and non-

metro areas of Illinois. BaP concentrations found in 30 samples from New York ranged from 7 to 4,740 µg/kg, with an arithmetic mean of 830 µg/kg and upper 95% confidence interval on the mean of 1,220 µg/kg (EPRI, 2003). Concentrations in 71 samples from Pennsylvania ranged from 7 to 7,920 µg/kg, with an arithmetic mean of 703 µg/kg and upper 95% confidence interval on the mean of 1,000 µg/kg (EPRI, 2008b). Soil BaP concentrations reported from 160 samples taken in Illinois ranged from 0.1 to 5,210 µg/kg, with an arithmetic mean of 70 µg/kg and upper 95% confidence interval on the mean of 92 µg/kg (EPRI, 2004). In another EPRI report that includes results from the above three studies, the average concentration in 318 total samples was 476 µg/kg with an upper 95% confidence interval on the mean of 578 µg/kg (EPRI, 2008a).

Prior Investigations of Dermal Absorption of BaP from Soil. Several studies have attempted to measure the absorption of BaP from contaminated soil or sediment, including *in vivo* studies performed on rats (Yang et al., 1989a) and rhesus monkeys (Wester et al., 1990), and *in vitro* models using the skin of rats (Yang et al., 1989a; Yang et al., 1989b; Roy et al., 1992), pigs (Abdel-Rahman et al., 2002), guinea pigs (Moody and Chu, 1995), and humans (Wester et al., 1990; Roy et al., 1992; Moody and Chu, 1995; Roy et al., 1998; Roy and Singh, 2001). Although fractional absorption is normally reported in these studies, a recent review calculated the average uptake flux based on experimental conditions and results from each study, reporting a range spanning six orders of magnitude (0.19 – 420,000 pg/cm2/h) (Spalt et al., 2009). This heterogeneity likely stems from significant differences in methodologies employed across prior studies—some of which contain obvious deficiencies (**Table 1**).

Critical Review of Wester et al. 1990. The current EPA default recommendation for dermal absorption fraction from soil for BaP and other PAHs is 0.13 (or 13%), based on data from *in vivo* studies of rhesus monkeys (Wester et al., 1990). This particular investigation

entailed loading contaminated soil to a 12-cm^2 shaved area of the animal's abdomen, placing four rhesus monkeys in chairs, and analyzing urine samples collected for seven days including the 24-hour exposure period. The total urinary excretion from the topical exposure was adjusted using the excreted fraction of an intravenously administered dose to obtain the dermal absorption dose estimate. Several shortcomings and/or ambiguous study conditions are worth noting, which may warrant caution in the interpretation of the estimated ABS value, and are indicative of the state of the available literature in general.

Table 1. Methodological considerations of prior studies investigating dermal absorption of BaP from soil (adapted from Spalt et al. 2009) [a,b]

Reference	Study Type	Monolayer load or less/% absorbed not reported	Concentration less than $C_{soil,sat}$	Particle size range includes fines	Soil-agent contact time reported and appropriate	Continuous contact assured
Yang et al. (1989)	*in vitro*	N	Y	Y	Y	Y
Yang et al. (1989)	*in vivo*	N	Y	Y	Y	ND
Wester et al. (1990)	*in vitro*	Y	Y	N	N	Y
Wester et al. (1990)	*in vivo*	Y	Y	N	N	N
Roy et al. (1998)	*in vitro*	N	N	Y	N	Y
Roy and Singh (2001)	*in vitro*	Y	Y/N	Y	Y	Y
Abdel-Rahman et al. (2002)	*in vitro*	N	N	ND	Y	Y

[a] Y, yes; N, no; ND, not determined due to incomplete reporting of methodological parameters; Y/N, questionable as concentration was very close to estimated saturation limit. [b] Footnotes from original table excluded; for more information see Spalt et al. 2009.

The Wester et al. study used a soil particle distribution of 180-300 µm, a relatively coarse particle size range which may not represent a realistic exposure scenario. The size and shape of the soil particles impact the mass of soil necessary for skin coverage, soil porosity, and other properties with important implications for absorption efficiency. Another concern related to the investigations of Wester et al. is the ambiguity of the soil-chemical contact time. A review of

literature suggests that many previous soil dermal absorption studies have employed very short soil-chemical contact times prior to application, presumably for convenience. It is not clear if Wester et al. allowed adequate time for the solvent vehicle to completely evaporate from the soil prior to application to skin, or if the chemical had adequate time to reach equilibrium with soil. A residual solvent could lead to permeation of chemical not yet sorbed into soil particles. Further, continuous contact between skin and contaminated soil is paramount for accurate measurement of dermal absorption from *in vivo* experimentation. The movements of non-human animals, however, are difficult to control and result in the possibility that skin contact with soil is interrupted or largely absent. In the Wester et al. studies, soil was applied to the abdomen of the anesthetized rhesus monkeys and covered in an apparatus with much larger total volume than the loaded soil. When the animals moved from the horizontal to the vertical position after application, it is likely that a large proportion of the coarse soil particles separated from the skin. Interruptions in skin-soil contact (*i.e.,* incomplete coverage of exposed skin) make interpretation of these results problematic. Lastly, direct recovery of chemical was not possible in live animals. Instead total chemical recovery from excreta following soil-dermal exposure was adjusted from recovery following an intravenous dose. Recovery from the latter was only 6.6%, however, requiring a large correction factor ($1/0.066 \approx 15$).

In summary, previous investigations to quantify dermal absorption have employed a variety of methodologies, and have mostly failed to account for one or more important methodological criteria in either reporting or execution (Spalt et al., 2009). These deficiencies, along with the lack of methodological standardization, render a review of available literature difficult and act as an impetus for further data collection.

Chapter 2. Dermal absorption of benzo[a]pyrene into human skin from soil: Effect of artificial weathering, concentration and exposure duration

Trevor K. Peckham[1], Jeffry H. Shirai[1], Annette L. Bunge[2], Yvette W. Lowney[3], Michael V. Ruby[4], John C. Kissel[1]

[1] Department of Environmental and Occupational Health Services, University of Washington, Seattle, WA 98195, USA [2] Chemical and Biological Engineering Department, Colorado School of Mines, Golden, CO 80401, USA [3]Exponent, Boulder, CO 80302, USA [4]Integral Consulting, Louisville, CO 80027, USA

ABSTRACT

In vitro assessments of ^{14}C-benzo[a]pyrene (BaP) absorption through human epidermis were conducted with the sub-63-μm fraction of four test soils, containing different amounts of organic and black carbon. Soils were artificially weathered and applied to epidermis at BaP concentrations of 3 and 10 mg/kg for 8 or 24 h. Experiments were also conducted at 24 h with unweathered soils and with BaP deposited onto skin from acetone at a comparable chemical load. For the weathered soils, absorption was independent of the amount of organic or black carbon, the mass in the receptor fluid was proportional to exposure duration but independent of concentration, and the mass recovered in the skin after washing was proportional to concentration and independent of exposure time. Results from the weathered and unweathered soils were similar except for the mass recovered in the washed skin, which was lower for only the higher concentration by less than 50%. The findings are consistent with concentrations that exceeded the BaP sorption capacity of all soils tested, and with BaP mass in the wash skin dominated by particles that were not removed by washing. Flux into and through skin from soils were lower by an order of magnitude from acetone-deposited BaP.

INTRODUCTION

Soil cleanup standards and assessment of human health risks at contaminated sites are based in part on predicted human exposure to soil contaminants, including from direct skin contact. Percutaneous absorption of soil-bound chemicals requires transfer from soil particles to the skin surface and then diffusion through the protective epidermis into the underlying dermis. Characterization of the rate of skin uptake is therefore important in predicting the absorbed dose.

Polycyclic aromatic hydrocarbons (PAHs) are commonly present in soil at or near hazardous waste sites and often drive risk and remedial decision making. Benzo[a]pyrene (BaP)—considered the primary risk driver under the current regulatory paradigm for PAH toxicity—ranked #8 on the Agency for Toxic Substances and Disease Registry (ATSDR) Priority List of Hazardous Substances in 2013, which is based on a combination of a contaminant's frequency, toxicity and potential for human exposure at National Priority List (NPL) sites (ATSDR, 2014). Several studies have attempted to measure the dermal absorption of BaP from contaminated soil or sediment, including *in vivo* studies performed on rats (Yang et al., 1989a) and rhesus monkeys (Wester et al., 1990), and *in vitro* experiments using skin of rats (Yang et al., 1989b; Yang et al., 1989a; Roy et al., 1992), pigs (Abdel-Rahman et al., 2002), guinea pigs (Moody and Chu, 1995), and humans (Wester et al., 1990; Roy et al., 1992; Moody and Chu, 1995; Roy et al., 1998; Roy and Singh, 2001; Stroo et al., 2005; Moody et al., 2007).

Fractional absorption is dependent on the mass of soil on the skin (the soil load) when the soil load covers the exposed area completely (*i.e.*, the *fraction* absorbed decreases as the soil load increases). Therefore, dermal absorption is best described in terms of gradient-driven flux, not percent absorption. Although fractional absorption has normally been reported by previous investigators, a recent review of dermal absorption studies of contaminated soils found that

average BaP uptake reported as flux from six of the studies listed above (Yang et al., 1989a; Wester et al., 1990; Roy et al., 1992; Roy et al., 1998; Roy and Singh, 2001; Abdel-Rahman et al., 2002) spanned a range of six orders of magnitude (0.19–420,000 pg/cm^2/h) (Spalt et al., 2009). A review of this literature is forthcoming (M. Ruby, personal communication) which notes that an important data gap in the existing literature is the effect of chemical concentration in the soil and soil characteristics.

To improve the general understanding of the potential for dermal absorption of PAHs from contaminated soil, *in vitro* assessments of absorption through human cadaver skin were conducted with four test soils spiked with radiolabeled BaP. For comparison with the soil measurements, absorption from BaP applied to skin in solvent was also evaluated. The present study was developed and performed with attention to important methodological criteria, including soil layering effects, appropriateness of particle size distribution employed, degree of chemical saturation of soil, and soil-chemical contact (*i.e.*, "aging") time. Prior experiments have mostly failed to account for one or more of these criteria in either reporting or execution (Spalt et al., 2009). Further considerations were implemented in the present study to produce conditions that represent realistic exposure scenarios; these included employing soil with BaP concentrations in a range that might reasonably be found at a contaminated site, and artificially weathering and aging spiked soils prior to experimental application.

METHODS

Chemicals. ^{14}C-BaP (specific activity = 26.6 μCi/μmol) in toluene was obtained from American Radiolabeled Chemicals (St Louis, MO). BaP-toluene stock solutions used to spike

soils were prepared with anhydrous toluene (99.8%, Sigma-Aldrich, St Louis, MO). For acetone-delivered experiments, toluene was removed by evaporation and BaP dissolved into acetone.

Study Soils. Soil experiments were conducted using the sub-63-μm fraction of four soils with varying total organic content (TOC) and black carbon (BC) as listed in **Table 1**. TOC was measured by combustion at 900°C after removal of inorganic carbon with hydrochloric acid, and BC content was measured using a chemo-thermal oxidation method (CTO-375) (Grossman and Ghosh, 2009). The CSU and ISU soils were collected from Colorado State University (Fort Collins, CO) and Iowa State University Agricultural Station (Ames, IA), respectively, and prepared following procedures described by Choate et al. (Choate et al., 2006a; Choate et al., 2006b). The Yolo soil, collected from the University of California (UC), Davis student farm (Reifenrath et al., 2002a) was acquired from the UC Davis soils lab, which was also the source of the Yolo County soil used by Wester et al. (1990; personal communication, R. Wester, 1994); see Supporting Information for additional details. The MTSS soil is a composite of soils collected from nine residences near the smelter in Anaconda, MT that has been used in oral bioavailability studies (Freeman et al., 1993; Freeman et al., 1995; Roberts et al., 2007).

Study Design. The study design is summarized in **Table 1**. Experiments were performed on weathered samples of all four soils and on unweathered samples of the MTSS and Yolo soils applied to skin from the same three donors. The soil load of approximately 30 mg/cm^2 was sufficient to cover the skin with multiple layers of particles. In the acetone-delivered experiments, approximately 80 ng/cm^2 of ^{14}C-BaP, which was similar to the mass applied in soils at the 3-mg/kg concentration, was deposited onto the skin surface in 50 μL of acetone. The experiments with BaP in weathered soil and acetone were randomized within two subsets (trials lasting 24 h and those lasting 4 or 8 h) that were performed in alternating weeks. The testing of

unweathered soils was completed in a single experimental run performed 2 days after the soil was prepared.

Soil Preparation. Soil concentrations of 3 and 10 mg/kg of BaP on 1.5 to 2 g of soil were achieved by adding 9 and 30 μL, respectively, of stock solution (325 μg/mL) per gram of the soils that were then subjected to weathering, and about 30 and 90 μL/g of stock solution at a concentration of 100 μg/mL to the soils tested unweathered. After spiking, the vials were placed onto a Labquake^{TM} rotator (Barnstead Thermolyne, Dubuque, IA) for 1 h, uncapped and placed in fume hood for 0.5-1 h to allow for volatization of toluene vehicle, and then recapped and placed back on the rotator for a total of 72 h of mixing to ensure sufficient homogenization of the radiolabeled BaP in the soils.

Artificial weathering of soils was conducted by adding 0.5 mL of deionized (DI) water (ACS reagent grade, Ricca Chemical Co, Arlington, TX) per gram of weathered soil once a week for 8 weeks to vials containing the eight test soils. The vials were then capped tightly for 3 days, after which the caps were removed and the samples were air dried for 2 days, followed by capping and mixing on the rotator for 2 more days. Experiments began 3 weeks after weathering was completed and were performed over a period of 14 weeks. During this time soils were stored at 3°C to limit any microbial activity. To test for homogenization, 5-mg aliquots of each test soil were taken in triplicate during each experimental run and analyzed; soil radioactivity compared across the experimental period showed no differences.

Skin Source and Preparation. Split-thickness human cadaver abdominal skin (~400 μm thick) was obtained from the National Disease Research Interchange (NDRI, Philadelphia, PA) and stored at –20°C until used. For highly lipophilic chemicals like BaP, the dermis can present a significant additional barrier to dermal absorption that is not present *in vivo*, because the dermis

is vascularized (Cross et al., 2003). Therefore, dermal absorption was measured through only the epidermis, which was prepared by placing skin samples, cut into usable sections while still partially frozen, into water at 60°C for one minute (Scheuplein, 1967). The epidermis was peeled carefully from the dermis and placed in DI water until it was positioned on the diffusion cells.

Diffusion Cell Experiments. Dermal absorption was measured using vertical flow-through Teflon^{TM} diffusion cells (9 mm, Series 1, in-line) from PermeGear (Bethlehem, PA), with a diffusion area of 0.64 cm^2 and a receptor volume of approximately 0.25 mL. The receptor fluid was 10 mM phosphate-buffered saline (PBS; 0.138 M NaCl; 0.0027 M KCl) with 4% bovine serum albumin (BSA) added to increase BaP solubility after degassing by vacuum filtration (0.45-μm pore size cellulose acetate membrane, Corning, Tewksbury, MA) to prevent bubbles in the system.

Twelve cells were used per experimental run. The epidermal membranes were mounted between the receptor chamber and donor chamber with the stratum corneum facing up. The twelve diffusion cells used in each experiment and sample collection system were housed within an environmental chamber in which temperature and relative humidity were controlled at 32°C and 40%, respectively. Once loaded with skin, the diffusion cells were equilibrated for approximately 12 h, with the receptor fluid flow rate delivered to each cell set to 0.6 mL/h. After equilibration, 20 mg of the test soils or 50 μL of the acetone-BaP solution were applied to the skin surface, and the receptor fluid flow rate to each cell from the multichannel Ismatec peristaltic pump (IDEX Health & Science, Oak Harbor, WA) was set to 1.5 mL/h and collected into borosilicate scintillation vials (VWR, Radnor, PA). The actual volume of receptor solution delivered to each cell was determined gravimetrically. The receptor fluid solution was collected at specified intervals throughout the 8- or 24-h exposure period (either 2, 4, and 8 h, or 3, 6, 12, and 24 h) and then mixed with 12 mL of scintillation cocktail.

At the end of each trial, 150 μL of DI water was pipetted into the donor chamber, and the skin surface was wiped with two dry cotton applicator tips (Puritan Medical, Guilford, ME). The moist soil was collected on the cotton, and the tips were clipped into scintillation vials ($\leq$2/vial). This wetting/wiping cycle was repeated twice per cell. The skin sample was then carefully removed from the receptor chamber with tweezers and rinsed by swirling the sample in DI water as a final cleaning step. The donor chamber was rinsed with Hionic-Fluor™ scintillation cocktail (Perkin Elmer, Waltham, MA), and the receptor chamber was wiped with a DI water-soaked cotton tip. All materials used in quantitating and loading the soils into the diffusion cells were rinsed with scintillation cocktail. Skin samples were solubilized in 2 mL of Soluene® 350 (PerkinElmer, Waltham, MA) with sonication (Branson Ultrasonics, Danbury, CT) for 2 h at 65° C and mixed with 10 mL of scintillation cocktail. Hionic-Fluor™ was used in all samples and rinses, except the receptor fluid and aqueous skin rinse solutions, which utilized Ultima Gold™ XR (also from Perkin Elmer).

Radiolabel Counting. Radioactivity of each sample vial was counted in a Beckman LS 6000SC liquid scintillation analyzer (Beckman Coulter, Brea, CA) five times over the course of 11 weeks. The averages of the five counts were used after adjusting for background from cocktail blanks (29.5 ± 0.46 disintegrations per minute (dpm), mean ± 95% confidence interval, corresponding to a detection limit of ~0.13 ng BaP). Vials containing high amounts of soil (*i.e.*, skin-washing materials and run-specific soil aliquots) experienced quenching during scintillation counting. For these vials 1-mL aliquots of well-mixed cocktail-soil solution were diluted with an additional 10 mL of cocktail and re-counted.

Data Analysis. Radioactivity counts in dpm were converted to BaP mass using 2.3×10^5 dpm/μg derived from the molecular weight of BaP (252.3 μg/μmol) and specific activity of the

^{14}C-BaP (26.6 μCi/μmol). Mass balances were calculated for each experimental trial. Experiments reported here were deliberately conducted at high soil loads to avoid issues with uneven distribution of soil on the skin surface, rendering direct reporting of fractional absorption inappropriate (Spalt et al., 2009; Kissel, 2011). The primary results normalized by skin surface area (A) were cumulative BaP mass in the receptor fluid (M_{rf}) and BaP mass recovered from the washed skin (including BaP that was in and/or on the skin) at the end of the exposure (M_{sk}), from which the average flux of BaP over the exposure duration into skin (J_{in}) and through skin and into the receptor fluid (J_{out}) were calculated. For risk assessment purposes, J_{in}, which includes chemical found in and/or on skin after washing and collected in the receptor fluid over the exposure period, is most relevant.

Statistical analyses were completed using Stata 12 (StataCorp, College Station, TX). One-way ANOVA and two-sample *t*-tests were employed to assess differences. Dixon's Q test was used to identify outliers at the 99% confidence level in BaP determinations of the receptor fluid and solubilized skin. Results are reported as mean with corresponding 95% confidence intervals (shown as error bars in figures, and as maximum errors on the mean in text and tables) calculated for all measurements.

RESULTS

The study results are summarized in **Table 2** and in the **Supporting Information** (**Tables S1 and S2**). Receptor fluid samples from two weathered-soil trials were rejected as outliers and all data from these trials were excluded in subsequent analyses. The average total radioactivity recovered was 101% (83%–117% range) for weathered-soil, 89% (85%–93%) for unweathered-soil and 75% (40%–98%) for acetone.

Weathered Soils. Results for M_{rf}/A, J_{out} and M_{sk}/A are presented in **Figure 1a–c.** No significant differences were seen among the four soil types for any of the endpoints measured. Accordingly, results for J_{in}, J_{out}, M_{rf}/A and M_{sk}/A presented in **Table 2** are averages across the test soils. Data for individual soil types are presented in **Tables S1 and S2**. No significant differences in M_{rf}/A were seen between the 3- and 10-mg/kg trials of the same duration (8-h: P=0.42; 24-h: P=0.79). At 24 h M_{rf}/A was greater than at 8 h by an amount that was proportional to the exposure duration (**Figure 1a**). As a result J_{out} was approximately constant across both exposure duration and soil concentration (**Figure 1b**). Within each soil concentration examined, no difference was seen in M_{sk}/A between 8-h and 24-h (3-mg/kg: P=0.55; 10-mg/kg: P=0.73). Values of M_{sk}/A in the 3 and 10-mg/kg trials were statistically different ($P \leq 0.0001$) by an amount that was approximately proportional to concentration.

Unweathered Soils. Results from trials with unweathered MTSS and Yolo soils are compared in **Figure 2** with results from the same two soils weathered. As with the weathered soils, no significant differences were seen between the two unweathered soils for any of the endpoints measured. After adjusting for the differences in the actual compared with nominal soil concentrations (multiplying by ratio of nominal to actual concentration), differences between the Yolo-MTSS average weathered and unweathered soils were not statistically significantly different for M_{rf}/A at either soil concentration or for M_{sk}/A in the 3-mg/kg trials ($P<0.90$). For soils at 10-mg/kg of BaP, M_{sk}/A was larger from the unweathered soils by a statistically significant difference (2.8±1.1 vs. 1.7±0.6 ng/cm^2, P=0.03). Driven by this greater recovery of BaP from skin, J_{in} was larger from weathered soils in the 10 mg/kg trials after adjusting for actual concentration (0.13±0.04 vs. 0.08±0.02 ng/cm^2/h, P=0.047).

Acetone Compared with Soils. Distribution of radioactivity observed in the acetone-delivered trials differed from the soil experiments: less mass was recovered in the skin surface wash, while a relatively larger mass was collected from the donor chamber (see **Table S1** for details). Compared with the weathered and unweathered soil experiments at similar BaP load (3-mg/kg concentration), M_{sk}/A for BaP delivered in acetone was approximately an order of magnitude greater (**Table 2**; P<0.0001) and did not vary with the length of the exposure for exposure times as small as 4 h. The appearance of BaP in the receptor fluid was similar from soil or acetone up to the 6 h measurement (**Figure 3**). After 24 h, M_{rf}/A was greater from the acetone-delivery experiments by approximately one order of magnitude compared to soil experiments at both concentrations. When delivered in acetone, J_{out} increased with time and was significantly greater than J_{out} in the soil experiments at 24 h (**Table 2**, P=0.002), which remained nearly constant for exposure periods of 6 h or greater.

DISCUSSION

Results from this study are discussed and then compared to prior studies and related to risk assessments.

Observations of this Study. Six significant outcomes have been identified, which taken together suggest that BaP contamination levels, although low, exceeded the sorbent capacity of the soils in this study and that the quantity of BaP measured in the skin is primarily attributable to residual soil not removed by the washing step.

(1) Absence of an Effect of Soil Concentration on Transfer to Receptor Fluid. Chemical penetration through skin is driven by thermodynamic activity, which, for a given vehicle, usually varies with concentration. A goal of this study was to examine the effect of BaP concentration on

dermal absorption within the constraints of adequate detection of BaP, which limited the lower soil concentration to 3 mg/kg, and the capacity of the test soils to sorb BaP (*i.e.*, the soil saturation limit), which is why a concentration higher than 10 mg/kg was not selected. The observation that M_{rf}/A did not vary with BaP concentration in either the weathered (**Figure 1**) or unweathered (**Figure 2**) soil experiments was unexpected. A plausible explanation for this finding is that the soil saturation limit for BaP was less than 3 mg/kg for all test soils, which caused the thermodynamic activity of BaP to be independent of soil concentration and soil type. Concentration invariance in dermal absorption measurements above the soil saturation limit has been demonstrated previously in experiments with methyl paraben on the 38-63 μm fraction of the same ISU and CSU soils tested in this study (Deglin, 2007).

To evaluate this possibility, the soil saturation limit ($C_{soil,sat}$) was estimated using Eq. (1), which has been proposed as suitable for non-ionizable lipophilic chemicals(Spalt et al., 2009):

$$C_{soil,sat} = TOC \times K_{oc} \times C_{w,sat} \quad (1)$$

In this equation $C_{w,sat}$ is the chemical saturation limit in water, and K_{oc} is the organic carbon water partition coefficient. For BaP, experimental values for $C_{w,sat}$ are reported to be 0.0016 mg/L (Miller et al., 1985), and 0.0038 mg/L (Mackay, 2001), and $\log K_{oc}$ (for K_{oc} in units of L/kg) is estimated to be 5.3 - 5.8 (USEPA, 2012a). Combining these numbers into Eq. (1) with the 1-4% TOC values of the test soils in this study, $C_{soil,sat}$ for BaP is estimated as 3 to 96 mg/kg, which is not too different from the soil concentrations in this study. Given the considerable uncertainty in the estimate of K_{oc} for BaP (Hassett et al., 1980; Means et al., 1980) and in the suitability of Eq. (1) for calculating $C_{soil,sat}$, soil saturation is a reasonable explanation of the observed results, especially as it is consistent with other observations described below.

(2) Absence of an Effect of Soil Characteristics on Uptake of BaP. These experiments were conducted using soils with a range of TOC and BC contents with the expectation that these characteristics would affect the sorbent capacity for BaP and hence, the thermodynamic activity and driving force for transfer from soil to and through the skin. No consistent effects of TOC or BC were observed for either the weathered or unweathered soils for any of the endpoints measured (**Figures 1 and 2**). This is consistent with the hypothesis that even the soils with the greatest expected sorbent capacity were saturated with BaP. Had sub-saturated soil concentrations been feasible within the limits of detection of the study, differences among soils might have been observed. A further consideration is that all soils were pre-sieved to the sub-63-µm fraction. Sorption on the increased surface areas associated with fine particles might have diminished the influence of carbon content (Deglin et al., 2010).

(3) Proportionality of Mass in Washed Skin to Soil Concentration. In both weathered and unweathered soils, M_{sk}/A varied directly with BaP concentration in the applied soil (**Figures 1c and 2b**). This observation could be explained by concentration-dependent transfer into the skin from soil, or by the amount of BaP found in the skin being primarily attributable to residual soil that was not removed by washing. The former explanation is not consistent with soil saturation. Saturation is supported by the observations that M_{rf}/A was not affected by differences in soil concentration or type described above. The latter explanation is further supported by a lack of time dependence of skin residues, which is necessary but not sufficient evidence (see next paragraph). If post-wash skin residues are attributable primarily to unrecovered soil, the amount of soil that would have to remain on the skin to yield these results would be on the order of 0.1 mg/cm^2, which is certainly plausible given initial soil loads of approximately 30 mg/cm^2 and an estimated monolayer load of about 1 mg/cm^2.

(4) Absence of Time–dependence on BaP in Skin Residues following Soil Exposure. If M_{sk}/A is dominated by unremoved soil, relevant processes should be relatively rapid in an *in vitro* system, *i.e.*, particles that cannot be cleaned from the skin surface probably adhere soon after the soil is applied. Therefore, M_{sk}/A should be insensitive to experimental duration. This is consistent with the observation of no difference in the 8-h and 24-h trials (**Figure 1c, Table 2**), although a more rigorous test would have been measurement M_{sk}/A after less than an hour of exposure.

(5) Absence of a Clear Effect of Weathering. No statistically significant difference was observed in M_{rf}/A from the weathered and unweathered soils (**Figure 2a**). This can be explained by saturation of the soils, which could have caused some readily available BaP to remain at the surface of each soil after weathering. Alternatively, the artificial weathering process applied here may have been insufficiently rigorous or carried out over too little time to see an effect. Indeed, in the only reliable study of BaP aging (but not weathering) Roy and Singh (2001) observed no effect after 45 days and only a 2-fold effect on M_{rf}/A after 110 days. Weathering did appear to effect M_{sk}/A at the 10 mg/kg concentration (**Figures 2b-c**). This could occur if more neat BaP is held less tightly on the outer surfaces of a saturated soil before weathering compared with after weathering. In this scenario, transfer of loosely held particles of neat BaP could increase the total amount of BaP in skin above that from soil particles left after washing. This effect would be greater for the soils contaminated with more BaP, which could explain the observation that M_{sk}/A was statistically significantly different in 10-mg/kg trials but not 3 mg/kg-trials.

(6) Vehicle-dependent Time Course of BaP Penetration to Receptor Fluid. The quantity of BaP in the receptor fluid at time periods less than 6 h was essentially the same from soil or acetone (**Figure 3**). This is again consistent with the hypothesis that soils were saturated. Free BaP on outer surfaces of soil grains in direct contact with the skin would behave similarly to BaP

deposited in acetone. Lack of increase after 6 h in the rate of uptake from soil, in contrast to uptake from acetone, likely reflects differences in direct skin contact to BaP. All BaP delivered by acetone is at the skin surface, whereas a large fraction of the BaP applied in multiple layers of soil is some distance from the skin surface.

Comparison with Prior Results. Prior investigations of dermal absorption of BaP from soil have been reviewed elsewhere (Spalt et al., 2009). The U.S. Environmental Protection Agency (USEPA) currently recommends that risk assessments assume the dermal absorption of PAHs from soils to be 13% of the total applied dose without consideration of differences in soils, exposure period, soil load or which PAH species (USEPA, 2004). This guidance relies on a subset of experiments reported by Wester et al. (1990) that were conducted *in vivo* using rhesus monkeys and BaP. The experiments by Wester et al. were similar to varying degrees with experiments that are reported in this study. In their soil experiments, Wester et al. used one of the four soils used here (Yolo) but sieved to 180–320 µm rather than to sub-63 µm. At this particle size fraction, their nominal soil load of 40 mg/cm^2, although larger than in this study, is estimated to cover the skin with only a single layer of particles (Duff and Kissel, 1996; Spalt et al., 2009). The initial BaP concentration of 10 mg/kg matched the high concentration in this study. Wester et al. also conducted *in vitro* experiments with human skin, and applied BaP in acetone as well as soil. Soil and acetone results from Wester et al., which were all 24-h exposures, are compared to this study in **Table 3** in terms of J_{in} and J_{out}.

Absorption measurements in the rhesus monkeys were calculated by dividing the amount of radiotracer collected in excreta over 7 days by 6.6%, which was the fraction collected over 7 days following intravenous administration. The result should represent BaP that penetrated through the skin in 24 h, plus residual BaP in the skin after washing at the end of the 24-h

exposure period and subsequently subject to systemic uptake. This corresponds to J_{in} equal to 2.2 ng/cm^2-h, or a cumulative uptake into and penetration through the skin of 52.8 ng/cm^2. The study's *in vitro* results using human skin were roughly one order of magnitude lower. Results obtained *in vitro* here are similar to the Wester et al. *in vitro* results with respect to J_{in} (*i.e.*, substantially lower than the Wester et al. *in vivo* results). Higher J_{out} in the current study compared with the *in vitro* experiments from Wester et al. for both soil and acetone experiments is consistent with the different skin sample preparations used in the two studies (*i.e.*, heat-separated versus dermatomed skin). The hydrophilic dermis layer in dermatomed skin has been shown to present an additional barrier to highly lipophilic chemicals like BaP that is missing entirely in the heat separated skin (Cross et al., 2003).

Questions have been raised previously regarding the Wester et al. *in vivo* trials (Spalt et al., 2009). Dermal soil experiments conducted *in vivo* in surrogate animals are limited by inherent difficulties in controlling animal behaviors. Soil was applied to the monkeys in the Wester et al. trials while they were anesthetized and lying on their backs. They were then positioned upright in metabolic chairs with restraints. Given the large particle sizes used (180 to 300 µm; fine to coarse sand) and the volume of the eye-guard used to cover the application site, it is unclear that soil-skin contact could reasonably be expected to be maintained for 24 h. However, if the soil was supersaturated and/or if the solvent used to deliver the BaP to the soil had not completely evaporated, initial transfer from soil surfaces or solvent could have rendered longer term soil contact irrelevant.

In vivo and *in vitro* J_{in} observed by Wester et al. subsequent to acetone-delivery of BaP are less disparate than the corresponding *in vivo/vitro* soil results, suggesting that much of the *in vivo/vitro* soil differential is attributable to something other than interspecies skin differences.

Acetone deposition experiments reported here were conducted at lower initial chemical load (80 ng/cm^2 compared with 500 ng/cm^2 in Wester et al.), which, as expected due to lower surface coverage, produced a proportionally lower J_{in}. When multiplied by the ratio of the chemical load in the two studies (*i.e.*, 500/80), the extrapolation of J_{in} from this study to the chemical load used by Wester et al. study is 7.5 ng/cm^2-h, which is between the *in vivo* and *in vitro* J_{in} (11 and 5, respectively) from Wester et al. Average J_{in} from acetone reported here is similar to the Wester et al. *in vivo* soil result, which further suggests that the latter may represent transfer from residual solvent rather than from soil.

Application to Risk Assessment. BaP is the index chemical for PAH risk assessment. In addition, in 2013, USEPA proposed, for the first time, a dermal carcinogenic slope factor for BaP (USEPA). That value is undergoing further review, but could potentially increase the importance of dermal exposures in evaluation of risks from contaminated sites. A careful review of the cancer bioassay studies upon which the proposed dermal slope factor was derived suggests that estimates of cancer risk should be based on absorbed rather than exposed dose, making the results of the experiments reported here directly relevant to the assessment of cancer risks from exposure to PAHs. In addition, the presumed primacy of the Wester et al. (1990) *in vivo* experiments is called into question. On the other hand, evidence is presented for rapid adherence of a portion of soil-borne contaminant. Some of that rapidly adhering mass may be on fine particles that are not easily removed. A health-protective assumption would be that non-removable particle-bound material is functionally equivalent to the same mass of neat compound in the outer layers of skin. Results presented here also highlight the importance of soil concentration relative to sorption capacity. Soils may be weak sorbents, with low-mg/kg levels of BaP representing soil saturation. Yet levels of BaP in soils are routinely found at

concentrations used in this study (EPRI, 2008a), suggesting soils in the environment might exist at super-saturated conditions. However, the saturation limit of soils might be influenced by source of the BaP: sorption capacity might increase if soil is amended with partitioning phases in the form of soot or other carbonaceous material. Experiments here used pure chemical to spike soils which might have contributed to exceeding saturation. Nevertheless, data reported here suggest that uptake from saturated soil is slower than a similar amount of BaP deposited from acetone.

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FIGURES AND TABLES

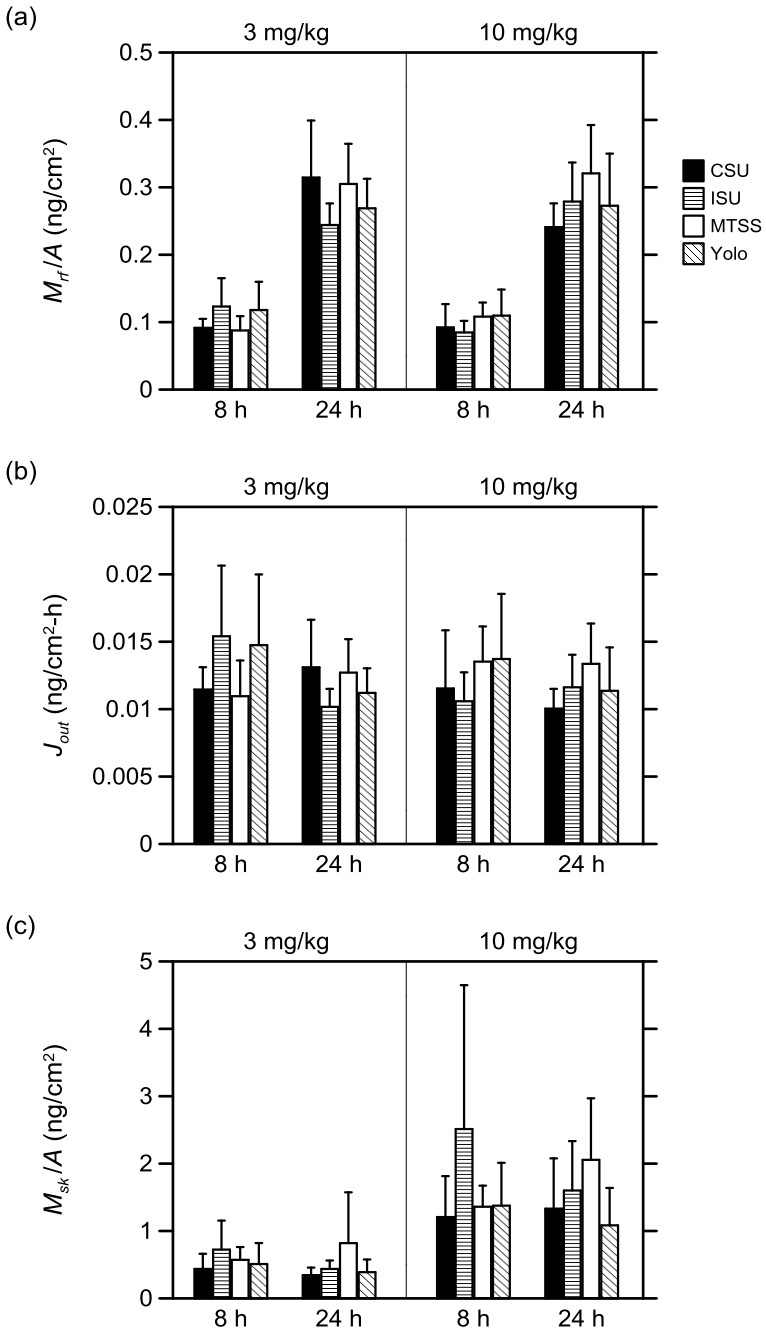

Figure 1. Results for four weathered soils at 3 and 10 mg/kg BaP concentration after 8- and 24-h exposures: (a) M_{rf}/A, (b) J_{out}, and (c) M_{sk}/A. Error bars represent 95% confidence intervals.

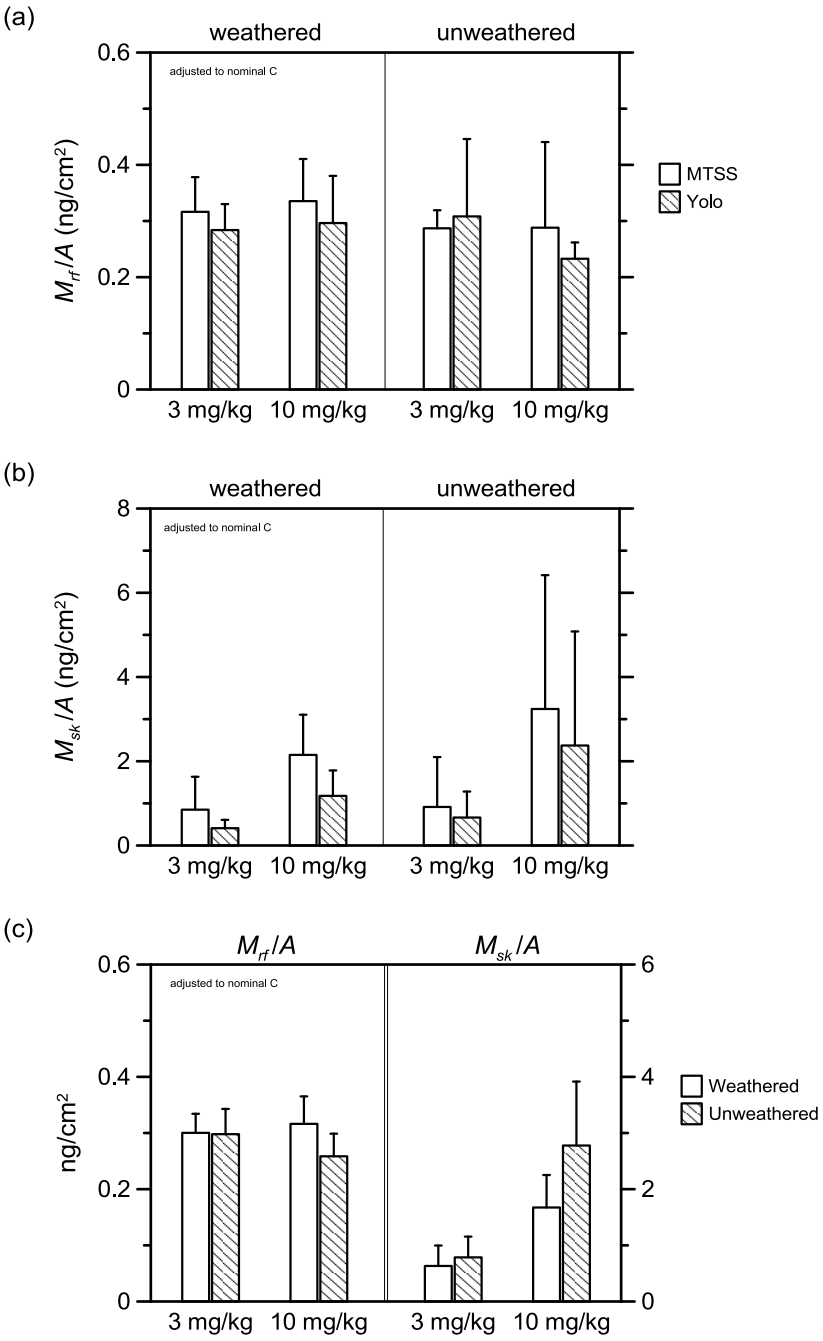

Figure 2. Results for weathered and unweathered Yolo and MTSS soils adjusted to the nominal BaP concentrations of 3 and 10 mg/kg after a 24-h exposure: (a) M_{rf}/A for each soil, (b) M_{sk}/A for each soil, and (c) average of Yolo and MTSS soils combined for M_{rf}/A (left axis) and M_{sk}/A (right axis); n = 3 and 6 for each unweathered and weathered soil, respectively. Error bars represent 95% confidence intervals.

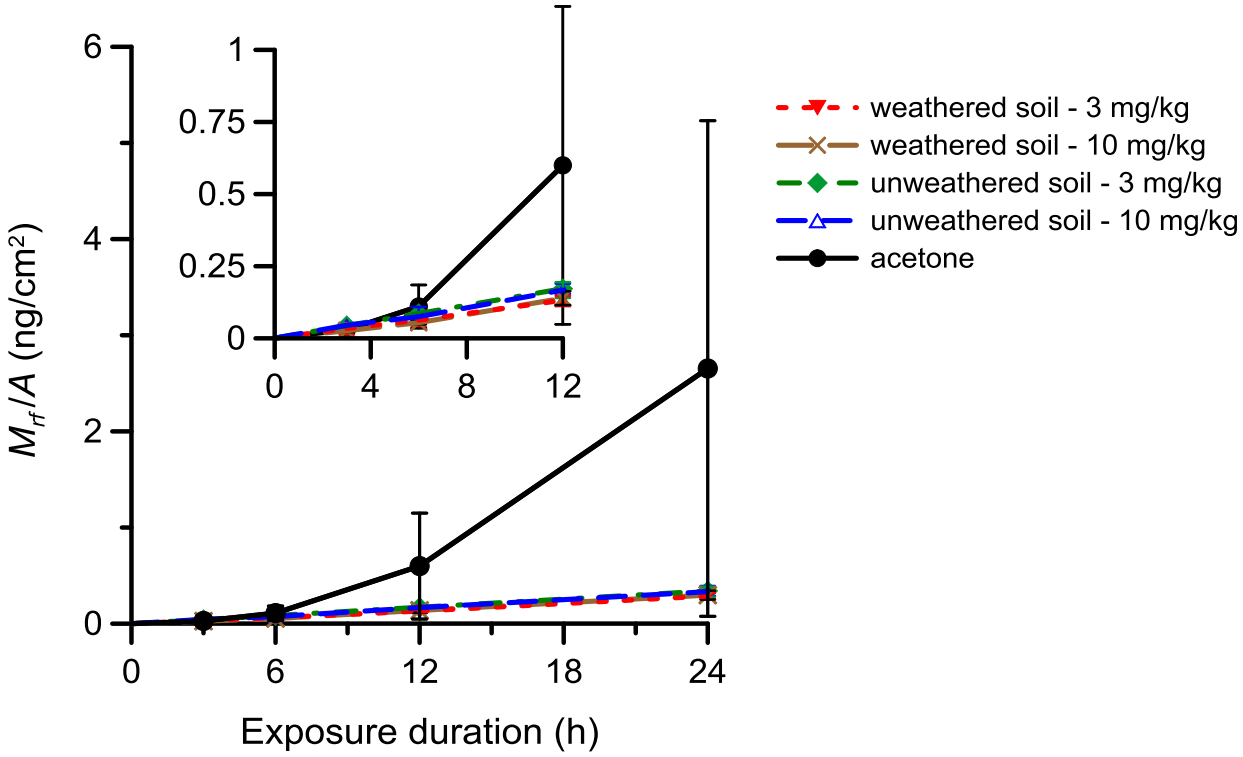

Figure 3. M_{rf}/A from BaP deposited in acetone and from BaP in the weathered and unweathered MTSS and Yolo soils at 3 and 10 mg/kg BaP concentrations. Lines connecting the results are drawn to guide the eye. Inset is an enlargement of the data to 12 h. Error bars represent 95% confidence intervals.

Table 1. Experimental matrix and carbon content of sub-63-μm fraction of the test soils

Vehicle	**Soils studied**	**Nominal C**	**Durations**	**Replicates**[a]
		mg/kg	h	
weathered soil	CSU, ISU, MTSS, Yolo	3, 10	8, 24	2
unweathered soil	MTSS, Yolo	3, 10	24	1
acetone	n/a	n/a	4, 8, 24	2
Soil	**CSU**	**ISU**	**MTSS**	**Yolo**
TOC[b] (%)	0.99	3.1	3.9	0.97
BC[b] (%)	0.14	0.23	1.2	0.09

[a] All studies were performed on the same 3 donors. [b] Determinations of total organic carbon (TOC) and black carbon (BC) are from U Ghosh (University of Maryland, Baltimore County)

Table 2. Summary results of BaP absorption into and through skin from soil or deposited onto the skin from acetone[a]

# of soils	n	Weathered?	t_{exp}	Nominal Soil *C*	Measured Soil *C*	BaP load	Mass balance	J_{in}	J_{out}	M_{sk}/A	M_{rf}/A
		Y/N	h	mg/kg	mg/kg	ng/cm^2	%	ng/cm^2-h	ng/cm^2-h	ng/cm^2	ng/cm^2
4	23	Y	8	3	2.7 (0.07)	82.7 (1.6)	103 (3.2)	0.083 (0.016)	0.013 (0.002)	0.56 (0.13)	0.11 (0.014)
4	24	Y	24	3	2.8 (0.05)	84.1 (1.6)	99.2 (2.6)	0.033 (0.008)	0.012 (0.001)	0.50 (0.17)	0.28 (0.025)
4	24	Y	8	10	8.8 (0.21)	271 (5.1)	103 (2.8)	0.21 (0.062)	0.012 (0.001)	1.6 (0.49)	0.10 (0.012)
4	23	Y	24	10	9.1 (0.17)	279 (5.0)	98.7 (2.4)	0.075 (0.014)	0.012 (0.001)	1.5 (0.32)	0.28 (0.027)
2[b,c]	12	Y	24	3	2.9 (0.05)	87.7 (1.7)	98.6 (4.3)	0.037 (0.015)	0.012 (0.001)	0.60 (0.35)	0.29 (0.032)
2[b,c]	12	Y	24	10	9.4 (0.16)	293 (5.9)	98.1 (1.7)	0.078 (0.023)	0.012 (0.002)	1.6 (0.54)	0.30 (0.046)
2[b]	6	N	24	3	3.4 (0.12)	104 (4.4)	89.7 (3.0)	0.052 (0.017)	0.014 (0.002)	0.90 (0.42)	0.34 (0.051)
2[b]	6	N	24	10	12.9 (1.0)	396 (24)	89.2 (2.0)	0.16 (0.062)	0.014 (0.002)	3.6 (1.5)	0.33 (0.052)
n/a[d]	6	--	4	--	--	81.7 (4.8)	72.0 (21)	6.4 (2.4)	0.011 (0.007)	25.6 (9.6)	0.044 (0.027)
n/a[d]	6	--	8	--	--	79.4 (4.3)	76.3 (24)	3.1 (0.88)	0.022 (0.017)	24.9 (7.0)	0.18 (0.14)
n/a[d]	6	--	24	--	--	76.7 (4.0)	77.2 (11)	1.2 (0.24)	0.11 (0.11)	26.1 (4.4)	2.7 (2.6)

[a] Experimental results presented as mean (maximum error of the mean at 95% confidence level); *n* = total number of experimental measurements, *C* = concentration; t_{exp} = time of exposure; J_{in} = average flux over exposure period into skin; J_{out} = average flux over exposure period through skin and into receptor fluid; M_{sk}/A = mass of BaP per skin surface area recovered from washed skin; M_{rf}/A = mass of BaP per skin surface area recovered from receptor fluid. [b] MTSS and Yolo soils only. [c] Data also included in results of weathered trials with all 4 soils; presented for comparison with unweathered soil experiments. [d] BaP delivered to skin surface via acetone-vehicle.

Table 3. Average BaP flux into and through skin over 24 hours from Yolo soil and deposited onto the skin with acetone measured in this study compared with results from Wester et al. (1990) [a]

Study/vehicle	Type	Species	n	Soil Weath-ered?	t_{exp}	Nominal Soil *C*	Nominal BaP load	J_{in}	J_{out}
					h	mg/kg	ng/cm^2	ng/cm^2-h	ng/cm^2-h
Soil									
Wester et al. 1990	*in vivo*	Monkey	4	N	24	10	400	2.2 (0.88)	n/a[b]
	in vitro	Human	6	N	24	10	400	0.24 (0.18)	0.0017 (0.0007)
This study	*in vitro*	Human	6	Y	24	10	300	0.057 (0.026)	0.011 (0.003)
	in vitro	Human	3	N	24	10	300	0.15 (0.16)	0.013 (0.002)
Acetone									
Wester et al. 1990	*in vivo*	Monkey	4	--	24	--	500	11 (7.3)	n/a[b]
	in vitro	Human	6	--	24	--	500	5.0 (2.1)	0.019 (0.012)
This study	*in vitro*	Human	6	--	24	--	80	1.2 (0.25)	0.11 (0.11)

[a] Experimental results presented as mean (maximum error of the mean at 95% confidence level); n = total number of experimental measurements, C = concentration; t_{exp} = time of exposure; J_{in} = average flux over exposure period into skin; J_{out} = average flux over exposure period through skin and into receptor fluid. [b] Not available; experimental protocol prevented determination of this number.

Table S1. Average BaP mass recovered from each experimental compartment and mass balance for all study conditions, including by individual soil type[a]

Vehicle	Weath-ered?	t_{exp}	nominal C		BaP mass loaded			mass recovered from: receptor fluid			washed skin			donor chamber rinse			skin washing[b]			cell wipe			% of applied dose recovered		
	Y/N	hr	mg/kg	n	ng/cm^2	± SD	± E	ng	± SD	± E	ng	± SD	± E	ng	± SD	± E	ng	± SD	± E	ng	± SD	± E	%	± SD	± E
Weathered Trials																									
CSU	yes	8	3	6	83.0	2.1	2.2	0.058	0.008	0.008	0.28	0.14	0.14	0.23	0.24	0.25	53.3	1.5	1.5	0.28	0.64	0.68	103	6.2	6.5
ISU	yes	8	3	6	76.8	3.3	3.5	0.078	0.025	0.027	0.46	0.26	0.27	0.25	0.09	0.09	51.7	3.0	3.2	0.28	0.50	0.52	108	8.9	9.3
MTSS[c]	yes	8	3	5	85.0	3.7	4.7	0.056	0.011	0.013	0.36	0.10	0.12	0.36	0.43	0.53	53.2	3.8	4.7	1.04	2.27	2.82	102	5.8	7.2
Yolo	yes	8	3	6	86.1	2.7	2.8	0.075	0.025	0.027	0.32	0.19	0.20	0.20	0.09	0.09	54.4	1.7	1.8	0.062	0.08	0.082	101	7.4	7.8
			Group:	**23**	**82.7**	**3.7**	**1.6**	**0.067**	**0.021**	**0.009**	**0.36**	**0.19**	**0.08**	**0.26**	**0.23**	**0.10**	**53.1**	**2.6**	**1.1**	**0.42**	**1.11**	**0.48**	**103**	**7.4**	**3.2**
CSU	yes	24	3	6	82.5	3.7	3.9	0.20	0.051	0.054	0.22	0.07	0.07	0.28	0.17	0.17	50.5	2.8	2.9	0.185	0.25	0.26	98	6.1	6.4
ISU	yes	24	3	6	78.3	2.5	2.6	0.16	0.017	0.018	0.28	0.08	0.08	0.13	0.05	0.06	49.9	3.9	4.1	0.098	0.13	0.14	102	5.0	5.3
MTSS	yes	24	3	6	87.9	3.1	3.2	0.19	0.036	0.038	0.52	0.46	0.48	0.19	0.09	0.09	55.6	2.7	2.8	0.008	0.00	0.003	101	7.1	7.4
Yolo	yes	24	3	6	87.6	2.6	2.7	0.17	0.027	0.028	0.25	0.11	0.12	0.19	0.15	0.15	52.8	4.1	4.3	0.032	0.03	0.033	96	5.9	6.1
			Group:	**24**	**84.1**	**3.8**	**1.6**	**0.18**	**0.037**	**0.015**	**0.32**	**0.26**	**0.11**	**0.20**	**0.13**	**0.05**	**52.2**	**3.9**	**1.7**	**0.081**	**0.15**	**0.063**	**99**	**6.1**	**2.6**
CSU	yes	8	10	6	278	8.5	8.9	0.061	0.020	0.021	0.77	0.37	0.39	0.84	0.53	0.55	174	7.0	7.3	0.49	1.15	1.21	100	3.9	4.1
ISU	yes	8	10	6	243	2.4	2.5	0.054	0.010	0.011	1.6	1.3	1.4	1.3	1.3	1.4	168	8.1	8.5	1.2	1.8	1.9	112	3.4	3.6
MTSS	yes	8	10	6	277	5.9	6.2	0.069	0.013	0.013	0.87	0.19	0.20	0.64	0.52	0.54	179	3.3	3.5	0.40	0.95	0.99	103	3.7	3.9
Yolo	yes	8	10	6	285	5.1	5.3	0.072	0.021	0.022	0.88	0.39	0.40	0.38	0.21	0.22	175	7.1	7.5	0.084	0.15	0.16	97	4.8	5.0
			Group:	**24**	**271**	**12.0**	**5.1**	**0.064**	**0.017**	**0.007**	**1.0**	**0.74**	**0.31**	**0.79**	**0.78**	**0.33**	**174**	**7.3**	**3.1**	**0.55**	**1.2**	**0.50**	**103**	**6.7**	**2.8**
CSU	yes	24	10	6	275	3.8	4.0	0.16	0.013	0.013	0.85	0.45	0.47	0.71	0.50	0.52	171	11.8	12.4	0.28	0.65	0.68	99	4.8	5.0
ISU[c]	yes	24	10	5	257	2.9	3.5	0.18	0.030	0.037	1.0	0.38	0.47	0.57	0.30	0.37	161	14.8	18.3	0.19	0.31	0.38	100	10.9	13.5
MTSS	yes	24	10	6	295	11.7	12.2	0.20	0.044	0.046	1.3	0.55	0.58	0.78	0.40	0.42	180	12.2	12.8	0.51	1.22	1.28	97	3.2	3.3
Yolo	yes	24	10	6	291	7.0	7.4	0.17	0.047	0.049	0.69	0.34	0.35	0.56	0.42	0.44	181	9.7	10.2	0.025	0.03	0.030	99	2.0	2.0
			Group:	**23**	**279**	**11.6**	**5.0**	**0.18**	**0.038**	**0.016**	**0.97**	**0.47**	**0.20**	**0.65**	**0.40**	**0.17**	**173**	**13.8**	**6.0**	**0.25**	**0.70**	**0.30**	**99**	**5.6**	**2.4**
Unweathered Trials																									
MTSS	no	24	3	3	98.7	1.5	3.8	0.20	0.009	0.023	0.64	0.34	0.83	0.41	0.28	0.69	56.1	1.9	4.8	0.075	0.105	0.260	91	1.7	4.2
Yolo	no	24	3	3	110	1.8	4.5	0.23	0.042	0.103	0.50	0.19	0.46	0.42	0.09	0.21	60.4	2.0	5.1	0.050	0.057	0.140	88	3.0	7.5
			Group:	**6**	**104**	**4.2**	**4.4**	**0.22**	**0.031**	**0.033**	**0.57**	**0.26**	**0.27**	**0.41**	**0.18**	**0.19**	**58.2**	**3.0**	**3.1**	**0.062**	**0.077**	**0.080**	**90**	**2.8**	**3.0**
MTSS	no	24	10	3	366	11.3	28.1	0.22	0.047	0.116	2.47	0.98	2.42	0.68	0.21	0.52	200	6.0	14.8	4.1	7.0	17.4	89	2.4	5.9
Yolo	no	24	10	3	427	2.9	7.2	0.20	0.010	0.025	2.08	0.96	2.37	0.81	0.068	0.17	239	7.5	18.7	0.024	0.019	0.048	89	1.9	4.8
			Group:	**6**	**396**	**22.5**	**23.7**	**0.21**	**0.031**	**0.033**	**2.28**	**0.89**	**0.93**	**0.74**	**0.15**	**0.16**	**219**	**21.9**	**22.9**	**2.1**	**5.0**	**5.2**	**89**	**1.9**	**2.0**
Acetone-vehicle Trials																									
BaP-acetone	--	4	--	6	**81.7**	**4.5**	**4.8**	**0.028**	**0.016**	**0.017**	**16.3**	**5.8**	**6.1**	**14.0**	**6.2**	**6.5**	**6.6**	**8.4**	**8.8**	**0.60**	**0.48**	**0.51**	**72**	**20.1**	**21.1**
BaP-acetone	--	8	--	6	**79.4**	**4.1**	**4.3**	**0.11**	**0.084**	**0.088**	**15.9**	**4.3**	**4.5**	**11.1**	**5.6**	**5.9**	**10.8**	**9.4**	**9.8**	**0.60**	**0.87**	**0.91**	**76**	**22.9**	**24.1**
BaP-acetone	--	24	--	6	**76.7**	**3.8**	**4.0**	**1.7**	**1.6**	**1.6**	**16.6**	**2.6**	**2.8**	**10.6**	**3.3**	**3.5**	**7.9**	**6.5**	**6.8**	**0.81**	**1.02**	**1.07**	**77**	**10.2**	**10.7**
Subset of Weathered Trials for Comparison with Unweathered Trials																									
MTSS	yes	24	3	6	87.9	3.1	3.2	0.19	0.036	0.038	0.52	0.46	0.48	0.19	0.09	0.09	55.6	2.7	2.8	0.008	0.003	0.003	101	7.1	7.4
Yolo	yes	24	3	6	87.6	2.6	2.7	0.17	0.027	0.028	0.25	0.11	0.12	0.19	0.15	0.15	52.8	4.1	4.3	0.032	0.031	0.033	96	5.9	6.1
			Group:	**12**	**87.7**	**2.7**	**1.7**	**0.18**	**0.033**	**0.021**	**0.38**	**0.35**	**0.22**	**0.19**	**0.11**	**0.07**	**54.2**	**3.6**	**2.3**	**0.020**	**0.024**	**0.016**	**98.6**	**6.8**	**4.3**
MTSS	yes	24	10	6	295	11.7	12.2	0.20	0.044	0.046	1.31	0.55	0.58	0.78	0.40	0.42	180	12.2	12.8	0.51	1.22	1.28	97	3.2	3.3
Yolo	yes	24	10	6	291	7.0	7.4	0.17	0.047	0.049	0.69	0.34	0.35	0.56	0.42	0.44	181	9.7	10.2	0.03	0.03	0.03	99	2.0	2.0
			Group:	**12**	**293**	**9.3**	**5.9**	**0.19**	**0.046**	**0.029**	**1.0**	**0.54**	**0.35**	**0.67**	**0.41**	**0.26**	**181**	**10.5**	**6.7**	**0.27**	**0.86**	**0.55**	**98.1**	**2.6**	**1.7**

[a] n = total number of experimental measurements; C = concentration; t_{exp} = time of exposure; SD = standard deviation; E = maximum error of the mean at a 95% confidence level. [b] Due to high amounts of soil in these vials, 1 mL aliquots of well-mixed cocktail-soil solution were added to an additional 10 mL of cocktail and re-counted. [c] One cell had receptor fluid values that failed outlier analysis (Dixon's Q test at 99% level).

Table S2. Average flux into and through skin for all experimental conditions, including by individual soil type[a]

Vehicle	Weathered?	t_{exp}	nominal		measured			average flux:									average mass/area:			
			C		C^b			J_{out}			J_{in}				M_{rf}/A			M_{sk}/A		
	(yes/no)	hr	mg/kg	n	mg/kg	± SD	± E	ng/cm2-hr	± SD	± E	ng/cm2-hr	± SD	± E	ng/cm2	± SD	± E	ng/cm2	± SD	± E
Weathered Trials																				
CSU	yes	8	3	6	2.7	0.13	0.14	0.011	0.002	0.002	0.066	0.026	0.028	0.092	0.013	0.013	0.44	0.21	0.23
ISU	yes	8	3	6	2.6	0.16	0.16	0.015	0.005	0.005	0.106	0.054	0.056	0.12	0.040	0.042	0.72	0.41	0.43
MTSS[c]	yes	8	3	5	2.8	0.16	0.20	0.011	0.002	0.003	0.082	0.020	0.025	0.088	0.017	0.021	0.57	0.15	0.19
Yolo	yes	8	3	6	2.8	0.14	0.15	0.015	0.005	0.005	0.078	0.036	0.038	0.12	0.005	0.005	0.51	0.30	0.31
			Group:	**23**	**2.7**	**0.17**	**0.07**	**0.013**	**0.004**	**0.002**	**0.083**	**0.038**	**0.016**	**0.11**	**0.033**	**0.014**	**0.56**	**0.29**	**0.13**
CSU	yes	24	3	6	2.8	0.13	0.13	0.013	0.003	0.004	0.027	0.007	0.007	0.31	0.081	0.085	0.35	0.11	0.11
ISU	yes	24	3	6	2.7	0.07	0.08	0.010	0.001	0.001	0.028	0.005	0.005	0.24	0.031	0.032	0.44	0.12	0.12
MTSS	yes	24	3	6	2.9	0.09	0.09	0.013	0.002	0.002	0.047	0.031	0.033	0.31	0.057	0.060	0.82	0.72	0.75
Yolo	yes	24	3	6	2.8	0.08	0.08	0.011	0.002	0.002	0.027	0.009	0.010	0.27	0.042	0.044	0.39	0.18	0.19
			Group:	**24**	**2.8**	**0.12**	**0.05**	**0.012**	**0.002**	**0.001**	**0.033**	**0.018**	**0.008**	**0.28**	**0.059**	**0.025**	**0.50**	**0.40**	**0.17**
CSU	yes	8	10	6	9.0	0.09	0.10	0.012	0.004	0.004	0.16	0.075	0.079	0.09	0.033	0.035	1.2	0.58	0.61
ISU	yes	8	10	6	8.1	0.16	0.17	0.011	0.002	0.002	0.32	0.26	0.27	0.08	0.016	0.017	2.5	2.0	2.14
MTSS	yes	8	10	6	9.0	0.31	0.32	0.014	0.002	0.003	0.18	0.039	0.041	0.11	0.020	0.021	1.4	0.30	0.31
Yolo	yes	8	10	6	9.3	0.19	0.19	0.014	0.005	0.005	0.19	0.080	0.084	0.11	0.037	0.039	1.4	0.61	0.64
			Group:	**24**	**8.8**	**0.49**	**0.21**	**0.012**	**0.004**	**0.001**	**0.21**	**0.15**	**0.062**	**0.10**	**0.028**	**0.012**	**1.6**	**1.2**	**0.49**
CSU	yes	24	10	6	9.1	0.08	0.08	0.010	0.001	0.001	0.066	0.030	0.031	0.24	0.034	0.035	1.3	0.71	0.75
ISU[c]	yes	24	10	5	8.6	0.26	0.32	0.012	0.002	0.002	0.078	0.025	0.032	0.28	0.047	0.058	1.6	0.59	0.73
MTSS	yes	24	10	6	9.6	0.20	0.21	0.013	0.003	0.003	0.099	0.036	0.038	0.32	0.068	0.072	2.1	0.87	0.91
Yolo	yes	24	10	6	9.2	0.11	0.12	0.011	0.003	0.003	0.057	0.024	0.026	0.27	0.074	0.077	1.1	0.53	0.55
			Group:	**23**	**9.1**	**0.39**	**0.17**	**0.012**	**0.003**	**0.001**	**0.075**	**0.032**	**0.014**	**0.28**	**0.062**	**0.027**	**1.5**	**0.75**	**0.32**
Unweathered Trials																				
MTSS	no	24	3	3	3.3	0.00	0.00	0.013	0.001	0.001	0.06	0.021	0.053	0.32	0.014	0.036	1.0	0.53	1.3
Yolo	no	24	3	3	3.5	0.00	0.00	0.015	0.003	0.007	0.05	0.014	0.034	0.36	0.065	0.162	0.78	0.29	0.73
			Group:	**6**	**3.4**	**0.11**	**0.12**	**0.014**	**0.002**	**0.002**	**0.05**	**0.017**	**0.017**	**0.34**	**0.049**	**0.051**	**0.90**	**0.40**	**0.42**
MTSS	no	24	10	3	12.0	0.00	0.00	0.014	0.003	0.008	0.18	0.065	0.160	0.35	0.074	0.18	3.9	1.5	3.8
Yolo	no	24	10	3	13.8	0.00	0.00	0.013	0.001	0.002	0.15	0.063	0.156	0.32	0.016	0.040	3.3	1.5	3.7
			Group:	**6**	**12.9**	**0.99**	**1.03**	**0.014**	**0.002**	**0.002**	**0.16**	**0.059**	**0.062**	**0.33**	**0.050**	**0.052**	**3.6**	**1.4**	**1.5**
Acetone-vehicle Trials																				
BaP-acetone	--	4	--	6	--	--	--	0.011	0.006	0.007	6.4	2.3	2.4	0.044	0.026	0.027	25.6	9.2	9.6
BaP-acetone	--	8	--	6	--	--	--	0.022	0.016	0.017	3.1	0.84	0.88	0.18	0.13	0.14	24.9	6.7	7.0
BaP-acetone	--	24	--	6	--	--	--	0.11	0.10	0.11	1.2	0.23	0.24	2.7	2.5	2.6	26.1	4.2	4.4
Subset of Weathered Trials for Comparison with Unweathered Trials																				
MTSS	yes	24	3	6	2.9	0.09	0.09	0.013	0.002	0.002	0.047	0.031	0.033	0.31	0.057	0.060	0.82	0.72	0.75
Yolo	yes	24	3	6	2.8	0.08	0.08	0.011	0.002	0.002	0.027	0.009	0.010	0.27	0.042	0.044	0.39	0.18	0.19
			Group:	**12**	**2.9**	**0.08**	**0.05**	**0.012**	**0.002**	**0.001**	**0.037**	**0.024**	**0.015**	**0.29**	**0.051**	**0.032**	**0.60**	**0.55**	**0.35**
MTSS	yes	24	10	6	9.6	0.20	0.21	0.013	0.003	0.003	0.099	0.036	0.038	0.32	0.068	0.072	2.1	0.87	0.91
Yolo	yes	24	10	6	9.2	0.11	0.12	0.011	0.003	0.003	0.057	0.024	0.026	0.27	0.074	0.077	1.1	0.53	0.55
			Group:	**12**	**9.4**	**0.24**	**0.16**	**0.012**	**0.003**	**0.002**	**0.078**	**0.037**	**0.023**	**0.30**	**0.072**	**0.046**	**1.6**	**0.85**	**0.54**

[a] n = total number of experimental measurements; C = concentration; t_{exp} = time of exposure; J_{in} = average flux into skin; J_{out} = average flux into receptor fluid; M_{sk}/A = mass of BaP per area in skin; M_{rf}/A = mass of BaP per area in receptor fluid; SD = standard deviation; E = maximum error of the mean at a 95% confidence level. [b] Due to high amounts of soil in these vials, 1 mL aliquots of well-mixed cocktail-soil solution were added to an additional 10 mL of cocktail and re-counted. [c] One cell had receptor fluid values that failed outlier analysis (Dixon's Q test at 99% level).

Chapter 3. Dermal Exposure Assessment from Contaminated Soil: Barriers and Opportunities for Improvement

ABSTRACT

Researchers have expressed concern with shortcomings in current guidance for the assessment of dermal exposure from contaminated soils, which calculates uptake by simply multiplying an experimentally determined fractional absorption value by total chemical loading on skin. Concern with this methodology includes two dimensions: (a) the simplistic method fails to account for the physical and chemical mechanisms actually driving dermal uptake of chemicals from soil; and (b) empirical fractional absorption values are susceptible to distortion by several methodological pitfalls which are common among existing soil-dermal absorption studies. A physics-based approach would improve characterization of exposure from this pathway; yet practical barriers exist that prevent adoption of a more sophisticated method. Until such an approach is available, exposure and risk assessors need to account for experimental conditions underlying fractional absorption (ABS) parameter values. One tangible recommendation is that experimental ABS values be adjusted for experimental soil loading conditions. The adjustment is easily implemented, empirically and theoretically supported, and represents a more health protective approach until further methodological improvements are feasible. Fractional absorption measurements from two soil-based dermal absorption studies with different experimental conditions are compared. This simple comparison shows clearly that experimental ABS values cannot be interpreted independently of experimental soil loading conditions.

INTRODUCTION

Current guidance for assessment of dermal exposures to contaminants in water and soil from the U.S. Environmental Protection Agency (EPA) is found in Part E of the Risk Assessment Guidance for Superfund (RAGS) (USEPA, 2004). The protocol for estimating absorbed dose of chemicals in water involves a two-compartment distributed model that describes absorption as a function of the path length of chemical diffusion (defined as stratum corneum thickness) and event duration. The model uses chemical-specific permeability coefficients (K_p), which can be estimated via a regression of experimentally determined values based on *in vitro* experiments of 90 different compounds using common methodology (human cadaver skin and steady-state conditions). The relatively large dataset allows for the estimation of permeability for unstudied compounds as a function of molecular weight and octanol-water partition coefficient (K_{ow}). In contrast, estimation of dermally absorbed dose to chemicals from soil involves multiplying total chemical loading on skin (*i.e.*, soil loading on skin x chemical-soil concentration) by an experimentally determined dermal absorption fraction (ABS). The ABS parameter is traditionally calculated as the gross percent of initial contaminant load absorbed in a fixed time frame.

Researchers have expressed concern with shortcomings in the soil protocol presented in RAGS Part E (Spalt et al., 2009)**.** Concern with this methodology includes two dimensions: (a) the simplistic method fails to account for the physical and chemical mechanisms actually driving dermal uptake of chemicals from soil; and (b) experimentally determined ABS values, considered fixed across study conditions, are susceptible to distortion by several methodological pitfalls that are common among existing soil dermal absorption studies.

Regarding simplicity of the current soil dermal exposure equation, one need only look to the EPA's water protocol to realize that the soil methodology is less sophisticated than is possible. Reliance on a fixed fractional absorption parameter to estimate uptake from soil neglects the fact that dermal absorption is best conceptualized as gradient-driven mass transfer through a membrane (Kissel, 2011). However, it is important to acknowledge the presence of several practical barriers that undermine the development of a physics-based methodology. The first challenge that must be considered is that the current method is exceedingly simple—essentially a one-step multiplication. Another potential factor may be that the soil-dermal pathway is not widely considered an important contributor to human health risk in typical exposure scenarios at contaminated sites, and thus there has not been an impetus to reevaluate current guidance. From a scientific perspective, another key barrier is paucity of reliable data. Compared to the water protocol, which is based on a relatively large database of *in vitro* experiments using common methodology, less than 40 compounds in soil have been investigated (with little standardization of methods).

Multiple alternative methodologies that are based in physics have been proposed (McKone and Howd, 1992; Bunge and Parks, 1998). However, adopting these models as a preferred option is attenuated by the complexity of the models, the small quantity of data available to assess their predictive abilities, and inherent model restrictions. A technical evaluation of these approaches is beyond the scope of this paper. It is important, though, to keep in mind that with adequate data it is certainly possible to develop a physics-based approach. Creating a more accurate and justifiable methodology to replace the current ABS-based method for assessing dermal exposure from soil is a worthwhile endeavor from a scientific standpoint; however, in the absence of such a method, improvements to the current approach should also be

sought. The rest of this paper will operate under the premise that, while a mechanistic approach is preferred, improvements in the selection and interpretation of experimentally determined ABS values are feasible.

The EPA has stated that "[e]mpirical values are used for the specific fraction of chemicals absorbed to compensate for the lack of data on soil matrix effects" (p.41) (USEPA, 2004). While a single ABS value simplifies assessment of exposure and risk, it doesn't account for effects soil loading conditions or other factors (*e.g.*, exposure duration, soil-chemical contact time) that might affect measurement of ABS. The state of existing ABS measurements has been mostly influenced by a small group of research teams. In fact, eight of 10 chemical-specific values recommended in RAGS Part E for use in exposure and risk assessments are from one group (USEPA, 2004). These prior investigations have employed a variety of methodologies, many of which do not appropriately consider relevant physical and chemical phenomenological processes (Spalt et al., 2009). Important phenomenological concepts and methodological considerations are reviewed in Spalt et al. (2009) and briefly described above (see Chapter 1).

Since experimentally determined ABS values are susceptible to affects from study conditions, it is inappropriate to take these measurements at face value—experimental conditions must be considered. One proposed method for improving assessment of dermal exposure from soil is to modify experimentally determined values of fractional absorption using correction factors. So far, corrections have been proposed to ameliorate one potential experimental pitfall: underestimation of ABS values due to experimental soil loading conditions that are greater than is required for complete skin coverage.

METHODS

Adjusting ABS values to account for experimental soil loading. Experiments with supramonolayer application of spiked soils will result in artificial suppression of apparent dermal availability of soil-bound chemicals (Spalt et al., 2009). Recognizing this, EPA's 1992 guidance for dermal exposure assessment (USEPA, 1992) discussed a correction factor that could be applied to experimental results obtained at soil loads greater than monolayer to appropriately scale ABS:

$$\boldsymbol{ABS_{scaled}} = \boldsymbol{ABS_{at\ SL_{experiment}}} \frac{\boldsymbol{SL_{experiment}}}{\boldsymbol{5\ mg/cm^2}} \quad (1)$$

where ABS is the fraction absorption efficiency, $SL_{experiment}$ is the soil load used in the experiment in mg/cm^2, and 5 mg/cm^2 is an estimate of the soil load that would nominally represent monolayer coverage. However, the mass of soil required for monolayer coverage is not constant and depends on soil particle size and density. Duff and Kissel (1996) proposed the following equation to estimate the soil loading representing monolayer coverage ($SL_{monolayer}$), assuming solid spherical particles and face-centered packing (Duff and Kissel, 1996):

$$\boldsymbol{SL_{monolayer}} = \frac{\boldsymbol{\rho_{particle}}\left(\frac{\boldsymbol{\pi d^3}}{\boldsymbol{6}}\right)}{\boldsymbol{d^2}} = \boldsymbol{\rho_{particle}}\left(\frac{\boldsymbol{\pi d}}{\boldsymbol{6}}\right) \quad (2)$$

in which $\rho_{particle}$ is the particle density of the soil in mg/cm^3 and d is particle diameter in cm. Median particle diameter can be estimated as equal to the square root of the product of the upper and lower particle size boundaries (*i.e.*, the approximate geometric mean of a lognormal distribution). The authors note that output from this equation is approximate, as soil particles are not actually uniformly sized. Given the relation of particle size and density to soil loading

required for monolayer coverage, Duff and Kissel (1996) recommend the following alteration to EPA's correction factor:

$$\boldsymbol{ABS_{scaled}} = \boldsymbol{ABS_{at\ SL_{experiment}}} \frac{\boldsymbol{SL_{experiment}}}{\boldsymbol{SL_{monolayer}}} \quad (3)$$

Studies evaluated. To illustrate these concepts, the proposed soil loading adjustment (Eq. 3) was applied to two studies that examined dermal absorption of benzo[a]pyrene (BaP) in soil. In the first study (Peckham et al., 2015), researchers developed and performed *in vitro* human cadaver skin experiments with attention to methodological criteria that might influence absorption. These considerations included employing a sub-63-µm particle size range, estimating degree of chemical saturation of soil (although results from the study suggest that the soil saturation limit may have been exceeded), and artificially weathering and aging spiked soils prior to application to skin. In an effort to achieve complete skin coverage and avoid depletion of chemical supply, soil loads applied to skin were substantially greater than the estimated $SL_{monolayer}$. Accordingly, Peckham et al. report their results in terms of flux. In the second study (Wester et al., 1990), *in vivo* experiments with rhesus monkeys and *in vitro* human cadaver skin experiments were performed. Each study used similar chemical-soil concentrations (10 mg/kg; Peckham et al. also studied soils spiked to 3 mg/kg), soil loads (30-40 mg/cm^2), and exposure durations (24 h; Peckham et al. also studied 8 h durations). Further details of each study design are revealed in **Table 1**. $SL_{monolayer}$ was estimated using Eq.2, based on nominal soil loading conditions reported in each study and an assumed $\rho_{particle}$ of 2.65 g/cm^3. ABS_{scaled} was determined using Eq. 3 for individual trials of each set of experiments and averaged across experimental condition.

RESULTS

Estimated $SL_{monolayer}$ and average ABS_{scaled} values for sets of experiments performed in each study are presented in **Table 1**. As noted above, Peckham et al. applied soil loads to skin that greatly exceeded estimated $SL_{monolayer}$. In terms of mass, these loads were similar to those used in Wester et al. experiments; however, the latter used relatively coarse soil particles, which led to experimental soil loading conditions near the estimated $SL_{monolayer}$. Experimental fractional absorption measurements in the Peckham et al. experiments are less than 1.2% in all study conditions. These results are similar to the Wester et al. *in vitro* experiments, but much lower than the 13% measured from the *in vivo* trials. When accounting for soil loading conditions, however, ABS_{scaled} values calculated from Peckham et al. are consistently larger than from Wester et al. experiments, from both *in vivo* and *in vitro* experiments, due to a much greater ratio of $SL_{experiment}$ to $SL_{monolayer}$ (~27 vs. 1.2, respectively).

Table 1. Comparison of study conditions and fractional absorption values from two studies of dermal absorption of BaP from soil[a]

Study	n	Soil weath-ered?	t_{exp}	Nominal Soil *C*	Nominal $SL_{experiment}$	Particle fraction	Estimated $SL_{monolayer}$	$ABS_{experimental}$	ABS_{scaled}
			hr	mg/kg	mg/cm2	um	mg/cm2	%	%
Peckham et al. 2015	23	Y	8	3	30	<63	1.10	0.82	24
	24	Y	24	3	30	<63	1.10	0.93	27
	24	Y	8	10	30	<63	1.10	0.65	19
	23	Y	24	10	30	<63	1.10	0.64	19
	6	N	24	3	30	<63	1.10	1.2	34
	6	N	24	10	30	<63	1.10	1.0	29
Wester et al. 1990	4	N	24	10	40	180-300	32.2	13[b]	16
	6	N	24	10	40	180-300	32.2	1.4	1.8

[a] *n* = total number of experimental measurements; *C* = concentration; t_{exp} = time of exposure. [b] Value determined from *in vivo* experiments using rhesus monkeys; all other values emanate from *in vitro* experiments using human cadaver skin.

DISCUSSION

Experimentally determined ABS values have the potential for distortion by a number of study conditions, including, but not limited to, layering effects (Spalt et al., 2009). Yet of all the recognized methodological pitfalls, the layering effect stands out in that: (a) the effect on the ABS measurement is known (*i.e.*, artificial suppression of ABS that could lead to an underestimation of risk); (b) a layering effect will reduce apparent ABS regardless of the presence of other study conditions that might contribute to additional uncertainty (*e.g.*, inappropriate particle size, chemical-soil concentrations exceeding saturation); (c) layering effects are relevant to nearly all experimental determinations of ABS (*i.e.*, studies usually employ supramonolayer soil loadings for practical experimental purposes); and (d) there are mathematically simple methods to correct and evaluate the potential impact of layering effects that can be easily implemented. Until a mechanistic model or equation is adopted in place of the current ABS-based method, it is recommended that exposure and risk assessors account for the effects of supramonolayer soil loadings by incorporating the simple correction factor proposed by Duff and Kissel (Eq. 3).

Guidance on dermal exposure assessment from soil in RAGS Part E (2004) acknowledges that ABS may be a function of soil loading and that soil characteristics such as particle size determine at what loading monolayer coverage will occur. The document also suggests that the equation to estimate $SL_{monolayer}$ (Eq. 2) from Duff and Kissel (1996) can be used to approximate an upper bound for adherence factor (AF) values appropriate in site-specific exposure assessment calculations (Appendix C) (USEPA, 2004). However, the guidance does not include either of the aforementioned correction factors, stating that the “absolute effect of soil loading on these parameters is not sufficiently understood to warrant adjustment of the

experimentally determined values" (pp. 3-18) (USEPA, 2004). Instead there is a suggestion that potential underestimation of ABS parameter values be acknowledged in the risk assessment as a relevant uncertainty. Reluctance to incorporate a correction factor of this nature into RAGS Part E, including their own proposed adjustment from previous guidance on dermal exposure assessment from soil (USEPA, 1992), may be the result of findings from two studies that failed to find a loading effect (Wester et al., 1996; Reifenrath et al., 2002b). While both studies reported ABS measurements at different soil loads did not significantly differ, analysis included in Spalt et al. (2009) suggest that these studies may not be well suited to test an effect of layering. This is primarily due to the researchers' selection of a range of soil loadings that did not likely exceed monolayer coverage, which merely confirm that there is no layering effect in the absence of layering (Spalt et al., 2009).

On the other hand, theoretical and empirical evidence of a layering effect is relatively robust (Yang et al., 1989b; Duff and Kissel, 1996; Roy and Singh, 2001; Touraille et al., 2005; Spalt et al., 2009). While the EPA had access to nearly all of these studies when developing and updating RAGS Part E, the thorough review by Spalt et al. (2009) was not yet available. The synthesis of available science included in the review is convincing evidence of the existence of the layering effect, and that the effect on parameter determination is an apparent reduction in measured ABS. The simple comparison presented here shows clearly that appropriate interpretation of $ABS_{experimental}$ varies across studies, and is consistent with the notion that experimental soil loading conditions—and study design, generally—may affect empirical measurements of fractional absorption. More importantly, given the current use of an ABS-based method of exposure assessment, results from Peckham et al. suggest that use of $ABS_{experimental}$ determined from Wester et al. *in vivo* experiments might lead to underestimation of risk.

To return to the larger picture, there are other, more important shortcomings inherent in an ABS-based method of exposure assessment than effects from soil loading conditions. The simplistic nature and absence of theoretical foundation are more fundamental reasons to propose reforming EPA's current guidance on dermal-soil exposure. However, advanced approaches have not taken hold, probably due to complexity, lack of data, and model limitations. Therefore, in addition to developing alternative methodologies, attention to improving the current guidance is warranted. The recommendation that a soil loading correction be applied to empirical ABS parameters is merely one step toward a larger goal that entails researchers and risk assessors carefully considering experimental conditions when determining or choosing parameter values for use in dermal risk assessment. Attention to these issues is necessary for avoiding underestimation of risk, accumulation of reliable data, and development of a more accurate and justifiable method for characterizing exposure from the soil-dermal pathway.

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Appendix A: Lay Summary

Pollution in the environment can be harmful to human health. Environmental health scientists study how chemicals from the environment enter the body and try to prevent this from happening. One way that chemicals can get into the body is through the skin. This thesis focuses on how chemicals in contaminated soil can be absorbed into the body. Both adults and children can get soil on their skin from activities like gardening or playing sports. Additionally, some workers can get dirt on their skin while doing their job. If the soil that gets on a person's skin is contaminated, chemicals in the soil might be able to travel through the skin into the body.

The U.S. Environmental Protection Agency (also called the "EPA") is the main federal governmental agency that works to protect people from environmental hazards, including chemicals in soil. Over 20 years ago, the EPA developed mathematical equations to estimate how much chemical can enter someone's body from contaminated soil on their skin. These calculations help us understand how likely it is that chemicals in soil will cause injury or illness to people if it is on their skin. This information is then used to make laws to protect people and to decide how much contaminated sites need to be cleaned. To make good decisions that protect people, we need to have good information about how chemicals get into the body.

The process of absorbing chemicals through the skin is complex, and requires understanding chemistry, physics, and mathematics, among other subjects. However, the current equations for estimating chemical absorption through skin from soil recommended by the EPA are very simple. In the years since these equations were developed, scientists have learned more about this process. Importantly, there are several factors that need to be considered when doing experiments to try to measure how much chemical from soil gets through the skin, including how long the soil is on the skin, how big the soil particles are, and how long the chemical has been in

the soil. Many experiments that have been done in the past did not account for these factors, making their results unreliable. Additionally, the current EPA equations do not account for these factors. This means that we might not have good information to make decisions that protect people from the chemicals at contaminated sites. We need more, better information on this topic.

The work done in this thesis hopes to improve our understanding of how people are exposed to chemicals in soil, and to try to move towards more advanced methods of estimating human health risks from contaminated soils. This research focuses on one particular chemical called benzo[a]pyrene. This chemical, also called BaP (pronounced "bee-aye-pee"), is commonly found in soil and can cause people to get cancer. The first part of this thesis describes scientific studies that have been done in the past on absorption through skin of BaP from soil. These studies were completed using many different methods, and they mostly got different results.

The second part of this thesis describes results from a new study that we performed. The study was designed to learn from mistakes of earlier studies to provide a better measurement of how much BaP gets through skin from soil. The study involved building a model in a laboratory to recreate a situation of soil on human skin, and was designed to better represent real-world conditions than previous studies. We found that more chemical absorption occurred when there was more chemical in the soil, and less absorption occurred when the soil was wetted and dried before we put it on the skin. Time of exposure did not matter, however, because some chemical on soil particles are not easily washed off the skin. We also found that absorption of BaP through skin occurs slower when it is applied in soil compared when it is applied directly on to the skin. The third part of this thesis recommends a simple change be made to the current equations used by the EPA to estimate risk from contaminated soils. This recommendation is easy to do and will help us make decisions that protect people from harmful exposures to chemicals in soil.

Appendix B: Standard Operating Procedures for *In Vitro* Experiments

STRATEGIC ENVIRONMENTAL RESEARCH AND DEVELOPMENT PROGRAM (SERDP) STUDY

Standard Operating Procedures

Section 1. Water-holding Capacity Measurement

1.1 OBJECTIVE

To develop an appropriate and consistent manner to determine the water-holding capacity of soils. Whole soil or fractionated soil samples can be tested in these water-holding capacity tests. These measured water-holding capacity values can then be used in subsequent weathering procedures of said or similar soils.

1.2 MATERIALS

centrifuge tubes (50-ml) and caps
Whatman Grade 2 filter paper (4.25 cm diameter)
pen
saw
utility knife
drill / drill bits
thick scrap wood (~2-3 cm)
test soil(s) (at least 2 g)
oven
"trial summary" worksheet from the "whc.xls" workbook
scoopulas
aluminum weigh boats or small glass beakers (10 ml)
disposable underpads
shallow metal baking pan
ASTM Type I deionized water
serological pipettes (10 ml)
serological pipettor
weight (~2-3 lbs)
empty pipette tip tray
beaker (50 ml)
Mettler balance

1.3 METHODOLOGY

1.3.1 Soil tube holders

1. From the top of a centrifuge tube, demarcate a line with a pen about 3.5 cm from the top of the tube (~37 ml mark). Using a saw, cut the centrifuge tube into two pieces at the demarcation (Figure 1). Dispose the longer portion of the tube.

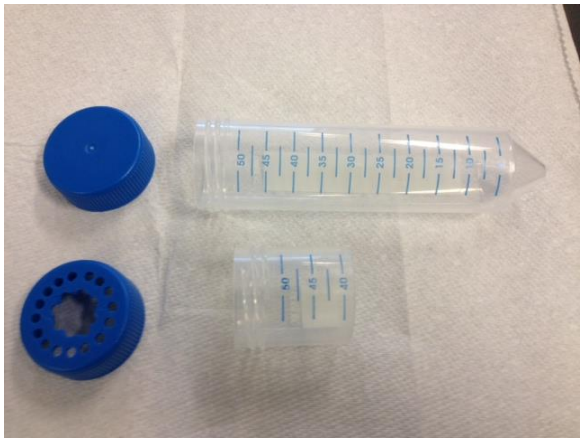

Figure S-1

2. Using a drill and a small bit (~1/8”), drill small holes in concentric circles into the centrifuge cap. The center portion of the cap can be carved out with a utility knife to maximize the hole opening area (see Figure 1). The cap can be placed atop scrap wood when drilling.

3. Repeat steps 1 and 2 for as many soil tube holders as are needed. Label each.

1.3.2 Pre Test (day before)

1. Obtain adequate masses of all test soil(s) and bake in oven overnight at 100°C.

2. The morning of the test day, take the soil(s) out of the oven and allow to cool at room temperature for at least 30-45 min.

1.3.3 Pre Test (day of test)

1. Print out a “trial summary” worksheet from the “whc.xls” workbook. Fill in the appropriate information (see Appendix).

2. Weigh a Whatman #2 filter only and the filter + tube + cap. Record measurements on the “trial summary” worksheet.

3. A completed soil tube holder with filter is shown in Figure 2. The filter is secured by placing it over the threaded side of the tube and fastening the modified cap.

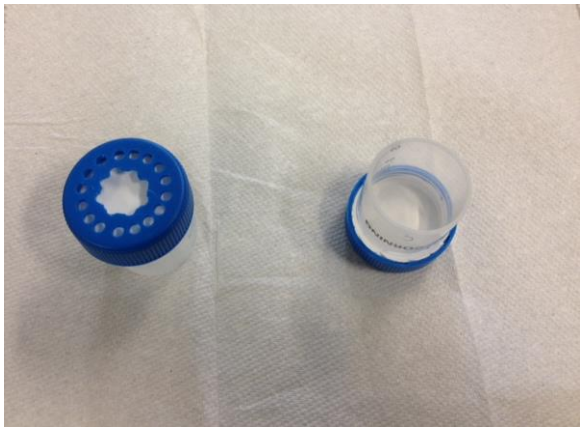

Figure S-2

4. Using a scoopula, carefully place a predetermined mass (*e.g.,* 2 g) of an oven-dried test soil onto a tared aluminum weigh boat or small beaker (*e.g.,* 10 ml). Adding a little above the target mass is ideal to compensate for the potential loss during transfer (Figure 3).

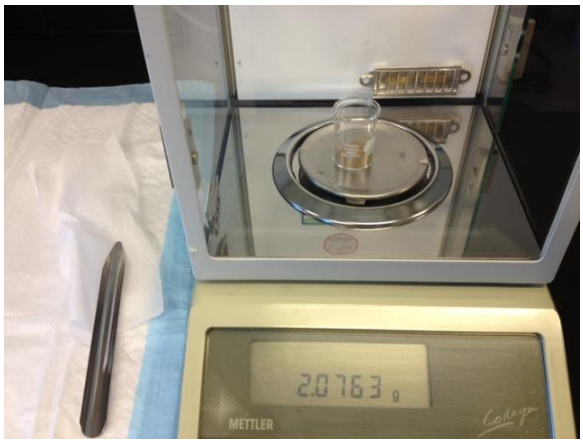

Figure S-3

5. Carefully transfer the test soil from the weigh boat/beaker to the preweighed filter + tube + cap soil tube holder (Figure 4). Weigh the filter + tube + cap + soil and record value on the "trial summary" worksheet.

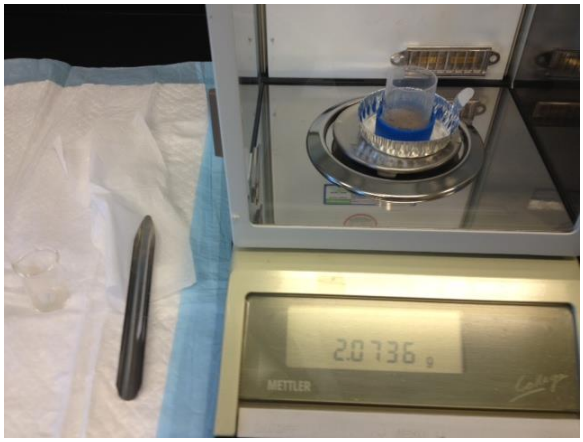

Figure S-4

6. Set aside weighed filter + tube + cap + soil soil tube holder atop a disposable underpad. Repeat steps 1-4 for however many test soils that are being assessed.

1.3.4 Soil soaking

1. Place all weighed filled soil tube holders into a shallow metal baking pan. Using a 10-ml serological pipette and pipettor7, put ASTM Type I deionized water into each filled soil tube holder, taking care not to splash the soil particles out of the holder. The soil should be slightly super saturated. Repeat for each filled soil tube holder (Figure 5).

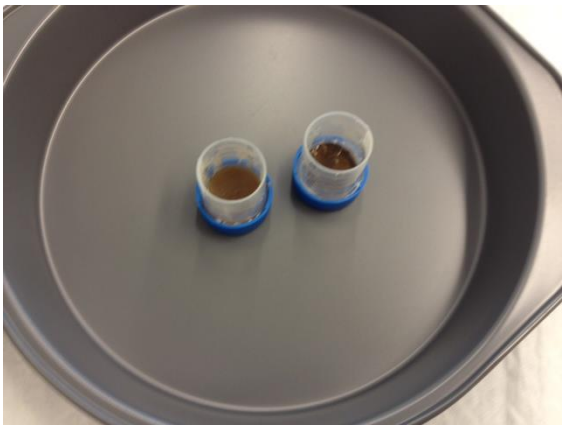

Figure S-5

2. Cap each water/soil-filled soil tube holders.

3. Arrange the water/soil-filled soil tube holders so that they can support an upside down empty pipette tip tray (Figure 6). Empty soil tube holders can be used to provide support if necessary.

Figure S-6 (A third, empty tube added for stability)

4. Position upside down empty pipette tip tray atop the water/soil-filled soil tube holders and place weight into tray cavity (Figure 7).

Figure S-7

5. Fill the shallow baking pan with ASTM Type I water (via a beaker to ease transfer) up to the highest water level within the water/soil-filled soil tube holders. This will ensure that the soil stays submerged under water and that all soil particles and channels between the particles will be sufficiently wet.

6. Allow the soaking process to run for two hours.

1.3.5 Soil drying

1. At the end of two hours, carefully remove the empty pipette tip tray and weight. Place the empty pipette tip tray – right-side up this time – atop a disposable underpad.

2. Take each of the water/soil-filled soil tube holders out of the baking pan and place atop the empty pipette tip tray. The caps should be left atop the open-end of the soil tube holders to prevent dust from falling inside. Caps can be placed slightly askew to allow air flow through the soil tube holders (Figure 8).

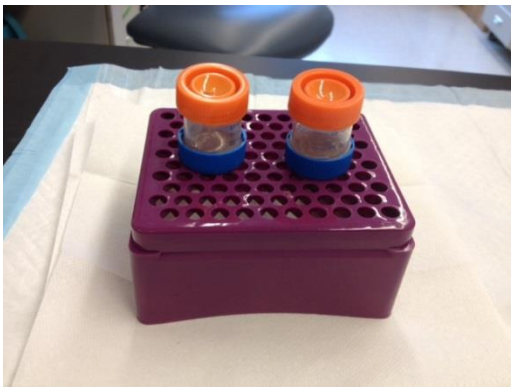

Figure S-8

3. Allow the water to drain (by gravity) out of the water-soil filled soil tube holders for four hours, taking mass measurements at the two, three, and four hour marks. Additional measurements at longer time points can also be taken if deemed necessary.

4. At each weighing time point, carefully remove the top cap and gently rotate the soil tube holder on a disposable underpad to allow any excess water to be absorbed by the pad. Make sure that there is no visible water on the exterior of the soil tube holder. Weigh each soil tube holder and record the mass on the "trial summary" worksheet. Repeat for all soil tube holders.

   NOTE: The above step requires attention to water droplets that may be present in between the filter and the modified centrifuge cap, or possibly between the outer edges cap and the side of the tube. Prior to each weight measurement, it is important that the same procedure is used to remove excess water (*e.g.*, rotating the tube holder on the pad three times in the same manner for all tubes).

1.3.6 Calculations

1. Use the following equation to calculate the water-holding capacity (whc):

$$\text{whc}\,(\%\ \text{of dry mass}) = \frac{S \cdot T \cdot D}{D} \cdot 100$$

Where:
S = mass of water-saturated soil + filter + tube + cap;
T = mass of filter + tube + cap; and
D = mass of oven-dried soil.

NOTE: This method is a modified version of the OECD/OCDE Annex 2 methodology sent to us by Roman Kuperman (Nov 2012). The source of that method is Annex C of ISO DIS 11268-2 (Soil Quality – Effects of pollutants on earthworms (*Eiseni* fetida). Part 2: Determination of effects on reproduction; 1996).

STRATEGIC ENVIRONMENTAL RESEARCH AND DEVELOPMENT PROGRAM (SERDP) STUDY

Standard Operating Procedures

Section 2. Soil Weathering

2.1 OBJECTIVE

To develop an appropriate and consistent manner to weather soil samples. Whole soil or fractionated soil samples can be weathered using this procedure. These weathered soils can then be used in dermal absorption experiments.

NOTE: the protocol described below is for small aliquots of soil (~2 g). The method can be scaled up for larger masses of soil.

2.2 MATERIALS

TraceClean® 20-ml vials (VWR #89093-838)
labels
"soil prep," "weathering soil aliquots," and "weathering schedule" worksheets from "SERDP dermal absorption study.xls" workbook
pen
test soils (≥ 2 g)
oven
radiolabel stock solutions (*e.g.,* benzo(a)yrene in toluene)
Hamilton syringes (10 µl and 50 µl)
tube rotator (Labquake®)
fume hood
small glass beakers (10 ml)
scoopulas / spatulas
fume hood
metal tray
ASTM Type I deionized water
pipettes (1 ml)
pipettor
disposable underpads
scintillation vials
scintillation cocktail (Hionic-Fluor®, PerkinElmer Inc.)
Mettler balance
Liquid scintillation counter (Beckman LS 6000)

2.3 METHODOLOGY

NOTE: this SOP describes the weathering protocol for two dry soil sample conditions: a low (3 ppm) and high (10 ppm) soil concentration, using a direct method of radiolabel application (chemical applied directly to soil and mixed). The radiolabeled chemical example used here is benzo(a)pyrene in toluene stock solution. Specifics of the protocol can be modified for other conditions/chemicals. For example, if wet soil (*e.g.,* a slurry) is to be used in place of the dry soil, a wetting procedure (*e.g.,* use of ASTM Type I deionized water and a 3-day mixing period on a tube rotator) can be incorporated into the protocol below.

2.3.1 Labeling vials

1. Label two 20-ml TraceClean® vials appropriately (*e.g.,* direct/3 ppm, direct/10ppm) (Figure 1).

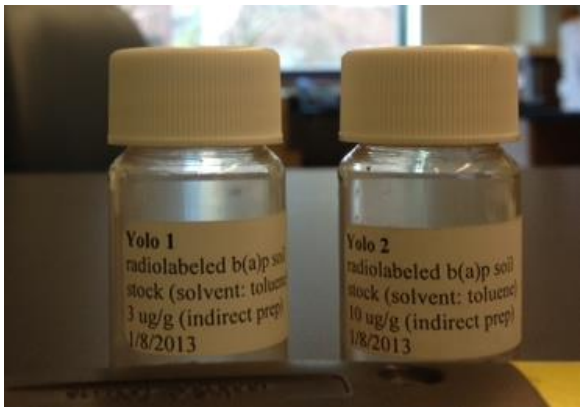

Figure S-9

2. Weigh each (including cap) and record masses on "soil prep" worksheet from the "SERDP dermal absorption study.xls" workbook (see Appendix).

2.3.2 Pre soil radiolabeling (day before)

1. Obtain adequate masses of all test soil(s) and bake in oven overnight at 100°C.

2. The morning of the test day, take the soil(s) out of the oven and allow to cool at room temperature for at least 30-45 min.

2.3.3 Radiolabeling the soil

1. Using the specific activity of the radiolabel (mCi/mmol), the target soil concentration (3 or 10 ppm), the soil aliquot mass (~2 g), molar mass of benzo(a)pyrene (252.31 g/mole), and measured radiolabel stock solution solvent radioactivity (mCi/ml), calculate the appropriate volume of radiolabel stock solution that will need to be placed into the vials:

$$\text{volume} = \frac{\text{target soil conc} \cdot \text{soil mass} \cdot \text{radiolabel specific activity} \cdot \text{conversion factor}}{\text{stock solvent radioactivity} \cdot \text{molar mass}}$$

2. **Direct method:** Use a scoopula to place ~2-gm aliquots of oven-dried a test soil into each tared labeled vial. Record each mass on the "soil prep" worksheet used for the labeled vials.

3. Using the appropriate Hamilton syringes, place the calculated volumes of radiolabel stock solution in vials that contain ~2 g of soil each. Cap each vial immediately following the transfer of the radiolabel stock solution (Figure 2).

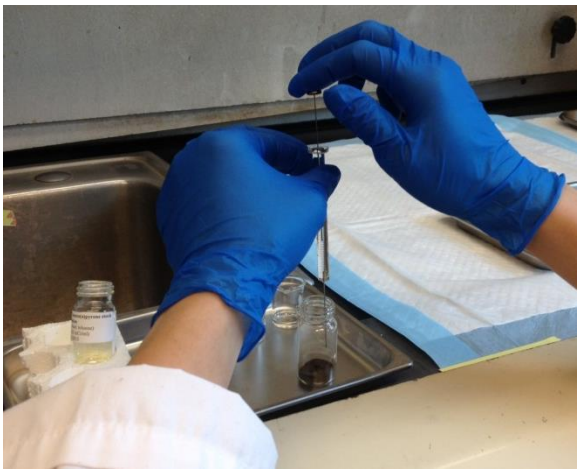

Figure S-10

4. Weigh both vials and enter pre-mixing vial masses onto the "soil prep" worksheet.

5. Place both vials onto a tube rotator. Run the rotator for 72 hours to complete the mixing process (Figure 3 shows this step for eight vials).

Figure S-11

6. A general note: be detailed in your notes of what you did. Times should be recorded, as should anything anomalous.

2.3.4 Weathering

1. Once the 72 hours of soil radiolabel mixing has been completed, remove the two vials from the tube rotator.

2. Properly label three scintillation vials for each TraceClean® vial of contaminated soil (*e.g.,* labels for scintillation vials for the initial (week 0) three aliquots of Yolo soil with 10 ppm BaP: Y10w0a, Y10w0b, and Y10w0c).

3. From each TraceClean® vial containing spiked soil, take ~ 5 mg of soil and place into a tared, labeled scintillation vial. Record weight in the "weathering soil aliquots" worksheet (see Appendix) from the "SERDP dermal absorption study.xls" workbook. Repeat until three aliquots are obtained for each spiked soil.

5. Add 10 ml of scintillation cocktail (Hionic-Fluor®) to each scintillation vial containing soil aliquots. Shake each scintillation vial vigorously for ~20 seconds.

6. Set aside all scintillation vials containing soil aliquots and scintillation cocktail to later run through a liquid scintillation counter.

7. **Wetting/Drying/Rotating Cycle:** Using a pipettor and a 1-ml pipette tip, put 60% of water-holding capacity of ASTM Type I deionized water into each TraceClean® vial containing spiked soil. Recap each vial securely. The procedure for determining water-holding capacity can be found in Section 1 of these SOPs. For a 2-g aliquot of <63 μm fraction of soil, we have determined that a reasonable estimate of 60% of water-holding capacity is ~1 ml of deionized water (whc ≈ 80% → 60% of 80% ≈ 50% → 50% of ~2 g → ~1 g of deionized water = 1 ml of deionized water).

8. Record the time of the wetting of the vials in the "weathering schedule" worksheet from the "SERDP dermal absorption study.xls" workbook (see Appendix).

9. Manually rotate the vials to help the deionized water moisten all of the soil. Take care not to spread the soil too wildly around the vial. You want the all of the soil to be at the bottom of the vial.

10. Leave the two capped vials in the fume hood for three days.

11. On Day 3, uncap the vials, and leave in the fume hood for another two days. (Figure 4 shows this step for eight vials).

Figure S-12

12. On Day 5, manually disaggregate the soil by breaking up the dried, clumped soil particles with a spatula. Take care not to break the glass vials. Scrape all soil particles that have adhered to the interior walls of the glass vials or are lodged in the crevices of the vial bottom edges. Once the soil has been disaggregated and visually resembles the original, dry < 63 μm soils), place the vials onto the tube rotator. Run the rotator for another two days.

13. Starting on Day 8, repeat steps #3 through #12 for the following week. Repeat this one-week wetting/drying/rotating cycle and soil aliquot procurement for a total of eight weeks.

    NOTE**:** In every week of the weathering process, there will be three aliquots of soil taken from each vial containing spiked soil. These aliquots will be measured for radioactivity via a liquid scintillation counter. The aliquots taken after steps #1 - #2 will document the concentration of the spiked soils before the weathering process has started. The following eight weeks of aliquots will serve as the last step of each week-long wetting/drying/rotating cycle described above (steps 7-13).

STRATEGIC ENVIRONMENTAL RESEARCH AND DEVELOPMENT PROGRAM (SERDP) STUDY

Standard Operating Procedures

Section 3. Operation of Dermal Chamber for Quantification of Average Flux of Radiolabeled Chemical from Soil through Human Cadaver Skin

3.1 OBJECTIVES

To develop an appropriate and consistent manner to determine the average flux of chemical contaminants in soil across human epidermis using flow-through diffusion cells. Whole soil or fractionated soil samples can be tested with these protocols.

3.2 MATERIALS

TraceClean® vial containing ≥ 1.5 g spiked, weathered soil ("test soils")
labels
radiolabel stock solutions (*e.g.,* benzo(a)yrene in toluene)
dermal chamber components (see Figure S-13)
receptor fluid
fume hood
small glass beakers (10 ml, 25 ml, 100 ml)
sterile 0.45-µm pore size cellulose acetate membrane filter
spatulas
tweezers
fume hood
metal tray
ASTM Type I deionized water
pipettes (5 ml, 10 ml)
pipettor
disposable underpads
scintillation vials
scintillation cocktail (Hionic-Fluor®, PerkinElmer Inc.; Ultima Gold®, PerkinElmer Inc.)
Mettler balance
Liquid scintillation counter (Beckman LS 6000)
pen
thermometer
hot plate
micropipettor

3.3 METHODOLOGY

NOTE: this SOP describes the protocol for an in vitro investigation of dermal absorption of radiolabeled chemical from contaminated soil using human cadaver skin and flow-

through diffusion cells. Radioactivity will be measured using a liquid scintillation counter. The radiolabeled chemical example used here is benzo(a)pyrene, which is loaded via spiked soil and using an acetone vehicle. Four test soils, with two concentrations each, are used here in 24- and 8-hour experiments, as well as 4-hour trials for acetone vehicle trials. Three different skin sources (donors) were used. Specifics of the protocol can be modified for other conditions/chemicals. For example, different chemicals, test soils, soil concentrations, and experimental time durations can be used.

3.3.1 Assembly of chamber apparatus

1. See Figure S-13 for main components and general set up of dermal chamber apparatus.

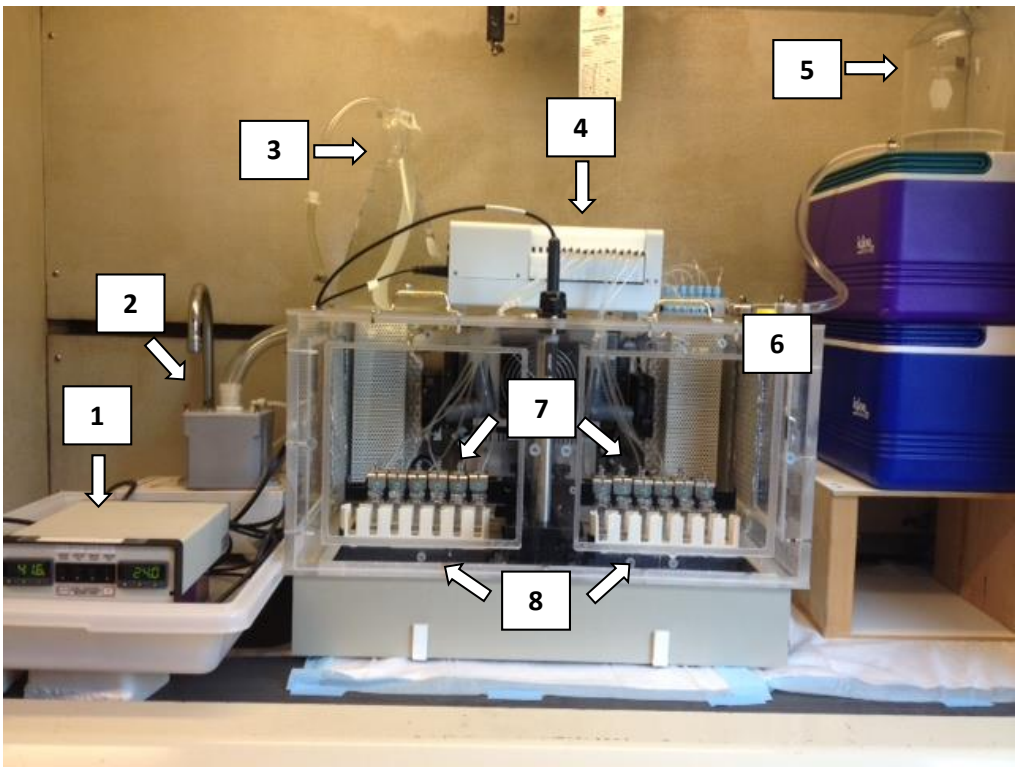

Figure S-13. Experimental apparatus for evaluating percutaneous absorption of chemical from soil. (1) Controller (heat, humidity, etc.); (2) humidifier; (3) humidifier reservoir; (4) peristaltic pump; (5) receptor fluid reservoir; (6) dermal chamber; (7) diffusion cell racks with diffusion cells connected to tubing; (8) scintillation vial racks containing receptor fluid scintillation vials.

3.3.2 Labeling and weighing of vials

1. Label vials and test tubes appropriately (see Table 1).

Table S1: Example list of vials/test tubes needed per run.

vial	vial description	container type

H#	hionic-fluor background #	scintillation vial
UG#	ultima gold background #	scintillation vial
Soil#a-c	soil # aliquot a-c	scintillation vial
#-B1	soil # transfer spatula wash and swab(s) a (& b)	scintillation vial
#-B2	soil # transfer beaker rinse and swabs a & b	scintillation vial
#-R12	cell # receptor fluid, hour 12 (24hr run)	scintillation vial
#-R24	cell # receptor fluid, hour 24 (24hr run)	scintillation vial
#-R3s	cell # receptor fluid, hour 3 sample (24hr run)	scintillation vial
#-R6s	cell # receptor fluid, hour 6 sample (24hr run)	scintillation vial
#-R12s	cell # receptor fluid, hour 12 sample (24hr run)	scintillation vial
#-R24s	cell # receptor fluid, hour 24 sample (24hr run)	scintillation vial
#-R2s	cell # receptor fluid, hour 2 sample (8hr run)	scintillation vial
#-R4s	cell # receptor fluid, hour 4 sample (8hr run)	scintillation vial
#-R8s	cell # receptor fluid, hour 8 sample (8hr run)	scintillation vial
#-D	cell # dermal chamber soak swabs a & b	scintillation vial
#-Db	cell # dermal chamber 2nd soak swabs a & b	scintillation vial
#-W1a	cell # skin swab 1a	scintillation vial
#-W1b	cell # skin swab 1b	scintillation vial
#-W2	cell # skin swabs 2a & 2b	scintillation vial
#-W3	cell # skin rinse	scintillation vial
#-C	cell # cell base swab	scintillation vial
Sb	solubilized skin blank	scintillation vial
S#	cell # skin aliquot	scintillation vial
Sol#	cell # solubilized skin	test tube

2. All scintillation vials involved in collecting receptor fluid (#-R3s, #-R6s, #-R12s, etc.) and test tubes involved in solubilizing skin (sol#) are weighed prior to the trial. Record masses.

3. Label 10-ml beakers for each cell in the trial, *e.g.*, 12 beakers for a trial of 12 dermal cells.

   NOTE: the Pre-trial Preparation Table below can be used as a reference in the lab for pre-trial preparation protocols.

Table S2. Pre-trial Preparation Table

vial	vial description	what to do	when
all vials		Label	Mon or Tues
S	skin aliquots	Weigh and record on mass sheet	Mon or Tues
R3s, R6s, R12 & R24 OR R2s, R4s, & R8s *	receptor fluid	Weigh and record on mass sheet	Mon or Tues
Sol	skin solubilization test tubes	Weigh and record on mass sheet	Mon or Tues
soil samples	soil aliquots	Take 3 aliquots of each soil (~5 mg each)	Mon or Tues
Acetone-BaP stock samples		Take 3 aliquots of each soil (~10 □l each)	Day of loading
B1	soil transfer spatula swish/DC swab swipe	Put 10 ml of Hionic-Fluor into each vial	Mon or Tues
D	donor chamber rinse	Label 25-ml beakers (1-12)	Mon or Tues
W3	skin dunk	Put 5.5 ml of ASTM Type I DI water into each vial	Breakdown day

* For 24- and 8-hour trials respectively

3.3.3 Preparation of Receptor Fluid

1. Fill 2L Erlenmeyer flask with 1 liter of ASTM Type I deionized water. Add one packet of phosphate-buffered saline (PBS; 0.138 M NaCl, 0.0027 M KCl, pH 7.4; Sigma-Aldrich product id#: P3813) and 40 grams of bovine serum albumin (BSA; Sigma-Aldrich product id#: A2153). Stir with stir plate and magnetic stir bar for two hours.

2. After mixing is complete, attach Corning 500 mL Bottle Top Filter (.45 µm CA, low protein binding, with 45 mm neck) to a 1L pyrex media bottle. Run all of the fresh receptor fluid through the filter while attached to vacuum hose (Figure 2).

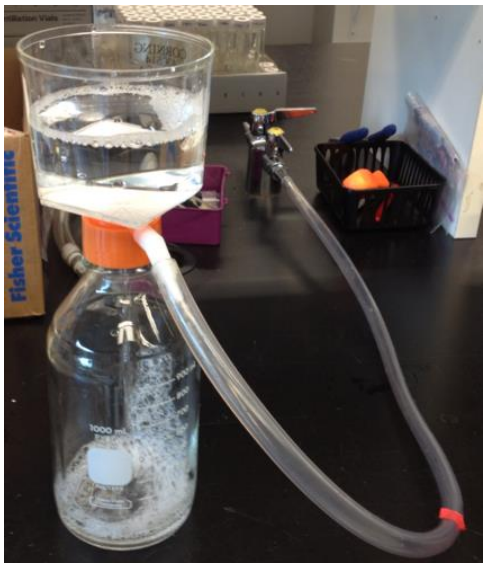

Figure S-14

3. After filtering, label media bottle including the date solution was prepared and store in refrigerator until use.

3.3.4 Preparation of skin samples/ Pre-exposure apparatus assembly (day before)

1. See schematic of inline diffusion cell (Figure 3).

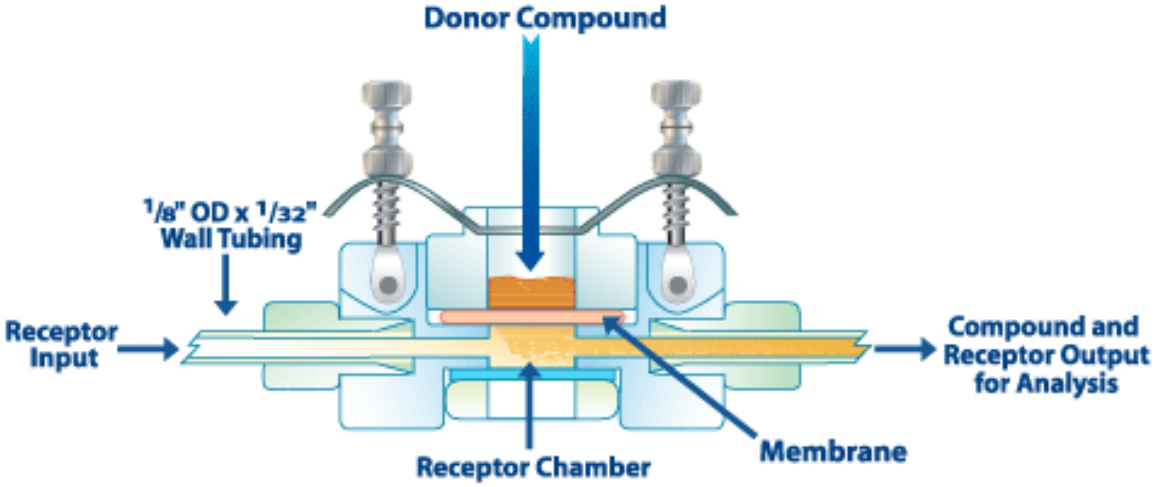

Figure S-15 **(http://www.permegear.com/inline.htm)**

2. Heat ~80 ml of DI water in a 100 ml beaker to 60°C and maintain temperature. Get skin from freezer; note identification number (ex. ND0068977-01). Fill baking pan tray with DI water so that the water is deep enough to completely submerge a diffusion cell.

3. Using a scalpel or scissors, cut the number of pieces (~1 cm x ~1 cm) needed for that week's run and skin donor (Figure 4). Cut one extra piece of skin to use as a blank (donor not important).

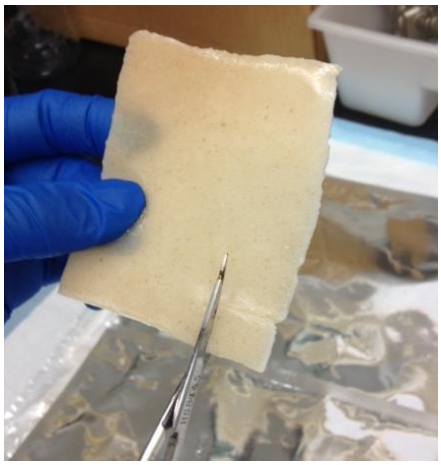

Figure S-16

NOTE: Minimize skin anomalies (*i.e.* leave out moles, navels, age spots, etc.)

4. One at a time, place a skin piece into the 60°C DI water for ~one minute. Remove skin from heated bath and place on metal tray. Using two tweezers, grab the edge of one corner and separate the dermis and epidermis (Figure 5). The epidermis will consist of the stratum corneum and some viable epidermis.

Figure S-17

5. Place separated skin in to baking pan tray with DI water floating stratum corneum side up. Immerse the diffusion cell, position epidermis over the receptor chamber aperture, and lift cell out of water, removing water from the chamber. Tweezers can be used to facilitate this process (Figure 6). Carefully place donor chamber into dermal cell, making sure that there is a seal made with the separated skin and there are no creases. Fix donor chamber in place using metal clamps; metal screws should be tightened so that the clip places adequate pressure atop donor chamber.

Figure S-18

6. Connect inline cells to proper pump channel tubing and place cell on rack accordingly.

   NOTE: to avoid tangling tubing, rotate inlet tubing from the pump counter-clockwise a few times before inserting into threading and tightening clockwise.

7. Remove bubbles from receptor chamber by performing the following steps: 1) using one hand, point spout of inline cell straight up; 2) turn on pump (scintillation vials should be in place to catch outgoing fluid from other cells); 3) press and release "MAX CAL" button repeatedly to push the bubble(s) out of the outlet. After the bubbles are removed, use a paper towel or kimwipe to wipe dry the diffusion cell and return to rack.

8. Put the remaining separated skin sample into test tube labeled "Sol b" and place in refrigerator. Properly dispose of leftover subcutaneous tissues.

9. Plug in all dermal chamber-related electrical equipment, and reconnect controller. If present, pour out old receptor fluid or water from receptor fluid reservoir jug; refill with new batch of receptor fluid. Refill flask connected to humidifier with DI water as needed.

10. Turn on heater and humidifier settings on controller. Turn on power to pump, set flow rate to 10.1 ul/min and start pump.

11. Record all pertinent data/information .

12. Cleanup notes:
    - Soak any "non-hot" skin-contaminated equipment in 10% bleach solution for 15 minutes (theoretically all equipment should have been wiped clean)
    - After soak, perform hot water/soap wash with DI water rinse
    - If there is any concern that something is hot, use Count-Off liberally and wipe area/equipment clean with kimwipes or paper towels; throw all wipes into the rad waste box; follow Count-Off clean with a soap water/water clean

3.3.5 Preparation of experimental trial

1. Obtain test soils from refrigerator and allow to acclimate to room temperature (place on counter for ~10 min).

2. Prefill all B1 vials with 10 ml of Hionic-Fluor and W3 vials with 5.5 ml of ASTM Type I deionized water

3. Once at room temperature, take three ~5 mg aliquots of each soil stock used in the current week's trial and place into prelabeled scintillation vials (*i.e.*, Soil#a-c); record masses on Trial Sheet. Store in refrigerator until after apparatus breakdown.

NOTE: This can be done prior to the day of the experiment.

4. Take one ~20 mg aliquot of each test soil corresponding to the current week's 12 cells, and place in prelabeled 10-ml beakers; record masses on Trial Sheet.

   NOTE: soil types, skin donor, and experiment duration for each week's experiments are randomized by random number generation.

5. Turn off pump. Record all pertinent pre-run data/information onto Experimental Run and Humidity/Temperature sheets. Refill flask connected to humidifier with DI water as needed.

6. If present, remove bubbles from all receptor chambers (see above 3.3.4 step #7).

7. Place wood block across side and frontal chamber doors, creating a flat surface. Take diffusion cell from rack and set on wood block. Remove metal clamps.

8. Acetone Vehicle Loading: Using a micropipettor, place 50 µl of acetone-BaP stock solution onto skin aiming for the center of the skin and being careful not to touch the sides of the donor chamber.

   Soil Loading: Using a spatula, angle down the 10 ml beaker containing the appropriate soil type and tap gently to load soil in donor chamber. Use spatula to maneuver soil to evenly cover skin.
   - Rinse tip of spatula in B1 vial containing 10 ml of Hionic-Fluor.
   - Rinse soil-transfer beaker with 10 ml of Hionic-Fluor; pour Hionic-Fluor into B2 vial. Wipe beaker with two dry Q-tips and clip cotton tips into B2 vial (Figure S-19).

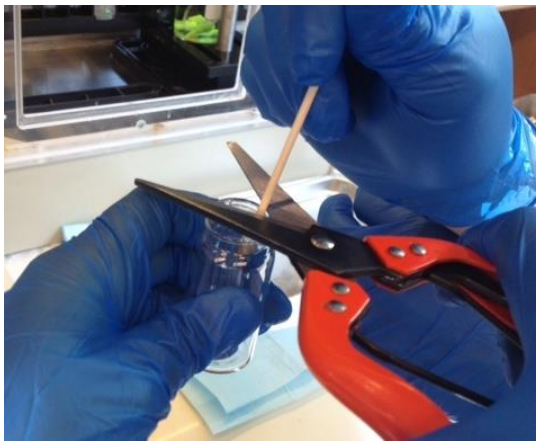

Figure S-19

   NOTE: clip off as little wood as possible while getting all of the cotton tip.

9. Dip a Q-tip into DI water and wipe the top of the donor chamber; clip cotton tip into B1 vial.

10. Carefully check under cell base to see if bubble is present and, if present, record on Trial Sheet. Fasten metal clips and place diffusion cell back on rack.

11. Repeat steps 7 through 10 for remaining cells.

12. Position R3s and R2s vials (for 24-hour and 8-hour experiments, respectively) under diffusion cells (Figure S-20).

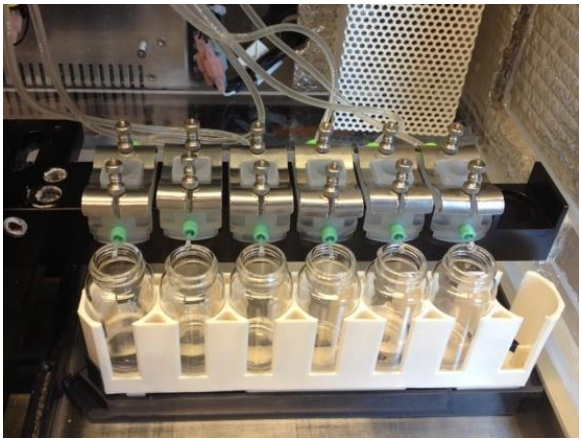

Figure S-20

13. Set pump to 25 ul/min and turn on. Record all pertinent data/information, including start time.

    NOTE: Observe receptor fluid outlet until all cells have produced at least one drop to assure that there is no blockage of fluid or other problems with flow of receptor fluid.

14. Switch receptor fluid-collecting vials at appropriate time marks.

    24-hour experiment:
    - RF3s vials to the RF6s at the 3-hr mark
    - RF6s vials to the RF12 tubes at the 6-hr mark
    - RF12 tubes to the RF24 tubes at the 12-hr mark

    NOTE: the difference between "RF" and "RFs" above; collection at 12 and 24 hour mark are in vials without "s".

    8-hour experiment:
    - RF2s vials to the RF4s at the 2-hr mark
    - RF4s vials to the RF8s at the 4-hour mark

15. Cleanup notes:
    - Soak any "non-hot" skin-contaminated equipment in 10% bleach solution for 15 minutes (theoretically all equipment should have been wiped clean)
    - After soak, perform hot water/soap wash with DI water rinse

- If there is any concern that something is hot, use Count-Off liberally and wipe area/equipment clean with kimwipes or paper towels; throw all wipes into the rad waste box; follow Count-Off clean with a soap water/water clean

3.3.6 Apparatus break down

1. Turn off pump. Record all pertinent pre-run data/information onto Experimental Run and Humidity/Temperature sheets.

2. Gather all materials necessary for cell breakdown (*e.g.*, metal trays/bins, tweezer, DI water beaker, Q-tips, waste tray, kimwipes, etc). Place metal tray in hood in front of chamber apparatus.

3. Check cell to see if there is a bubble; record if present. Disconnect pump tube, and place tube outlet into beaker; place cell on metal tray and remove metal clamp.

4. Using micropipettor, insert 150 ul of ASTM Type I deionized water into donor chamber. Use one dry Q-tip to wipe up soil and water; clip cotton tip into W1 vial.

5. If W1b is present, repeat above step; clip cotton tip into W1b vial.

6. Repeat step 4 using two Q-tips to wipe up soil and water, clipping both into W2 vial.

7. Remove donor chamber and place into 100 ml beaker labeled with cell number. Put 10 ml of Hionic-Fluor into beaker and set aside.

8. With tweezers, take skin from cell base and dip into W3 vial containing 5.5 ml of ASTM Type I deionized water. Swish around in water as to rinse surface of skin (Figure S-21). Place skin into pre-labeled and pre-weighed test tube (Sol#).

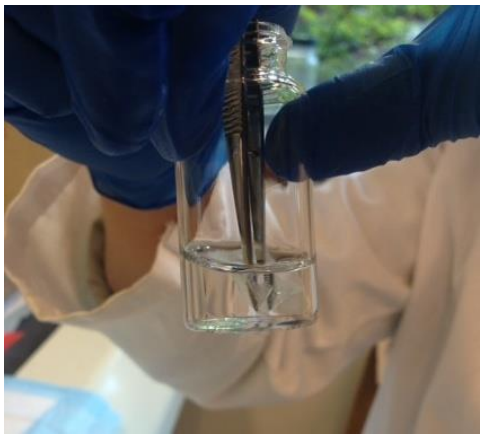

Figure S-21

9. Wipe the cell base with a DI water Q-tip; clip cotton tip into C vial.

10. Repeat steps 3 through 8 for remaining cells.

11. Return to 100 ml beaker with donor chamber; using a Q-tip, hold donor chamber in place and pour Hionic-Fluor rinse into D vial (Figure S-22). After pouring, use Q-tip to mechanically wipe donor chamber and sides of beaker; clip into D vial. Repeat mechanical wiping with another dry Q-tip; clip into D vial.

Figure S-22

12. Pour an additional 10 ml of Hionic-Fluor into beaker containing donor chamber. Let soak for at least 30 min. Repeat step 10 using Db vial.

    NOTE: The Wipe Details table below can be used as a reference in the lab during the apparatus breakdown.

Table S3. Wipe details.

Vial	vial description	swab wipe protocol
B1	soil transfer spatula swish/DC swab swipe	DI water swab on top of donor chamber after soil transfer (remove metal clip before soil transfer)
D	donor chamber soak/rinse (5-15 min)	Two dry swabs (following 10 ml of HF into 25-ml beaker holding DC)
Db	donor chamber soak/rinse (15+ min)	Two dry swabs (following 10 ml of HF into 25-ml beaker holding DC)
W1	skin swabs 1a & 1b	Two dry swabs (following 150 □l DI water into DC)
W1 and W1b	skin swabs 1a & 1b (separate vials)	Two dry swabs (following 150 □l DI water into DC)
W2	skin swabs 2a & 2b	Two dry swabs (following 150 □l DI water into DC)
C	cell base swab	DI water swab

3.3.6 Post-experiment processing of vials

NOTE: These steps were executed on the day of the apparatus breakdown for 24-hour trial and the day after breakdown for 8-hour trials.

1. Position sonicator with heating coil and test tube tray. Fill sonicator with DI water up to fill line; turn on heater and bring water bath to 65°C.

2. Add 2 ml of Soluene 350 to each test tube containing skin samples, including skin blank.

3. Place test tubes into sonicator water bath and turn on sonicator (Figure S-23). Record start time. Allow a minimum of 2 hours for solubilization of skin.

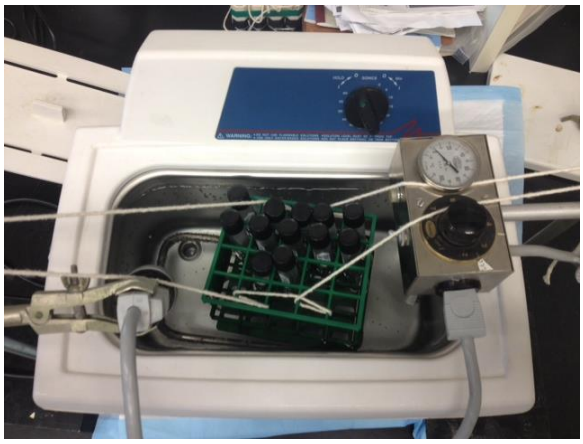

Figure S-23

4. When solubilization is done, remove test tubes from sonicator water bath and allow to cool to room temperature (~10-15 min). When cooled, take post solubilization masses and record.

5. Transfer contents of test tubes into appropriate S vials. Take post-aliquot masses of Sol test tubes and record on Mass Sheet.

6. Weigh all RFs and RF vials; record post-experiment masses on Mass Sheet.

7. For 24-hour runs, aliquot 5.5 ml of receptor fluid from 12 and 24 hour RF vials into appropriate RFs vials (*e.g.*, 1-RF12 → 1-RF12s). Take post-aliquot masses of RF vials and record on Mass Sheet.

8. Add 12 ml of Ultima Gold to the RFs and W3 vials. Add 10 ml of Hionic-Fluor to all other vials (Soil, B1, B2, D, Db, C, W1, W2, Sol and S).

9. Cleanup Notes: See 3.3.5.15 above.
NOTE: The Scintillation Cocktail Details table below can be used as a reference in the lab during the post-experiment processing of samples.

Table S4. Scintillation cocktail details.

vial	vial description	cocktail	cocktail volume (ml)
soil samples		Hionic-Fluor	10
Acetone-BaP stock sample		Hionic-Fluor	10
B1	soil transfer spatula swish/DC swab swipe	Hionic-Fluor	10
B2	transfer beaker rinse	Hionic-Fluor	10
D	donor chamber soak/rinse	Hionic-Fluor	10
Db	donor chamber soak/rinse	Hionic-Fluor	10
W1	skin swabs 1a & 1b	Hionic-Fluor	10
W1 and W1b	skin swabs 1a & 1b (separate vials)	Hionic-Fluor	10
W2	skin swabs 2a & 2b	Hionic-Fluor	10
W3	skin dunk	Ultima Gold	12
C	cell base swab	Hionic-Fluor	10
S**	skin aliquots	Hionic-Fluor	10
R3s, R6s, R12s & R24s	receptor fluid hour ** samples	Ultima Gold	12

3.3.7 Liquid Scintillation Counting

1. Place all vials for the week's trial into liquid scintillation counter in the appropriate order.

2. Vials are to be counted in triplicate at 2.5 minutes/vial.

   NOTE: The quench limits (normal range of the H#) are 112.07-145.96. Luminescence should be no more than 10%.

Appendix G

Additional Project-Related Work Product

Environmental Science & Technology

Critical Review

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Selective Soil Particle Adherence to Hands: Implications for Understanding Oral Exposure to Soil Contaminants

Michael V. Ruby*,† and Yvette W. Lowney‡

†Integral Consulting Inc., 285 Century Place, Suite 190, Louisville, Colorado 80027, United States

‡Exponent, 4141 Arapahoe Avenue, Suite 101, Boulder, Colorado 80303, United States

ABSTRACT: Over the last 30 years, there has been extensive research designed to quantify the extent of oral bioavailability and bioaccessibility of organic and inorganic contaminants in soil. One aspect of this research is the soil particle size selected to represent environmental exposures, which may affect study results and comparability across studies. Different research groups have studied soil particle sizes ranging from <45 μm to <2000 μm. This article reviews the historical and technical considerations that pertain to the selection of an appropriate particle size fraction for evaluating the relative oral bioavailability of chemicals from soil, which include (1) how the resultant data will be used in human health risk assessment, (2) soil fractions historically used in oral bioavailability studies, (3) studies of soil adherence to human hands, (4) the distribution of contaminants in soils as a function of particle size, and (5) the effect of differential bioavailability as a function of soil particle size and geochemical matrix. These factors are first discussed from a general perspective, applicable to all contaminants in soil, and then more specifically for polycyclic aromatic hydrocarbons (PAHs) in soil. Based on this review, a specific soil particle size of <150 μm is recommended for future studies on the oral bioavailability and bioaccessibility of PAHs in soil.

INTRODUCTION

One of the assumptions incorporated into studies to characterize human exposures to contaminants in soil is the notion that the nature of the matrix studied is representative of the material to which humans are actually exposed. One aspect of this assumption is the particle size fraction of the soil that is used in studies of the oral bioavailability and bioaccessibility of chemicals in soils (use of the terms "oral bioavailability" and "bioaccessibility" herein are consistent with those in use by U.S. EPA).[1] Historically, a wide variety of soil particle sizes has been used by different research groups (ranging from <45 to <2000 μm) thereby making it difficult to directly compare reported results across studies. This article reviews factors that affect decisions regarding the particle size that best represents oral exposures to humans, including studies of soil adherence on hands and distribution of contaminants in soils as a function of particle size, the historical context regarding selection of soil particle sizes, and considerations of how the resultant data will be used in risk assessment. These factors are discussed from a general perspective, one that applies to all contaminants in soil, and then specifically with respect to oral exposures to polycyclic aromatic hydrocarbons (PAHs) in soil. It is not the intent herein to address the amount of soil being ingested, but rather the size of the soil particles ingested. The reader is referred to USEPA[2] for a thorough discussion of soil mass ingested by children and adults.

APPLICATIONS TO RISK ASSESSMENT

Under the current regulatory paradigm developed by the U.S. Environmental Protection Agency (U.S. EPA), contaminant remediation standards or goals that are developed for direct contact with soil are based on the assumption that incidental ingestion of soil provides the primary pathway for human exposure to soil contaminants.[3] The general understanding is that this incidental ingestion is largely the result of hand-to-mouth contact (or hand-to-food contact and subsequent ingestion of that food) after loading of soil onto the hands during normal daily activities (including play, for young children). Although other mechanisms exist that could contribute to soil ingestion, such as ingestion of soil particulates adhering to vegetables or inhalation of soil particulates, followed by ingestion of particulates after mucosiliary clearance, the available information suggests that these are minimal contributors to ingestion exposures. Because soil ingestion by adults is lower than that for children,[2] ingestion of soil particulates on vegetables and ingestion of particulates subsequent to inhalation and mucociliary clearance do not appear to be significant

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contributors given that adult consumption of vegetables, and inhalation volumes for air, are higher than those for children.[2] In addition, mechanistic modeling to evaluate the contribution to soil ingestion from mouthing behavior yields soil exposure estimates that are similar to values from soil ingestion tracer studies (68 vs 100 mg/day), with about 90% assumed to be contributed from hand-to-mouth contact.[2]

This pathway of incidental ingestion is distinct from the soil ingestion that might be incurred by a child who exhibits pica behavior for soil. In the instance of pica, larger masses of bulk soils may be ingested, and the risks in these instances may include shorter-term toxicity.[4] The focus of this paper is to better understand the nature of the particles contributing to long-term exposure to soil, associated with loading onto hands and subsequent, inadvertent, ingestion.

Under the conventional paradigm for human health risk assessment outlined by the National Academy of Sciences,[5] risks from exposure to contaminants in soil are estimated by combining information regarding the potential toxicity of the chemical(s) of interest together with information regarding human exposure to those chemicals. Exposure from contaminated soil is calculated according to the following formula:

$$\text{Exposure}_{\text{ingestion}} = \frac{C_s \times \text{IR}_s \times \text{RBA}}{\text{BW}}$$

where

- C_s = concentration of contaminant in soil (μg/g)
- IR_s = soil ingestion rate (g/day)
- RBA = relative bioavailability adjustment (unitless)
- BW = body weight (kg)

Thus, ingestion exposures are expressed in terms of mass (units of micrograms of chemical per kilogram of body weight per day). As a result, it is the mass of the chemical that defines the level of exposure and hence the associated potential for toxicity, or "risk". Therefore, in refining our understanding of exposures to chemicals in soils from incidental ingestion, the ultimate goal remains to understand what controls the mass of the chemical ingested. This distinction is important for two reasons. First, although small particles (e.g., clay and silt size) are generally more abundant on hands than larger particles (e.g., fine sand), the mass of soil adhering may reside in the larger particles. If those larger particles contain a significant mass of contaminant, then they may contribute to exposure (i.e., mass of contaminant ingested). Second, most studies of contaminant enrichment in soil report the enrichment as a function of concentration when it is actually enrichment as a function of mass that is important for selecting a soil particle size for characterizing human exposures.

In the context of assessing exposures to contaminants in environmental media, U.S. EPA provides guidance for how to select inputs to exposure assessments. Risk Assessment Guidance for Superfund[6] states that "some intake variables may not be at their individual maximum values, but when in combination with other variables will result in estimates of the Reasonable Maximum Exposure (RME)." Thus, some intake factors will be set at high-end values, while some are estimates of mean values, such that the overall estimate of intake represents an RME. Review of U.S. EPA's Exposure Factors Handbook[2] confirms that recommendations for inputs to exposure calculations can vary from mean to high-end values; however, it does not specifically discuss soil particle sizes ingested. In light of this approach to characterizing exposures, we have selected to present the particle size cutoffs that would account for 50% and 90% of the mass adhering to hands (termed the 50th and 90th percentile values herein) for each of the studies for which these values are reported or can be calculated. The selection of the most appropriate value for characterizing exposure is discussed below.

HISTORICAL PERSPECTIVE

The earliest published studies on the oral bioavailability of contaminants in soil were conducted for dioxins/furans in the mid-1980s. Two studies performed at the National Institute of Environmental Health Sciences, by McConnell et al.[7] and Lucier et al.,[8] used the <250 μm (60 mesh sieve) soil fraction to investigate oral bioavailability of soils from the Times Beach and Minker Stout, Missouri, sites. During the same time period, Bonaccorsi et al.[9] and Shu et al.[10] used 30−74 μm and <420 μm soil size fractions, respectively, for studies of dioxin bioavailability from soil at Seveso, Italy, and Times Beach, Missouri. None of these authors provide rationales for why they chose a particular size fraction for their studies.

Subsequently, in the 1990s, several research groups began conducting studies on PAH bioavailability from soils. These studies generally used finer particle sizes—Goon et al. (crushed to <100 μm),[11] Rozett et al. (studied a range of particle sizes with the smallest being <150 μm and the largest being <850 μm),[12] and Weyand et al. (<150 μm)[13]—although sometimes larger soil size fractions were used (e.g., Koganti et al., <1000 μm).[14] Once again, these authors did not provide any rationale for the particle sizes selected to represent oral exposures.

In the early 1990s, U.S. EPA began developing a swine model for the assessment of soil lead bioavailability. The earliest version of this model used a <149 μm (100 mesh sieve) particle size.[15] However, this particle size was increased to <250 μm in the final version of U.S. EPA's swine model.[16] The reason for the change from <149 to <250 μm is not documented. U.S. EPA has also developed a swine model for determination of relative arsenic bioavailability from soil, which uses a <250 μm soil fraction.[17] U.S. EPA's selection and use of the <250 μm soil fraction most likely influenced later research groups, which generally have used the <250 μm particle size for oral bioavailability research models (Maddaloni et al., lead in humans;[18] Roberts et al., arsenic in primates;[19,20] Budinsky et al., dioxins/furans in rats and swine;[21] Finley et al., dioxins/furans in rats[22]). Recently, James et al. developed a swine model for PAH bioavailability from soil using a <45 μm soil fraction.[23] The selection of this particle size was based on the research of Siciliano et al.[24] (discussed below) and was selected as a soil fraction that would be enriched in clay and silt size particles because "it is commonly thought that metallic toxicant concentrations will be higher in the clay fraction and organic toxicant concentrations higher in the silt fraction."

The <250 μm soil fraction has also been used in environmental health studies for lead and arsenic that have been conducted at mining sites around the western U.S. (Anaconda, Montana;[25] Butte, Montana;[26] Midvale, Utah[27]) and in U.S. EPA's Urban Soil Lead Abatement Demonstration Project (also known as the "Tri-City Lead Study") conducted in the late 1980s to evaluate the effect of lead-contaminated soil removal on lead in children's blood.[28,29] It is also the basis upon which U.S. EPA's Integrated Exposure Uptake Biokinetic (IEUBK) model, for estimating blood lead concentrations in children, is validated because the environmental health lead studies used for that purpose relied on soil samples sieved to <250 μm.[30] Recommendations for conducting bioavailability studies to support blood lead modeling with the IEUBK specify that it is "critical to sieve soil

Table 1. Summary of Soil Adherence Studies

study	year	data reporting method	study type and conditions	particle size cutoff accounting for 50% of adhering mass	particle size cutoff accounting for 90% of adhering mass
Duggan et al.[33]	1985	number of particles adhering in each size range	field	NM[a]	NM
Duggan and Inskip[34]	1985	mass of soil adhering for each size fraction	laboratory	57 μm	130 μm
Que Hee et al.[35]	1985	mass of house dust adhering for each size fraction	laboratory	NA[b]	NA
Driver et al.[38]	1989	mass of soil adhering for each size fraction	laboratory	NR[c]	NR
Sheppard and Evenden[39]	1994	enrichment ratios of the mass of specific size fractions adhering	laboratory	NR	NR
Kissel et al.[40]	1996	mass of soil adhering for each size fraction	laboratory: dry soil	62 μm	210 μm
			Laboratory: Wet soil	150 μm	350 μm
Choate et al.[41]	2006	mass of soil adhering for each size fraction	laboratory: low moisture soil	33 μm	110 μm
			laboratory: medium moisture soil	44 μm	120 μm
			laboratory: high moisture soil	80 μm	220 μm
Yamamoto et al.[42]	2006	mass of soil adhering for each size bin	field	67 μm[d]	134 μm[d]
Siciliano et al.[24]	2009	mass of soil adhering in each size fraction	field	40 μm	130 μm
			laboratory: agricultural soils	40 μm	370 μm
			laboratory: brownfield soils	125 μm	760 μm
Bergstrom et al.[43]	2011	estimated mass adhering for each size fraction	laboratory	NR	NR

[a]NM = No mass-based estimate of soil adherence. 90% of particles were <10 μm. [b]NA = Not applicable (study used house dust not soil). [c]NR = Not reported or not calculable from data presented. [d]Average value for the population of children (three of nine) with the largest soil particles adhering.

samples to <250 to more closely represent the size of soil particles that would be expected to adhere to children's hands".[31] Finally, U.S. EPA's Superfund Lead-Contaminated Residential Sites Handbook specifies analysis of the <250 μm size fraction for evaluation of childhood exposures to lead in residential soils.[32]

The selection of the <250 μm soil size fraction for assessment of human exposure to metals (and metalloids) in soil appears to have emerged from the environmental health studies described above. At that time (late 1980s), there were few data available to consider when selecting a particle size cutoff (see Table 1 for list and dates of studies). Given the data published in the last 15 years (discussed below), it appears that the <250 μm soil size fraction may not be the optimum soil fraction for assessing human exposures due to direct contact with soil.

SOIL PARTICLE SIZES ADHERING TO HUMAN HANDS

A number of studies on the size of particles that adhere to human hands have been conducted (summarized in Table 1). Some of the primary distinctions between these studies are (1) whether they studied adherence under field conditions or in the laboratory, (2) the methods by which the hand was exposed to soil (press, contact by inversion of soil container over hand, crumbling of soil in hand, or rubbing soil into digits), (3) the methods by which soil was removed from the hand (wash, wipe, or abrasion), (4) the particle size measurement method (optical microscopy, mass in different sieved fractions, or particle size analyzer), (5) whether they studied the effects of moisture content of the soil or the hand (a variable controlling particle size adhesion), and (6) whether they reported the results as the distribution of the number of individual particles or the distribution of soil mass adhering as a function of particle size. Only one study, Duggan et al., reported results based on the former measure, and their results are clearly anomalous when compared to all of the other studies (discussed below).[33]

Studies that have measured soil particle sizes adhering to hands are reviewed below and are broken out as those studies that report the number of particles adhering as a function of particle size versus those that report the mass adhering as a function of particle size because of the importance of this distinction.

Studies Reporting the Number of Particles Adhering to Hands as a Function of Size. Duggan et al. studied soil particle sizes naturally adhering to 20 children's hands following play activities.[33] The authors used a hand wipe method followed by particle sizing by optical microscopy (50 particles sized per wipe). They observed a mean particle diameter of 4.5 μm and a maximum particle diameter of 100 μm. This study has been cited widely as the basis for selecting particle size fractions to which humans are exposed through hand-to-mouth activity. While it has the advantage of having studied children in their natural environment, it has the limitation of having reported only the number of particles in each size class adhering to the children's hands, and not the soil particle sizes that contributed most greatly to the mass of material adhering.

Studies Reporting the Mass of Soil Adhering to Hands as a Function of Size. Duggan and Inskip report on a small study (one subject and four soil samples) in which 20 mg of prefractionated soil (fractions were 0−53, 53−100, 100−150, and 150−500 μm) were rubbed into the thumb and forefingers, removed by gentle abrasion, and then weighed.[34] Under these conditions, the authors observed 48, 28, 16, and 8% of the mass in the 0−53, 53−100, 100−150, and 150−500 μm fractions adhered, respectively (data normalized to adherence for the total mass from all four fractions). Based on these data, the 50th and 90th percentile values are 57 and 130 μm, respectively (Table 1, Figure 1). The authors state that "if the hand-mouth route is the

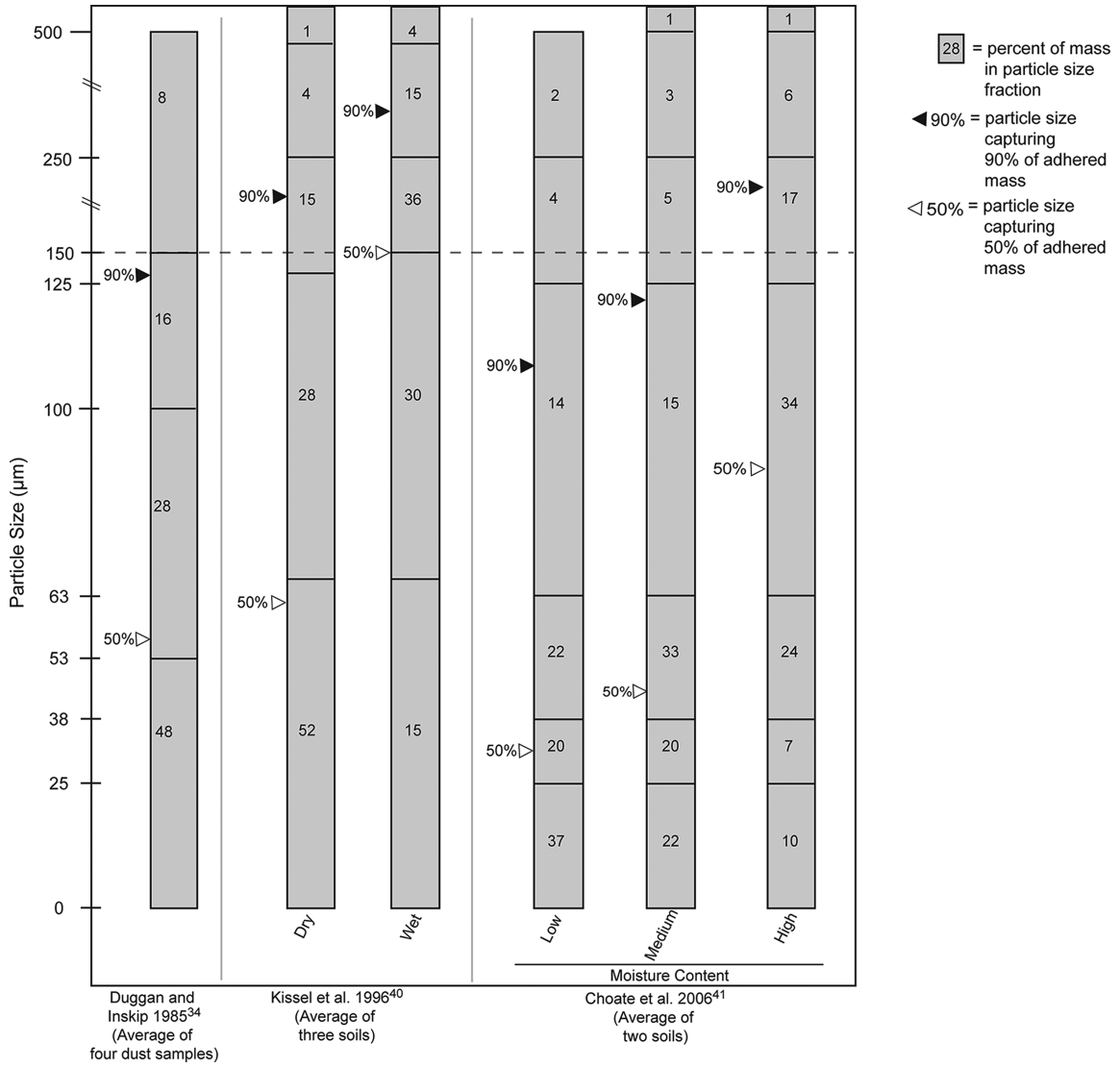

Figure 1. Summary of studies presenting mass of soil adhering to hands as a function of particle size and soil moisture content. Black arrow (▶) indicates particle size capturing 90% of adhering mass, white arrow (▷) indicates particle size capturing 50% of adhering mass.

important one for children, then there would be some merit in analyzing only those particles of diameter less than, say, 200 μm." The rationale for the selection of a 200 μm particle size is not discussed in the publication, but it would capture about 95% of the mass adhering to hands.

Que Hee et al. conducted a study that involved only a single volunteer and a single house dust sample.[35] The house dust was prefractionated into fractions of <44, 44−149, 149−177, 177−246, 246−392, and 392−833 μm. The hand of a "small adult" was placed lightly over a dish containing 5 g of each individual soil fraction. The hand and dish were inverted and then reinverted. The mass adhering was calculated by difference in weight of dust in the containers pre- and posthand contact. These authors report that for all materials in the <246 μm fractions an equal mass adhered to the palm of the volunteer. This result stands in contrast to all of the other studies reported herein, which have all shown that smaller particle sizes preferentially adhere to hands for dry soils. The use of house dust, as opposed to soil, may be the cause of this discrepancy. House dust is composed of a large amount of organic material (e.g., insect parts, food particles, exfoliated skin cells, hair, and small organisms[36,37]) relative to soil. If this organic material occurred in the size fractions <246 μm in this study, it could have altered the adherence characteristics of the different size fractions. As a result, this study is not considered to be comparable to soil adherence studies.

Driver et al. conducted a study that involved the adherence of 11 soils fractionated into 3 particle sizes (<150 μm, <250 μm, and bulk) to the hand of a single volunteer (soils were a combination of both top soil and subsurface soil).[38] Using hand press trials (each test in triplicate), these authors measured the mass of material adhering to one subject's hand from each size fraction of material for each soil. The mass adhering was calculated by difference from the weight of the soil container pre- and postloading, and results were reported as the mass adhering per square centimeter of skin surface. The mass of soil adhering was greatest for the <150 μm fraction (average of 1.40 mg/cm^2), followed by the <250 μm fraction (0.95 mg/cm^2), and then bulk soil (0.58 mg/cm^2). Due to the manner in which the study was performed, the data could not be used to calculate the relative distribution of mass adhering from each size fraction. Across the 11 soils tested, there was more than 2-fold variability in the masses adhering for the <150 μm and the <250 μm fractions.

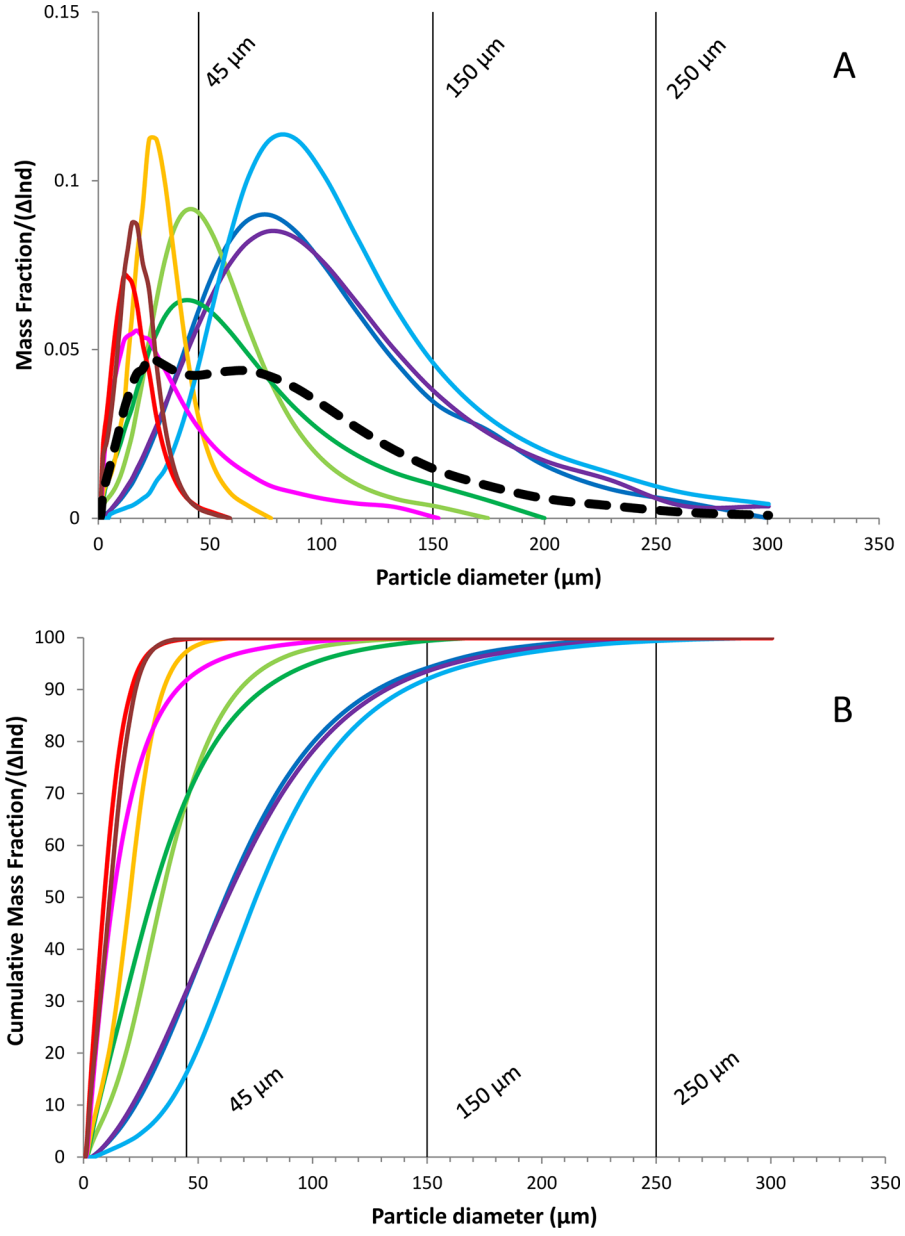

Figure 2. Mass fraction of soil adhering to children's hands as a function of particle size (data from Yamamoto et al.).[42] Panel A presents distributions for individual children (colored lines) and the average distribution for all nine children (solid dashed line). Panel B presents the cumulative mass fraction adhering as a function of particle size.

Sheppard and Evenden conducted hand press trials for 11 different soils, selected to represent a range of soil types, followed by washing to remove the soil.[39] Particles sizes were measured in solution using a particle size analyzer and the particle mass in each size class (the mass fraction) was calculated based on the assumptions that all particles were spherical and had the same density (2.54 g/cm^3). These authors found that particles less than about 50 μm preferentially adhered to hands relative to particles in the 50−100 μm size range. The authors did not report the mean or maximum size adhering: the particle size analyzer had a measurement limit of 100 μm, so the entire range of adhering particles was most likely not measured. These authors also studied the soil material not removed by the washing procedure and concluded that there was strong adhesion of clay particles (<2 μm), which are similar in size to skin surface roughness characteristics. This has implications for hand-to-mouth transfer of soil and suggests that some portion of the clay fraction that adheres to hands may not be ingested due to difficulty of removal once it has adhered to skin.

Both Kissel et al. and Choate et al. studied the effect of soil moisture content on particle size adherence to hands and found that increased soil moisture could substantially increase the adhering particle sizes.[40,41] Kissel et al. studied two soil moisture contents (1−6% and 14−19%) and used hand press trials with three soils, followed by washing and sieving. This work indicated that for dry soils (<6% moisture content), the bulk of adhering soil mass (about 80%) was in the sub-135 μm size fraction (Figure 1; data interpolated from Figure 1 of Kissel et al.).[40] However, for wet soils (14−19% moisture content), the <135 μm size fraction accounted for only about 45% of the mass adhered, and the >135 μm fraction became the dominant contributor to mass adhered (55%). Choate et al. observed

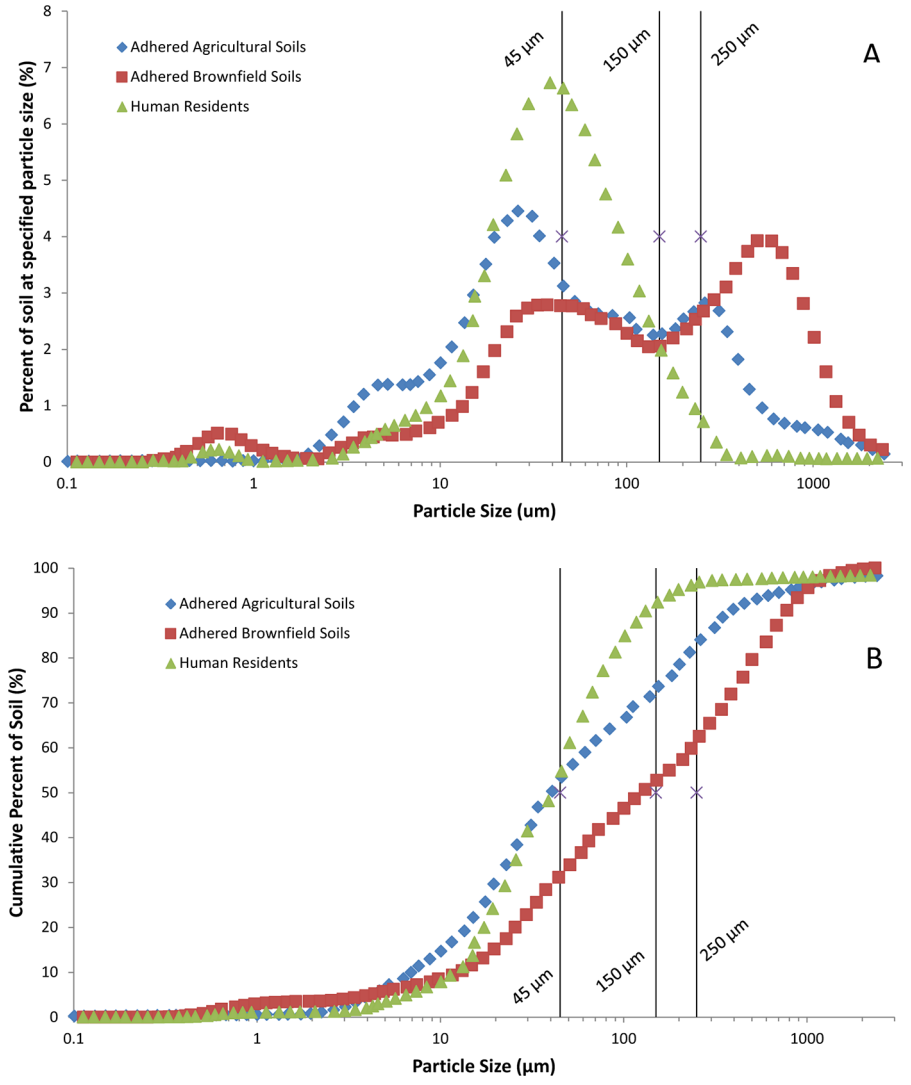

Figure 3. Soil mass adhering to hands as a function of particle size (data from Siciliano et al.).[46] Panel A presents the distributions for the three soil/exposure conditions studied and panel B presents the cumulative distributions for those three studies.

similar results when studying the effect of three soil moisture contents—low (1−2%), medium (3−4%), and high (9−10%)—on adherence of two soils that were contacted by volunteer's palms (the hand was placed over an open-ended container of soil and the container was inverted 10 times), washed with deionized water, and then wet sieved. These authors report that for dry and moderately moist soils, 75−80% of the adhering mass was in the <63 μm size fraction (Figure 1; data interpolated from Figures 1 and 2 of Choate et al.).[41] However, for the moist soil (9−10% water content), the 63−125 and 125−250 μm fractions constituted (on average) 34 and 17%, respectively, of the adhering mass.

Yamamoto et al. studied the distribution of particle sizes on the hands of young children (n = 9; average of 4 years of age) following play activities at a nursery school.[42] This is the first published study to evaluate adhering particle size distribution, as a function of soil mass adhered, under real-life conditions. Children's hands were washed and the particles were analyzed with a particle size analyzer. Results were converted to mass of material adhering for each of 46 particle size bins based on the assumption that all particles were spherical and had equivalent density (2.54 g/cm^3). Figure 8 of Yamamoto et al. is reproduced here as Figure 2a (figures in this paper were generated from the raw data provided by Dr. Yamamoto; the y-axis nomenclature [mass fraction/Δ ln d] stands for the mass fraction per particle size bin; the bins sizes were designed so that taking their natural log resulted in equivalent bin sizes), and Figure 2b provides a cumulative distribution of mass fraction for each of the nine children.

Yamamoto et al. found a 6-fold difference in the mode particle size adhering to the hands of different children, consistent with previous studies that have shown large variability among individuals or study populations. The mode diameter (i.e., the value that occurs most frequently in a distribution) was used to characterize the central tendency of the distributions and varied from approximately 15 to 90 μm for different children (Figure 2a) (this is essentially the particle size at which the maximum mass of material is adhering). The maximum size particles observed were in the 100−300 μm range. In addition, increasing hand moisture was associated with an increased mass of soil adhered to hands and a slight increase in mean particle size.

This is a particularly useful study because it provides data on individual study participants. Of the children studied, 30% of the population (3 of 9 children) had considerably larger particles adhering to their hands than the other children (Figure 2a). If these larger particles, in the range of 50−150 μm, contain

significant contaminant mass, then they will contribute to exposure and it is important that they be included in the size fraction that is used for characterizing oral exposures (as described below, the 50−150 μm fraction does contain an appreciable mass of the PAHs in soil). For this reason, the 50th and 90th percentile values were calculated for the three children with the largest particles adhering (Figure 2a). This results in 50th and 90th percentile values of 67 and 134 μm, respectively (Table 1). This approach to interpreting the data is consistent with guidance for conducting risk assessment, discussed above, as it captures a high percentile of mass adhered for all individuals, and ensures that meaningful data from a complex data set is not overlooked.

Siciliano et al. conducted a laboratory study of soil adherence to human hands for 13 agricultural soils and 17 soils from a brownfield site.[24] In this study, volunteers crumbled a handful of soil, excess soil was lightly brushed off, the hand was washed with a dilute nitric acid solution, and particle sizes were determined with a particle size analyzer. For these two types of soils, the mean particle sizes adhering were reported as 34 and 105 μm, for agricultural soils and brownfield soils, respectively. When hand-washing trials were conducted in the field on 19 residents of Iqaluit, Canada (age range of 4 to 62 years with a median age of 23 years), the mean particle size adhering was 36 μm. Figure 2 from Siciliano et al. is reproduced herein as Figure 3a (data reproduced by digitizing the original figure) and are also presented as cumulative percent of soil mass adhering (Figure 3b). The bimodal distribution in Figure 3a for the agricultural soils and brownfield soils suggests a large degree of variability between the different soils tested (curves are aggregation of data for 13 and 17 different soils, respectively). For applications to risk assessment, preference is given to the data collected in the field, which resulted in 50th and 90th percentile values of 40 and 130 μm, respectively (Table 1). Less weight is given to the laboratory derived values, which generally produced larger soil particles adhering to hands (the agricultural and brownfield soils had 90th percentile values of 370 and 760 μm, respectively; Table 1). Siciliano et al. recommend, based on these data, that soils should be sieved to <45 μm for evaluating human exposures to contaminants in soil. In reviewing the study data, we find that selection of the <45 μm value would exclude nearly 50% of the mass adhered to the hands of residents. This is not consistent with our goal to identify the particle size fraction that captures the bulk of the soils contributing to ingestion exposures, while excluding, to the extent possible, those particle sizes that are not relevant for exposure.

The most recent paper to evaluate the particle sizes of soils adhering to hands, Bergstrom et al., conducted a laboratory study on the hand adherence of particulates from nine geologic media derived from mining, smelting, and quarrying activities.[43] The mining and smelting samples were primarily collected from the banks and sand bars/beaches of rivers that had received mining and smelting wastes and thus likely contained tailings and slag, while the quarried materials were described as "gravel products". As a result, these materials were distinctly different from typical soil and consisted primarily of very fine to very coarse sand. Each sample was sieved to four different size fractions (<63, 63−150, 150−250, and 250−2000 μm), and the mass of material and metals concentrations were determined for each fraction. Six volunteers actively handled each unfractionated medium under wet and dry conditions (wet conditions ranged from 3.5% to 14.7% moisture content, depending on the water holding capacity of the media, and dry conditions were <0.25% moisture content). Subjects' hands were washed with deionized water and the collected material was dried and analyzed for metals concentrations. Adhering particle sizes were not directly measured. Rather, proportions of adhering particle size fractions were estimated using a maximum likelihood estimation (MLE) technique. Fractions of adhering mass attributed to each size fraction were allowed to vary to minimize a function representing the difference between predicted and observed concentrations of metals in adhering media. Based on the MLE analysis, the authors report that greater than 60% of the adhered mass was <63 μm in the dry media (7 of 9 samples). In the trials with wet media, the <63 μm fraction was estimated to account for less than 25% of the adhered material (8 of 9 samples), and the largest particle size category (>150 um) dominated the mass adhered for 7 of the 9 media tested. These results suggest that, particularly for the wet media, larger particles are preferentially adhering relative to the studies on soils discussed above. This may be due to the unique character of the materials tested and the fact they consisted primarily of relatively coarse materials.

The studies discussed above are summarized in Table 1, in terms of the type of study, the data reporting method, and the particle size cutoff that would account for 50% or 90% of the adhering mass. This last metric either could not be determined, or was not reported, for a number of the studies for various reasons, including (1) the authors did not report their results on the basis of mass adhering,[33] (2) the authors studied house dust, not soil,[35] (3) the authors did not fractionate their soils into sufficiently fine fractions to support this calculation,[38] and (4) the manner in which data were reported would not support this calculation.[39] The 50th and 90th percentile values are also not presented for Bergstrom et al. because the size of adhering particles was inferred from modeling and not directly measured, and because the authors caution that the media tested "are not conventional soils".[43]

DISTRIBUTION OF CONTAMINANTS IN SOIL AS A FUNCTION OF PARTICLE SIZE

If a contaminant is not evenly distributed across soil particle sizes, then preferential ingestion of a specific particle size fraction may either increase or decrease the mass of contaminant ingested for a given mass of soil ingested. For example, if a chemical is enriched in the fine fraction of soils and this fraction is preferentially ingested, then the mass of chemical ingested will increase relative to what would have been ingested if the chemical were evenly distributed in soil. There is an extensive body of literature regarding the enrichment of both organic and inorganic compounds in soils and a thorough review of this topic is beyond the scope of this paper. In general, the concentration of both organic and inorganic compounds is enriched in the fine fraction of soils,[24,43−46] although site-specific exceptions occur. Unfortunately, most authors report only enrichment as a function of concentration, when reporting distributions in terms of both concentration and calculated mass would be more useful for overall evaluation of oral exposures. The distributions of organic and inorganic compounds in soil are dependent on the source of the contamination (e.g., size of contaminant particles released to soil) and the redistribution of the organic chemicals or inorganic elements into different sorption domains or mineral phases during weathering in the soil environment. The literature addressing this issue for PAHs is discussed in detail below.

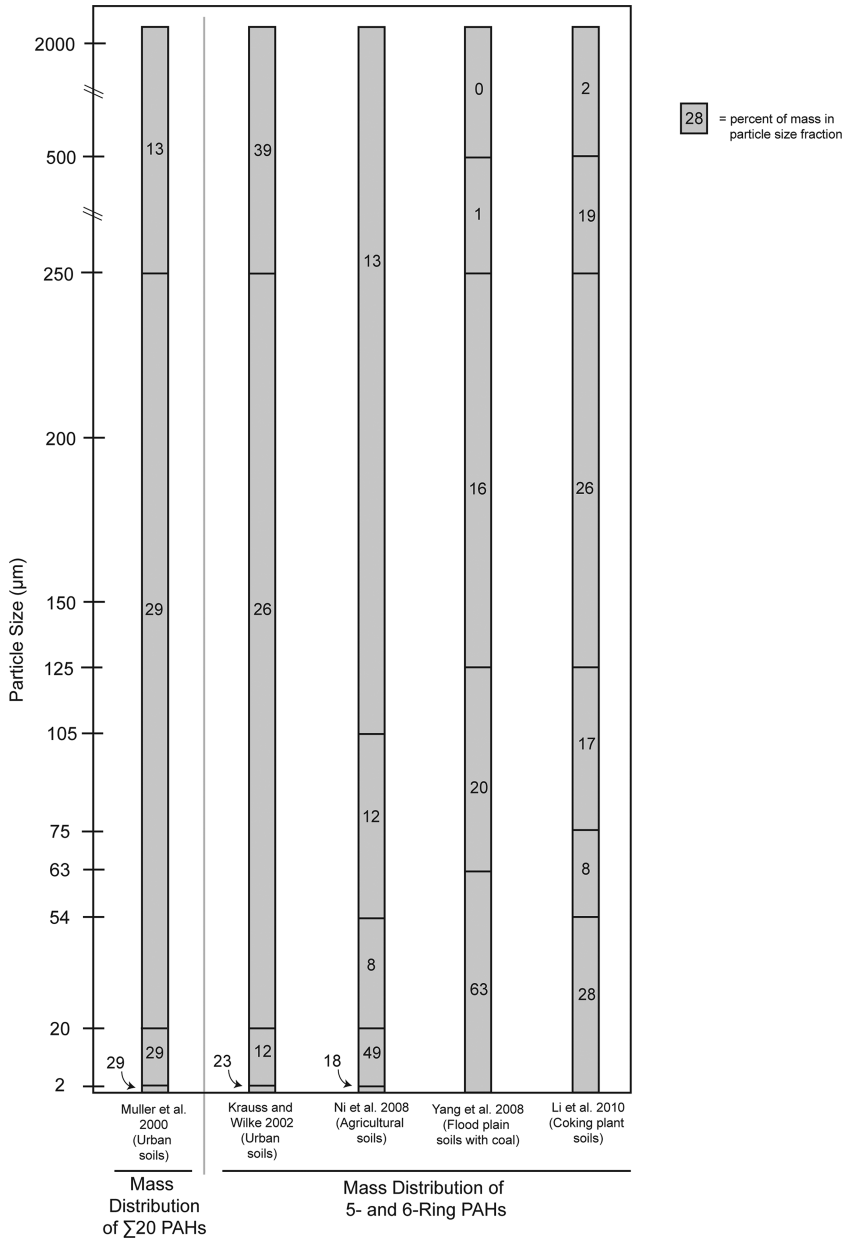

Figure 4. Distribution of the mass of PAHs in different soil particle size fractions, based on reanalysis of data from the five studies indicated.

DIFFERENTIAL BIOAVAILABILITY AS A FUNCTION OF PARTICLE SIZE AND GEOCHEMICAL MATRIX

Both the size and the chemical composition of an ingested particle can affect the oral bioavailability of organic and inorganic chemicals. This issue is reviewed in detail for inorganic chemicals in Ruby et al.[47] Basically, mineral forms that are more stable under acidic conditions (e.g., the stomach) yield lower oral bioavailability, and smaller particles yield greater bioavailability because dissolution occurs faster and more extensively during passage through the gastrointestinal tract. The morphology of the mineral phases, particularly the rinding or encapsulation of primary mineral phases by secondary alteration phases, will also impact the oral bioavailability of inorganic elements.[47]

For hydrophobic organic chemicals (HOCs), there are fewer data on the factors that control oral bioavailability than there are for inorganic elements. It appears that the form in which the HOC enters a soil (e.g., matrix effects), the structure of the matrix particles (e.g., porosity), and the organic carbon−water partition coefficient (K_{oc}) values of the different organic carbon phases in the soil (e.g., natural organic matter, kerogen, and black carbon forms such as soot and char) will determine the tendency of a soil to sequester HOCs.[48,49] Studies indicate that differences of up to 2 orders of magnitude exist between the K_{oc} values of natural organic matter and black carbon forms in soil.[48] Based on this, we postulate that the extent and type of black carbon in soils will control the oral bioavailability of HOCs to humans. There is only one published study on the effects of black carbon on the oral bioavailability of an HOC in soil or sediment. Saghir et al. evaluated the ability of lampblack soot to reduce the oral bioavailability of hexachlorobenzene in soil when dosed orally to rats;[50] other studies that attempt to address this have only looked at total organic carbon as a variable effecting oral bioavailability. However, studies using ecological receptors (e.g., earthworms, benthic invertebrates) have demonstrated decreased HOC uptake from soils and sediments containing elevated levels of black carbon, coal, coke, kerogen, and biochar.[48,51,52] The

differential oral bioavailability of PAHs as a function of particle size and geochemical matrix is discussed below.

CONSIDERATIONS SPECIFIC TO PAHS

The following analysis is specific to the selection of a particle size for oral bioavailability and bioaccessibility studies for PAHs in soils.

Consistent with the body of research on other contaminants, a variety of particle sizes has been used for oral bioavailability studies of PAHs in soils, ranging from <100 μm to <1000 μm.[14,53] A review of the literature regarding PAH bioaccessibility (i.e., in vitro testing) over the last 10 years (17 publications) reveals a pattern that is similar to that observed for oral bioavailability studies. Although <250 μm was the size fraction most commonly used (seven publications), bioaccessibility data for four other particle size fractions have also been reported (<2 mm, <1 mm, 125−250 μm, and <45 μm).[46,54−56]

Distribution of PAHs among Soil Particle Size Fractions. To accurately characterize oral exposures to PAHs in soils, it is important to understand how PAHs are distributed among the different particle size fractions. For example, if PAHs are present only in the finest particles, then exposure will occur only from ingestion of those fine particles. However, if PAHs are evenly distributed across all particle sizes that are ingested, then any of those particles will constitute an exposure.

A number of publications have evaluated the distribution of PAHs in soils as a function of particle size.[45,46,57−61] Of these seven studies, only five report the data required to establish the distribution of PAHs as a function of mass (rather than just as a function of concentration) in surficial soils and are, therefore, the focus of our review. There are a similar number of publications that evaluate PAH distribution as a function of particle size in sediments. These papers are also not reviewed here because of the distinct nature of soils versus sediments.

A study by Muller et al. evaluated the distribution of PAHs as a function of soil particle size fraction in 10 urban surface soils from Bangkok, Thailand.[59] Each sample was separated into 4 size fractions: <2, 2−20, 20−250, and 250−2000 μm, and each fraction was analyzed for 20 PAHs ranging in size from 2 to 6 rings. Geometric mean concentrations of total PAHs, across all 10 samples, decreased in the following order: 2−20 μm (219 mg/kg) > <2 μm (201 mg/kg) > 20−250 (139 mg/kg) > 250−2000 μm (51 mg/kg). However, when these results are converted to a mass distribution, by correcting for the mass of soil in each size fraction of each sample, the geometric mean mass of total PAHs is found to be evenly distributed across the three smallest size fractions, with a distribution of <2 μm (29% of total mass) = 2−20 μm (29%) = 20−250 (29%) > 250−2000 μm (13%) (Figure 4). For these soils, expressing PAH distribution on a mass basis shifts the distribution to a larger particle size fraction, relative to the distribution based on PAH concentrations. These authors did not report the concentrations of individual PAHs in different particle size fractions, so the mass distribution of carcinogenic (five- and six-ring) PAHs cannot be determined for this study.

Krauss and Wilcke studied the distribution of PAHs in 11 urban top soils from in and around the city of Bayreuth, Germany, and reported on the same 4 particle size fractions (<2, 2−20, 20−250, and 250−2000 μm) and for the same 20 PAHs as Muller et al.[45] The study samples represented a diversity of soil types and land uses, including a forested area, road-side, garden, alluvial grassland, agricultural soils, a landfill, and a former gasworks site. The authors report the concentrations of five- and six-ring PAHs, relative to the sum of all 20 PAHs (as a percent). Using these data (interpolated from Figure 1a of the article) and the average distribution of soil mass in the different size fractions, the mass-distributions of five- and six-ring PAHs were calculated. On a concentration basis, the five- and six-ring PAHs were distributed relatively evenly across the four size fractions (range of 3.8−5.1 mg/kg). As result, the mass-based distribution was primarily influenced by soil texture (i.e., the distribution of mass in the different soil fractions) and yielded a result of 250−2000 μm (39% of total mass) > 20−250 μm (26%) > <2 μm (23%) > 2−20 μm (12%) (Figure 4).

Ni et al. studied the distribution of PAHs as a function of soil size fractions for nine agricultural soils from Zhejiang province, China.[60] Each soil was separated into 5 size fractions: <2, 2—20, 20—54, 54—105, and 105—2000 μm, and each fraction analyzed for the 16 U.S. EPA priority pollutant PAHs. The authors report the percent of soil mass in each particle size fraction for each soil, and also the average percent of five- and six-ring PAHs in each size fraction. From these data, the mass distribution of five- and six-ring PAHs was calculated for each of the five particle size fractions. These results indicate that the mass of five- and six-ring PAHs are predominantly in the clay (<2 μm) and fine silt (2−20 μm) fractions of these soils (total of 67% of mass; Figure 4). This outcome is influenced by both the soil texture and the concentrations in the different soil size fractions, because both of these variables span a wide range in these samples.

Yang et al. evaluated the distribution of PAHs in 16 river floodplain soils from the Mosel river in Germany that were known to be impacted by coal and coal-derived particles from coal mining and coking operations.[61] Each soil was fractionated into 5 fractions: <63, 63−125, 125−250, 250−500, and 500−2000 μm, and each fraction was analyzed for 19 PAHs ranging in size from 2 to 6 rings. When the geometric mean percent of five- and six-ring PAHs was calculated (data interpolated from Figure 4 of the article), as a function of total PAHs in each fraction, and was corrected by the mass of material in each size fraction, the mass distribution of five- and six-ring PAHs is <63 μm (63%) > 63−125 μm (20%) > 125−250 μm (16%) > 250−500 μm (1%) > 500−2000 μm (0%) (Figure 4). It should be noted that the floodplain soils evaluated in this study were fine grained materials (59% of mass was <63 μm), and that, as with the Ni et al. study, the mass distribution of five- and six-ring PAHs was partially controlled by the soil texture. The authors noted a strong positive correlation between total PAH concentrations and black carbon (e.g., coal, r^2 = 0.98, p < 0.005) and concluded that the distribution of PAHs in these soils is largely controlled by the particle size distribution of coal and coal-derived particles, which are both the primary PAH sources to these soils and the geosorbents that will sorb any PAHs in the soils most strongly.

Li et al. reports on a study that evaluated the distribution of PAHs in 15 samples from a former coke oven plant in Beijing, China.[58] Each soil was separated into 6 size fractions (<50, 50−75, 75−125, 125−250, 250−500, and 500−2000 μm), and the concentrations of the 16 U.S. EPA priority pollutant PAHs were determined in each fraction. The results indicated (data interpolated from Figure 2 of article) that the geometric mean concentrations of the five- to six-ring PAHs were distributed relatively evenly across the <50 μm and the 125−2000 μm size particles, with considerably lower concentrations in the 50−125 μm particles. When these data are corrected for the mass of soil in each size fraction, the mass-distribution of PAHs becomes <50 μm (28%) > 125−250 μm (26%) > 250−500 μm (19%) > 75−125 μm (17%) > 50−75 μm (8%) > 500−2000 μm (2%) (Figure

4). As in the Yang et al. publication,[61] the mass distribution of carcinogenic PAHs is controlled by the particle size distribution of the PAH source materials (coal tar and coal tar pitch in this case). The results also indicate that total PAH concentration in soils was strongly correlated with black carbon content (r^2 = 0.92) and less strongly with total organic carbon content (r^2 = 0.73).

Several trends emerge from the five studies reviewed above, despite varying particle size cutoffs used and the different data reporting approaches. Whether the data are reported for total PAHs, or for carcinogenic PAHs, particles up to 250 μm contain an appreciable mass of PAHs (Figure 4). Thus, particles up to 250 μm in size can contribute to oral exposures. In all of these studies, the soil texture is an important determinant of overall PAH mass distribution, because in most cases the concentrations of PAHs are relatively evenly distributed across the different size fractions. It is also clear that for sites with solid PAH source materials, the particle size of that source material will dictate the particles that contain the carcinogenic PAHs. Therefore, it is not necessarily correct to assume that PAHs will always be heavily enriched in the clay and silt fraction of soils.

Oral Bioavailability of PAHs from Soil as a Function of Particle Size. There is only one published study that addresses the effect of soil particle size on the oral bioavailability of PAHs from soil. Rozett et al. (published as an abstract only) studied the oral bioavailability of pyrene and genotoxic PAHs from one contaminated soil from a manufactured gas plant site.[12] The soil was fractionated into seven different particle size fractions ranging from <150 to <1000 μm, blended with different batches of rodent diet, and dosed to mice. The absorption of pyrene was quantified based on the urinary excretion of pyrene metabolites, and the absorption of genotoxic PAHs by the presence of DNA adducts in lung and forestomach tissue. The <150 μm soil size fraction produced the greatest excretion of pyrene metabolites and formation of adducts, relative to the coarse size fractions. As a result, the authors concluded that oral bioavailability of PAHs from soil is enhanced in the finest particle size fraction that they studied.

There is also only one published study of the solubility of PAHs in soil in a bench-scale "bioaccessibility test" from multiple soil particle size fractions. Siciliano et al.[46] evaluated two different soil particle sizes (<45 and <4000 μm) and found that the fraction of total PAHs extracted in this test was greater for the <4000 μm size fraction than for the <45 μm size fraction; however, this study evaluated only one particle size that is relevant to oral exposures, because a <4000 μm size fraction (particles up to fine gravel) is not expected to contribute to long-term incidental soil ingestion. From this data set, it is not possible to conclude whether the larger PAHs (five- and six-ring compounds that are the PAHs associated with carcinogenic activity) will consistently have higher or lower bioavailability in the fine fraction of soils relative to the coarser fractions of relevance.

Selection of a Soil Particle Size Fraction for PAH Bioavailability and Bioaccessibility Studies. The goal of this analysis is to identify the soil particle size fraction that best represents oral exposures due to hand-to-mouth transfer of particulate material, and then to select a particle size cutoff that provides the best representation of incidental oral exposures, for use in in vivo and in vitro experimental work. Because the fractionation occurring due to hand adherence of soil versus sieving creates different particle size distributions, it is not possible to select a sieve size that will produce a distribution identical to hand-adhered particles for all soils. For example, for a clay loam soil, in which the soil mass is contained primarily in particles <50 μm, sieving with a 250 μm sieve would result in a particle size distribution similar to that which adheres to hands (there are not very many large particles to adhere to hands or to be sieved out). In contrast, sieving a sandy soil, in which the soil mass is contained primarily in larger particles (50−2000 μm) with a 250 μm sieve would result in a sample in which large particles are over-represented relative to what would adhere to hands (many large particles that would not adhere to hands pass through the sieve and are included in the sample).

Given that a number of studies on soil adherence to hands have recently emerged in the literature and that they report a range of results, a weight-of-evidence approach was taken for selecting a particle size fraction that would best represent soil exposure due to incidental ingestion. Because it is unknown (i.e., existing information is contradictory) whether PAH bioavailability will be greater from large or small particles (as discussed above), and also because the data indicate that significant mass of five- and six-ring PAHs may be present in soil particles up to 250 μm, it seems unwise to exclude a significant proportion of soil particles that would potentially be ingested. For these reasons, and to be consistent with risk assessment guidance for selecting high-end versus mean values for exposure parameters, we have selected the 90th percentile values for characterizing the mass of soils adhering to hands and contributing to ingestion exposures. In addition, we have given preference to the two studies that assess exposures by residents or children to soil under conditions of natural contact.[24,42]

Based on these considerations, a size cutoff of 150 μm was selected as one that would include the bulk of particles adhering to hands while not overemphasizing large particulate material that would not be ingested. The 150 μm particle size captures 92% of the mass of soil adhering in both the Yamamoto et al. (Figure 2, population of children with the largest particles adhering)[42] and in field trials reported by Siciliano et al. (Figure 3).[24] It also captures between 80% and 95% of the hand-adhered material in the Duggan and Inskip, Kissel et al., and Choate et al. studies (with the exception of the Kissel et al. wet soil for which a 150 μm particle size cutoff would capture only 45% of the hand adhered material) (Figure 1).[34,40,41] The 150 μm cutoff would exclude about 30% and 50% of the mass reported by Siciliano for the agricultural and brownfield soil studies, respectively; however, in comparison to data from individuals studied in the field, those studies appear to have anomalously large particles adhering to hands. As a result, the 150 μm particle size cutoff would capture the bulk of the soil mass observed to be on the hands of children and adults, including both small and large particles, but does so without creating a sieving-induced bias toward larger particles than would generally adhere to hands.

FUTURE RESEARCH

For the purposes of assessing incidental oral exposures of children and adults to carcinogenic PAHs in soil, a definitive study of target soil particle sizes of relevance has yet to be conducted. Such a study would utilize both field and laboratory components (adherence to two- to three-year old children's and adult hands in both settings) to understand the effect of age-dependent characteristics (physical and behavioral), and field versus laboratory studies, and to further assess differences in intersubject variability. The distribution of PAHs as a function of particle size in these soils would be determined, and reported on both a concentration and mass basis for individual PAHs in

different soil particle size fractions. Because published data on the distribution of PAHs in soil are limited, it would be useful to develop these data for a variety of soils that contain PAHs from different sources (e.g., soot from diesel exhaust, char from pyrolysis, coal tar and coal tar pitch from manufactured gas plant sites or coking facilities, and nonaqueous phase liquids such as diesel or fuel oil) and a range of soil textures.

The results reported by Sheppard and Evenden suggest that some fraction of the clay size material (<2 μm) that adheres to a hand is not ingested because it remains adhered to hands following hand-mouthing behavior in children.[39] Because the one study conducted to date reports a hand-to-mouth transfer efficiency of only 11 to 22%, depending on mouthing type activity (thumb sucking, finger mouthing, or palm licking),[62] it is unclear whether the strong adherence of clay particles to hands is creating a particle size fractionation during hand-to-mouth transfer. A study of particle size fractionation during hand-to-mouth transfer would be a valuable addition to the understanding of the soil particle sizes contributing to incidental soil ingestion because no such study currently exists.

Although it may be tempting to further characterize the bioavailability of chemicals from different particle size fractions, the utility of such data would be limited: they may allow for a better understanding of the bioavailability data that have been reported to date, but such a retrospective evaluation likely has little merit given the other differences and limitations of the existing database on bioavailability. Moving forward, it does not matter what the bioavailability is from different particle sizes if the fraction used in the bioavailability study is the fraction that is being ingested. Therefore, future research would be best focused on determining the particle size range contributing to oral exposures. Based on the information presented above, a reasonable estimate of the upper range of the soil particle size that contributes to soil ingestion is 150 μm.

AUTHOR INFORMATION

Corresponding Author

*Phone: (720) 465-3314, ext. 14; fax: (303) 404-2945; e-mail: mruby@integral-corp.com.

Notes

The authors declare no competing financial interest.

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Oral Bioavailability, Bioaccessibility, and Dermal Absorption of PAHs from Soil—State of the Science

Michael V. Ruby,[†] Yvette W. Lowney,[*,‡] Annette L. Bunge,[§] Stephen M. Roberts,[∥] Jose L. Gomez-Eyles,[⊥,∇] Upal Ghosh,[⊥] John C. Kissel,[○] Priscilla Tomlinson,[¶] and Charles Menzie[□]

[†]Integral Consulting Inc., Louisville, Colorado
[‡]Exponent, Boulder, Colorado
[§]Colorado School of Mines, Golden, Colorado
[∥]University of Florida, Gainesville, Florida
[⊥]University of Maryland, Baltimore County, Maryland
[∇]Integral Consulting Inc., Seattle, Washington
[○]University of Washington, Seattle, Washington
[¶]Washington Department of Ecology, Seattle, Washington
[□]Exponent, Alexandria, Virginia

[*]Corresponding Author

Phone: 303-589-9955. E-mail: YLowney@Alloy-llc.com

Supporting Information

Ten pages, including five tables and one figure.

Reference numbers shown in Supporting Information tables correspond with those presented in the manuscript.

Table S1. Summary of Oral Bioavailability Studies for PAHs in Soil

Publication[a]	Site Type/PAH Source	Test Soil Concentration	Particle Size Tested	TOC of Soil(s) (%)	Test Species	PAHs Evaluated	Dosing Medium	PAH Dose(s)	Reference Material	Endpoint Measured	Relative Bioavailability/Comments
Studies from Peer-Reviewed Literature, with Sufficient Detail that the Quality of the Study Can Be Evaluated											
Kadry et al. 1995 (*2*)	Two uncontaminated soils ^{14}C-PN in presence of soil dosed by gavage (PN not adsorbed onto soil)	140 mg/kg of PN	NR	Sandy soil: 4.4 Clayey soil: 1.6	Sprague Dawley rat (female)	PN	Test soil in solution by gavage	69.6 µg/kg bw PN	^{14}C-PN in aqueous solution dosed by gavage	AUC in plasma, urinary excretion, fecal elimination, and tissue distribution of ^{14}C radiolabel	From AUC measurements: −Sandy soil: 91% −Clayey soil: 85% From UEF measurements: −Sandy soil: 110% −Clayey soil: 109% From fecal recovery measurements: −Sandy soil: ≤86% −Clay soil: ≤79% Presence of soil in solution with PN did not reduce RBA of PN
van Schooten et al. 1997 (*6*)	One soil from an industrial site (coal tar and pitch)	12 mg/kg BaP 35 mg/kg pyrene 4 mg/kg anthracene	"Pulverized" soil	4.1	Lewis rat (male)	BaP, pyrene, and anthracene	Test soil in solution by gavage	18.4 µg/kg bw BaP 17.5 µg/kg bw pyrene 7.2 µg/kg bw anthracene	BaP, pyrene, and anthracene in sunflower oil by gavage	Parent PAHs, and hydrolylated metabolites of BaP, pyrene, and anthracene in blood, urine, and feces	Inter-animal variability in AUC data was too large to calculate reliable RBA values. Excretion of parent PAH in feces: −BaP: ≤47% −Pyrene: ≤45% −Anthracene: ≤87% Excretion of hydroxlyated metabolite in feces: −BaP: ≤61% −Pyrene: ≤33% Excretion of hydroxlyated metabolite in urine: −Pyrene: 5.6%
Koganti et al. 1998 (*7*)	Ten soils containing coal tar from three MGP sites	0.7 to 118 mg/kg BaP 0.11 to 986 mg/kg cPAH 0.57 to 3,120 mg/kg tPAH	<1,000 µm	NR	B6C3F1 mouse (female)	EPA 16 priority pollutant PAHs	Soil in rodent chow	0.006 to 32.8 mg tPAH/mouse; BaP dose not reported or calculable	Organic extracts of soils in diet	Urinary excretion of 1-hydroxypyrene; DNA adducts in lung tissue	Excretion of 1-hydroxyprene: −Site A: 21% to 75% −Site B: 8.5% to 31% −Site C: 26% to 111% Lung DNA adducts: −Site A: 8% to 47% −Site B: 15% to 32% −Site C: 20% to 76%
Fouchécourt 1999 (*8*)	One soil from a coking facility (coal tar) One uncontaminated soil spiked with BaP and PN	21 to 120 mg/kg BaP 120 to 736 mg/kg tPAH	<5,000 µm	NR	Fisher 344 rat (male)	Suite of 13 PAHs	Test soils mixed with cage litter	Not reported or calculable	Sand mixed with cage litter	Parent PAHs in lung and liver tissue; DNA adducts in lung and liver tissue; enzyme induction in liver tissue	Not reported or calculable
Bordelon et al. 2000 (*10*)	One uncontaminated soil spiked with 5% coal tar from an MGP site Spiked soil aged for 90 days	80 to 85 mg/kg BaP 3,134 to 3,242 mg/kg tPAHs	<2,000 µm	NR	Fisher 344 rat (male)	Suite of 28 PAHs	Soil in rodent chow	Total doses over 17 days: 4.3 to 5.6 mg/kg bw BaP 179 to 212 mg/kg bw tPAHs	Neat coal tar	DNA adducts in lung and liver tissue	Not reported or calculable Aging for 90 days did not change extent of DNA adducts in lung or liver tissue
Koganti et al. 2001 (*12*)	Three soils containing coal tar from MGP sites Four neat coal tar samples	64 to 118 mg/kg BaP 1,230 to 2,050 mg/kg tPAH	NR	NR	A/J mouse (female)	Suite of 19 PAHs	Soil or coal tar in rodent chow	0.006 to 32.8 mg tPAH/mouse; BaP dose not reported or calculable	Organic extracts of soils in diet	DNA adducts in lung tissue	RBA values not reported or calculable No BaP adducts detected following soil dosing for two of the three soils
Reeves et al. 2001 (*13*)	One uncontaminated soil amended with coal tar (MGP source) Amended soil aged for 9 months	76 to 85 mg/kg BaP 478 to 536 mg/kg cPAHs	<1,000 µm	NR	Fisher 344 rat (male)	Suite of 12 PAHs	Soil in rodent chow	Not reported or calculable	Coal tar in feed Coal tar/sand in feed	Urinary excretion of 1-hydroxypyrene; PAH concentrations in liver tissue	Not reported or calculable No clear effect of aging (1-hydroxyprene and liver tissue results yielded opposite trends)
Roos 2002 (*14*)	Ten soils from industrial sites	2.1 to 110 mg/kg BaP 4.6 to 487 mg/kg five- and six-ring PAHs 59 to 4,649 mg/kg tPAHs	<200 µm	NR	Sprague Dawley rat (male)	EPA 16 priority pollutant PAHs	Soil in rodent chow	25 to 2,290 µg/kg bw five- and six-ring PAHs 287 to 20,190 µg/kg bw tPAHs	"Clean" soil	Enzyme induction in duodenum, liver, and kidney tissue	Not reported or calculable Enzyme induction in duodenal tissue increased with increasing dose of five- and six-ring PAHs
Roos et al. 2002 (*15*)	Three soils from industrial sites	22 to 271 mg/kg BaP 102 to 1,059 mg/kg five- and six-ring PAHs 756 to 3,805 mg/kg tPAHs	<1,000 µm	NR	Goettingen mini pig	EPA 16 priority pollutant PAHs	Soil mini-pig feed	11 to 1,400 µg/kg bw BaP 50 to 5,300 µg/kg bw five- and six-ring PAHs 380 to 19,000 µg/kg bw tPAHs	Non-exposed animals	Enzyme induction in liver, duodenum, lung, kidney, and spleen tissue	Not reported or calculable Enzyme induction in liver tissue increased with increasing dose of five- and six-ring PAHs
Ataria 2007 (*18*)	One soil each from a fuel-loading depot and an MGP site One uncontaminated soil spiked with BaP and benzo[*a*]anthracene	Target concentrations of 0.2, 20, and 100 mg/kg BaP or benzo[*a*]anthracene in spiked soil; PAH concentrations in site soils not reported	<5,000 µm	5.2 (spiked soil)	C57BL/6 mouse (female)	BaP and benzo[*a*]anthracene	Spiked soil, or soil from fuel depot or gasworks on floor of cage	Not reported or calculable	"Clean" soil on floor of cage	PAHs in carcass; enzyme induction in liver tissue; immune response	Not reported or calculable
Ounnas et al. 2009 (*19*)	One uncontaminated soil spiked with BaP, pyrene, and PN Spiked soil aged for 40 days	100 mg/kg of BaP, pyrene, and PN in same soil	<2,000 µm	5	Alpine goat	BaP, pyrene, and PN	Soil in goat feed	Not reported or calculable	BaP, pyrene, and PN in oil/feed mixture	Excretion of hydroxylated metabolites of pyrene and PN in urine and milk of lactating goats	Excretion of hydroxylated pyrene metabolites: −Urine: 50% −Milk: 61% Excretion of hydroxylated PN metabolites: −Urine: 100% 3-hydroxy BAP not detectable in urine or milk

Table S1. Summary of Oral Bioavailability Studies for PAHs in Soil

Publication[a]	Site Type/PAH Source	Test Soil Concentration	Particle Size Tested	TOC of Soil(s) (%)	Test Species	PAHs Evaluated	Dosing Medium	PAH Dose(s)	Reference Material	Endpoint Measured	Relative Bioavailability/Comments
Studies from Peer-Reviewed Literature; Details Not Sufficient to Fully Evaluate the Quality of the Study											
Goon et al. 1991 (*1*); additional details provided in Magee et al. 1996 (*3*)	Two uncontaminated soils (one sandy and one clayey) spiked with ^{14}C-BaP Spiked soils aged for 1, 7, and 30 days and 6 and 12 months	100 mg/kg BaP	<100 µm (sandy soil was "ground")	Sandy soil: 0.04 Clayey soil: 1.4	Sprague Dawley rat (male)	BaP	Test soils in solution by gavage	1 µg/kg bw BaP	^{14}C-BaP in aqueous solution by gavage	AUC of ^{14}C-BaP radiolabel in blood	Values based on average of 6- and 12-month results: −Sandy soil: 57% −Clayey soil: 37% Aging for 6 or 12 months reduced RBA of sandy and clayey soils by 14% and 27%, respectively, relative to soils aged 1, 7, and 30 days
Stroo et al. 2000 (*11*)	Two soils containing coal tar (MGP site) One soil containing lamp black (MGP site)	0.7 to 30 mg/kg BaP 7.7 to 1,040 mg/kg tPAH	NR	0.08, 2.4, and 4.5	Fisher 344 rat	BaP, chrysene, pyrene, and PN	Soil in rodent chow	Total dose over 10 days: 0.2 to 27 µg/kg bw BaP 2.3 to 3,370 µg/kg bw tPAH	No reference material	Fecal excretion of parent PAHs	Coal tar soil: ≤109% Lampblack soil: ≤61% Bioavailability calculated as mass of PAH dosed minus mass in feces
Pu et al. 2004 (*16*)	Four uncontaminated soils spiked with PN Spiked soils not aged	Each soil at 200 and 400 mg/kg PN	<2,000 µm	0.52 to 1.74	Sprague Dawley rat (male)	PN	Soil/water slurry by gavage	400 and 800 µg/kg bw	PN in corn oil by gavage	AUC of PN in blood	Soil — 400 ppm — 800 ppm Bloomfied — 203% — 138% Milford — 65% — 90% Toronto — 83% — 113% Heiden — 61% — 94% RBA values greater than 100% due to absolute bioavailability of reference material determined to be 24% (note: this value is inconsistent with other studies) No relationship between soil TOC and RBA values
Stroo et al. 2005 (*17*)	Four soils containing lampblack (MGP site)	NR	<6,400 µm	NR	"Mouse"	BaP and PN	Unclear	NR	NR	Fecal excretion of parent PAHs	PN: ≤0.6% to 1.1%
James et al. 2011 (*20*)	Three soils containing coal tar (MGP sites) Three soils from wood-treating sites Two soils from petroleum sites	0.17 to 650 mg/kg BaP potency equivalents	<45 µm	2.5 to 8.5	Juvenile swine (female)	cPAHs	Soil in swine feed	NR	No reference material	Parent PAH concentrations in serum, 2 hours post-dosing	RBA not reported or calculable Reports absorption as mass of cPAHs in serum at 2 hours post-dosing/mass of cPAHs in soil dosed
Duan et. al. 2014 (*21*)	Eight uncontaminated soils spiked with BaP Spiked soils aged for 50 or 90 days	50 mg/kg BaP	<2,000 µm	0.7 to 7.5	Juvenile swine (male)	BaP	Soil in swine feed	NR	BaP spiked onto sand and dosed in diet	AUC of BaP in plasma	~40% to ~110% (reported in figures only) Assumed no loss of BaP during weathering for calculation of RBA values; no difference in RBA values for 50 vs. 90 days of aging; RBA values appear to correlate with both (silt+clay)/TOC and pore size distribution of test soils
James et al. 2016 *(22)*	Fourteen soils from contaminated sites: MGP sites (12 soils) Wood-treating site (1 soil) Unspecified, United Kingdom (1 soil)	2.5 to 290 mg/kg BaP; benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and chrysene also reported	Not specified	1.3 to 33	Juvenile swine (female)	Benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and chrysene	Soil in swine feed	NR	EPA 16 priority pollutant PAHs, intravenously in glycerol trioctanoate vehicle	AUC of parent PAH in serum (48-hour)	RBA not reported or calculable Crossover study design; uses unconventional definition of bioavailability, reported as 48-hour mass in blood/mass in dosed soil
Studies Available as Abstracts Only											
Rozett et al. 1996 (*4*); additional details provided in Magee et al. 1996 (*3*)	One soil containing coal tar (MGP site) Soil sieved to obtain eight different particle size fractions	14 to 186 mg/kg pyrene	Various particle size fractions, ranging from <150 µm to <1 mm	NR	CD1 mouse (female)	Pyrene and cPAHs	Soil in rodent chow	NR	Pure MGP residue in diet	Urinary excretion of 1-hydroxy pyrene; DNA adducts in lung and forestomach tissue	Not reported or calculable Based on excretion of pyrene metabolites and formation of DNA adducts, absorption of pyrene from <150 µm size fraction was about 4 to 5 times greater than absorption from larger size fractions
Weyand et al. 1996 (*5*)	Soils containing coal tar (MGP sites)	NR	<150 µm	NR	B6C3F1 mouse (female)	cPAHs	Soil in rodent chow	NR	Organic extracts of soils in diet	DNA adducts in lung tissue	For cPAHs: 7% to 17%
Magee et al. 1999 (*9*)	Three residential yard soils near a waste site	9 to 70 mg/kg BaP potency equivalents 66 to 388 mg/kg tPAH	<250 µm	NR	B6C3F1 mouse (female)	BaP and cPAHs	Soil in rodent chow	NR	Organic extracts of soils in diet	Urinary excretion of 3-hydroxy BaP; DNA adducts in lung tissue	Urinary excretion of 3-hydroxy BaP: 7% to 27% DNA adducts: 8% to 36%

Notes:
AUC = area under the curve
BaP = benzo[*a*]pyrene
bw = body weight
EPA = U.S. Environmental Protection Agency
MGP = manufactured gas plant
NR = not reported
PAH = polycyclic aromatic hydrocarbon; cPAH = carcinogenic PAH; tPAH = total PAHs;
PN = phenanthrene
ppm = parts per million
RBA = relative bioavailability
TOC = total organic carbon
UEF = urinary excretion fraction

[a]Reference numbers correspond with those presented in the manuscript.

Table S2. PBET Study Methods, Parameters, and Results

Publication[a]	Site Type/PAH Source	PAHs Evaluated	PAH Concentrations	Particle Size	TOC/ Other Soil Characterization	Gastric Phase pH/Time/Composition	Small Intestinal Phase pH/Time/Composition	Colon Phase pH/Time/Composition	Lipid, Food, or Infinite Sink Added to Test	Separation of Supernatent	Bioaccessibility/Comments
Hack and Selenka 1996 (*23*)	Thirty-one samples of soil, sewage sludge, asphalt, metal scrap, and blast sand residue	EPA 16 priority pollutant PAHs	20 to 5,000 mg/kg tPAHs	NR	NR	2.0 2 hours HCl, mucin, pepsin, NaCl	7.0 6 hours Bile (3 g/L), pancreatin, trypsin	NA	Whole milk powder	Centrifuged (10 minutes at 7,000 x g)	For tPAHs: 5% to 14% without milk powder 23% to 66% with milk powder Addition of whole milk powder increased bioaccessibility by 4x to 8x
Jin et al. 1999 (*24*)	Two clean soils spiked with naphthalene; aged 135 days	Naphthalene	20 and 200 mg/kg naphthalene	<250 µm	2.4% and 39%	1.0 Variable times HCl, NaCl	6.7 Variable times NaCl	NA	No	Centrifuged (10 minutes at 1,150 x g)	NR
Sips et al. 2001 (*25*)	Seven clean soils spiked with BaP; no indication of whether spiked soils were aged	BaP	Each soil spiked with 20, 40, 120, or 200 mg/kg BaP	<2,000 µm	1.5% to 30%	1.0 2 hours HCl, mucin, BSA, pepsin, urea, various salts (included salivary phase)	5.5 2 hours Chicken bile (0.9 g/L), BSA, pancreatin, lipase, various salts	NA	No	Centrifuged (5 minutes at 3,000 x g)	For BaP: 2% to 50% depending on soil type and BaP concentration BaP bioaccessibility inversely related to TOC
Holman et al. 2002 (*26*)	Nine diesel and crude oil–contaminated soils (heavily weathered)	Analyzed for TPH	NR	<1,000 µm	0.7% to 10.8%	NA	6.5 4 hours Mixture of 10 conjugated bile salts that are representative of human bile (200 mM)	NA	Mixture of 5 common lipids (8 mL/L) added to fed-state tests	Centrifuged (45 minutes at 1,100 x g), filtered at 0.45 µm	Crude oil-contaminated soil: −Fasted state: 0.5% to 2.5% −Fed state: 2.5% to 8% Diesel contaminated soil: −Fasted state: 4% to 11% −Fed state: 7.5% to 32% TPH bioaccessibility inversely related to TOC
Oomen et al. 2004 (*27*)	Three field soils (no source cited) Synthetic soil (70% sand, 20% kaolin, 10% peat moss) spiked with BaP (2-week aging time)	BaP	NR for field soils 20 and 200 mg/kg BaP for spiked soils	NR	NR	1.0 2 hours HCl, mucin, BSA, pepsin, urea, various salts (included salivary phase)	8.0 2 hours Evaluated pig, chicken, and ox bile (0.9 g/L), BSA, pancreatin, lipase, various salts	NA	No	Centrifuged (5 minutes at 2,750 x g)	For BaP: 4% to 44% depending on soil and bile type Compositions of pig and ox bile most similar to human bile
Pu et al. 2004 (*16*)	Four clean soils spiked with PN; no indication of whether PN spiked soils were aged	PN	Each soil spiked with 400 or 800 mg/kg PN	<2,000 µm	0.52% to 1.7%	3.0 2 hours HCl, mucine, urea, various salts (included salivary phase)	7.0 2 hours Chicken bile (0.9 g/L), BSA, pancreatin, lipase, various salts	NA	No	Centrifuged (5 minutes at 3,000 x g)	For PN: 18% to 70% for 200 mg/kg soils 53% to 89% for 400 mg/kg soils PN bioaccessibility inversely related to TOC
Van de Wiele et al. 2004 (*28*) (Simulator of the Human Intestinal Microbial Ecosystem [SHIME] method)	One urban soil Atmospheric deposition of industrial PAH sources	Suite of 10 PAHs	5.9 mg/kg BaP 49 mg/kg tPAH	NR	3.3%	1.5 2 hours HCl, pepsin, NaCl	6.3 5 hours Ox bile (6 g/L), pancreatin	5.9 to 6.3 18 hours Fecal material	Nutrilon (baby mush)	Centrifuged (5 minutes at 1,500 x g)	For tPAHs: 0.1% to 1.4% depending on extraction conditions Values lower than in other similar studies
Stroo et al. 2005 (*17*)	MGP site/lampblack (7 soils)	BaP and PN	NR	<1,000 µm	NR	NA	6.5 4 hours Mixture of 10 conjugated bile salts that are representative of human bile (200 mM)	NA	Mixture of common lipids (8 mL/L)	Centrifuged (45 minutes at 1,100 x g), filtered at 0.45 µm	For BaP: 0.2% to 5.0% For phenathrene: 0.8% to 15%
Minhas et al. 2006 (*29*)	Clean soil spiked with ^{14}C-chrysene; aged 6 or 12 months	Chrysene	NR	125 to 250 µm size fraction	15.2% (as NOM)	2.5 1 hour HCl, pepsin, organic acids, NaCl	7.0 Variable times Bile salts (4 g/L), pancreatin, $CaCl_2$	NA	Caco-2 cells or EVA thin film	Filtered with glass fibre filters (3.1 and 0.7 µm)	Bioaccessability values not reported Aging for 6 or 12 months did not appear to reduce chrysene bioaccessibility
Tang et al. 2006 (*30*)	Thirteen soils from gas stations, roadsides, bus stops, kindergarten, schools, and residential locations	EPA 16 priority pollutant PAHs (except naphthalene)	0.11 to 27.8 tPAHs	< 250 µm	0.75% to 6.2%	1.5 1 hour HCl, pepsin, organic acids, NaCl	7.0 4 hours Porcine bile extract (1.2 g/L), pancreatin	NA	No	Centrifuged (10 minutes at 7,000 x g), filtered at 0.45 µm	For tPAHs: 9.2% to 60.5%
Grøn et al. 2007 (*31*)	MGP sites Fish net tarring site Urban soil close to highway Porcelain factory ash Urban soil (7 soils)	BaP and dibenzo[*a,h*]anthacene	5.6 to 270 mg/kg BaP 0.77 to 43 mg/kg dibenzo[*a,h*]anthacene	<2,000 µm	1.8% to 5.0%	1.3 2 hours HCl, mucin, BSA, pepsin, urea, various salts (included salivary phase)	8.2 2 hours Chicken bile (0.9 g/L), BSA, pancreatin, lipase, various salts	NA	Chicken/mashed potato mush	Centrifuged (5 minutes at 3,000 x g)	For BaP: 5.7% to 38% For dibenzo[*a,h*]anthracene: 12% to 40%
Vasiluk et al. 2007 (*32*)	Two clean soils spiked with ^{14}C-BaP; aged for 4 months	BaP	NR	125 to 250 µm size fraction	11% and 29% (as NOM)	2.5 1 hour HCl, pepsin, organic acids, NaCl	7.0 Variable times Bile salts (4 g/L), BSA, pancreatin, $CaCl_2$	NA	Caco-2 cells or EVA thin film	Filtered with glass fibre filters (3.1 and 0.7 µm)	BaP bioaccessibility inversely related to TOC
Hurdzan et al. 2008 (*33*)	PN adsorbed to cutin and cutan (NOM surrogates)	PN	NR	NR	NR	1.0 2 hours HCl, mucine, BSA, pepsin, urea, various salts (included salivary phase)	8.0 2 hours Chicken bile (0.9 g/L), BSA, urea, pancreatin, lipase, various salts	NA	C18 disk	Centrifuged (5 minutes at 2,750 x g)	PN bioaccessibility varied with type of NOM substitute (cutin > cutan)
Khan et al. 2008 (*34*)	Ten wastewater irrigated soils	EPA 16 priority pollutant PAHs (except naphthalene)	1.5 to 6.9 mg/kg tPAH	<250 µm	1.7% to 6.2%	1.5 1 hour HCl, pepsin, organic acids, NaCl	7.0 4 hours Porcine bile extract (1.2 g/L), pancreatin	NA	No	Centrifuged (10 minutes at 7,000 x g), filtered at 0.45 µm	For tPAHs: 20% to 46%

Table S2. PBET Study Methods, Parameters, and Results

Publication[a]	Site Type/PAH Source	PAHs Evaluated	PAH Concentrations	Particle Size	TOC/ Other Soil Characterization	Gastric Phase pH/Time/Composition	Small Intestinal Phase pH/Time/Composition	Colon Phase pH/Time/Composition	Lipid, Food, or Infinite Sink Added to Test	Separation of Supernatent	Bioaccessibility/Comments
Lu et al. 2009 (*35*)	One residential soil	EPA 16 priority pollutant PAHs (except naphthalene)	~78 mg/kg BaP 880 mg/kg tPAHs	<250 µm	7.5%	Variable (1 to 2) Variable (1 to 3 hours) HCl, pepsin, organic acids, NaCl	Variable (6.8 to 8.5) Variable (4 to 6 hours) Porcine bile extract (1.2 g/L), pancreatin	NA	No	Centrifuged (10 minutes at 4,000 x g), filtered at 45 µm	For tPAHs: 30% to 44% across range of pH values and extraction times tested Extent of bioaccessibility most sensitive to a combination of gastric pH and solid:fluid ratio
Cave et al. 2010 (*36*) (Fed ORganic Estimation human Simulation Test [FOREhST] method)	MGP site/coal tar and pitch (11 soils)	Suite of 6 PAHs	2 to 68 mg/kg BaP 10 to 300 mg/kg tPAHs	<250 µm	1% to 13%	1.3 2 hours HCl, mucine, BSA, pepsin, urea, various salts (included salivary phase)	8.1 2 hours Chicken bile (0.9 g/L), BSA, pancreatin, lipase, various salts	NA	Sunflower oil	Centrifuged (5 minutes at 3,000 x g)	For tPAHs: 12% to 62%
Lu et al. 2010 (*37*)	Twenty soils from residential, industrial, business areas, scenic areas, agricultural areas, and public areas	EPA 16 priority pollutant PAHs	0.083 to 8.84 mg/kg tPAHs	<250 µm	3.4% to 6.6%	1.5 3 hours HCl, pepsin, organic acids, NaCl	7.5 6 hours Porcine bile extract (1.2 g/L), pancreatin	NA	No	Centrifuged (10 minutes at 4,000 x g), filtered at 0.45 µm	For tPAHs: 15% to 63% Bioaccessibility increased with PAH size; inversely related to TOC
Siciliano et al. 2010 (*38*) (SHIME method)	Eighteen soils from along roadways and from residential properties	EPA 16 priority pollutant PAHs	3.7 mg/kg tPAHs in <45 µm fraction (average for all soils)	<45 µm and <4,000 µm	NR	1.5 2 hours HCl, pepsin, NaCl	6.3 5 hours Oxgall (6 g/L), pancreatin	5.9 to 6.3 18 hours Fecal material	No	Centrifuged (5 minutes at 1,500 x g)	NR
Tao et al. 2010 (*39*)	Four soils with PAHs resulting from aerial deposition	EPA 16 priority pollutant PAHs	NR	NR	0.63%, 1.1%, 1.6%, and 2.9%	1.5 2 hours HCl, pepsin	7.5 12 hours Bile salts (20 g/L), pancreatin, lipase	6.9 10 hours α-amylase	No	Centrifuged (10 minutes at 7,600 x g)	NR Bioaccessibility of tPAHs inversely related to TOC
James et al. 2011 (*20*)	MGP sites/coal tar (3 soils) Wood-treating/creosote (3 soils) Petroleum sites (2 soils)	Suite of 13 PAHs	0.17 to 650 mg/kg BaP potency equivalents	<45 µm	2.5% to 8.5%	1.5 1 hour HCl	7.0 4 hours Bovine bile (1.8 g/L), pancreatin	NA	C18 disk	Filtered at 0.45 µm	For BaP: 0.5% to 7.9% Addition of C18 membrane increased PAH bioaccessibility by 5x
Tao et al. 2011 (*40*)	Three industrial soils	EPA 16 priority pollutant PAHs	NR	NR	0.18%, 0.77%, and 1.5%	1.5 2 hours HCl, pepsin	7.5 12 hours Bile salts (range of 2 to 20 g/L), pancreatin, lipase	6.9 10 hours α-amylase	No	Centrifuged (10 minutes at 7,600 x g)	Bioaccessibility constant over range of 2 to 20 g/L bile salts Bioaccessibility of tPAHs inversely related to TOC
Tilston et al. 2011 (*41*) Colon-extended PBET (CE-PBET) method	Synthetic soil (70% sand, 20% kaolin, 10% peat moss) spiked with PAHs; aging time not reported	Suite of 7 PAHs	96 mg/kg pyrene	<200 µm	NR	2.5 1 hour HCl, pepsin	7 4 hours Bile salts (1.8 g/L), pancreatin	6.5 8 hours Bile salts, mucin, cysteine haemin, various salts	Dietary components: potato starch, casein, tryptone, yeast extract, pectin, and xylan	Centrifuged (10 minutes at 3,000 x g)	Addition of colon compartment increased PAH bioaccessibility by approximately 2x Addition of food increased pyrene bioaccessibility by 64%
Wang et al. 2011 (*42*)	Evaluated ^{14}C-phenanthrene sorption to carbon nanotubes under different gastrointestinal tract conditions	PN	50 mg/kg PN	NA	NA	2.0 NR HCl, pepsin, NaCl	7.5 NR Bile salts (0.5 g/L for fasted state; 5 g/L for fed state)	NA	Carbon nanotubes	Centrifuged	NA
Gouliarmou and Mayer 2012 (*43*)	Wood soot	Suite of 6 PAHs	40 mg/kg BaP	<150 µm	NR	Used cyclodextrin as carrier of PAHs from soot to silicone rod; 2-week extraction time			Silicone rod	PAHs extracted from silicone rod using acetone	Presence of silicone rod increased BaP bioaccessibility by 3x to 24x
Lorenzi et al. 2012 (*44*) (FOREhST method)	MGP site/coal tar and pitch (6 soils)	EPA 16 priority pollutant PAHs	54 to 68 mg/kg BaP	<250 µm	NR	1.3 2 hours HCl, mucine, BSA, pepsin, urea, various salts (included salivary phase)	6.0 2 hours Chicken bile (0.9 g/L), BSA, pancreatin, lipase, various salts	NA	Sunflower oil	Centrifuged (5 minutes at 3,000 x g)	For BaP: 15% to 41%
Gouliarmou et al. 2013 (*46*) (CE-PBET method)	Kindergarten soil Wood soot	Suite of 6 PAHs	NR	<250 µm for soil <150 µm for soot	NR for soil 24% (soot)	2.5 1 hour HCl, pepsin, various organic acids	7.0 4 hours Bile salts (1.8 g/L), pancreatin	6.5 16 hours Bile salts, mucin, cysteine haemin, various salts	Dietary components: potato starch, casein, tryptone, and yeast extract, pectin, and xylan Silicone rod	PAHs extracted from silicone rod using methanol	For BaP: ~Kindergarten soil ~30% ~Soot ~15%
Duan et al. 2014 (*21*)	Silica sand spiked with BaP; aged 50 or 90 days	BaP	50 mg/kg BaP	<2,000 µm	0.72% to 7.5% Also results for pH, surface area, mesopore volume, pore size, and soil mineralogy	Used simple chemical extractions with butanol and cyclodextrin			No	Butanol extracts centrifuged; cyclodextrin extracted with hexane	For BaP in butanol extractions: ~25% to 75% after 1 day of aging ~20% to 60% after 50 days of aging ~10% to 55% after 90 days of aging Extractability with butanol and cyclodextrin decreased with aging time
Cave et al. 2015 *(47)*	Twenty-six soils from three gasworks sites and a domestic garden	Nineteen PAHs including EPA 16 priority pollutant PAHs	From ~0 to 7 mg/kg in domestic garden to ~1,000 mg/kg	<250 µm	0.54% to 34.0%	1.3 2 hours HCl, mucine, BSA, pepsin, urea, various salts (included salivary phase)	8.1 2 hours Chicken bile (0.9 g/L), BSA, pancreatin, lipase, various salts	NA	Vegetable oil	Centrifuged (5 minutes at 3,000 x g)	For BaP: ~0% to 60%

Table S2. PBET Study Methods, Parameters, and Results

Publication[a]	Site Type/PAH Source	PAHs Evaluated	PAH Concentrations	Particle Size	TOC/ Other Soil Characterization	Gastric Phase pH/Time/Composition	Small Intestinal Phase pH/Time/Composition	Colon Phase pH/Time/Composition	Lipid, Food, or Infinite Sink Added to Test	Separation of Supernatent	Bioaccessibility/Comments
Li et al. 2015 *(48)*	Five clean soils spiked with pyrene One PAH-impacted agricultural soil	Pyrene EPA 16 priority pollutant PAHs	Clean soil spiked with 10 mg/kg pyrene Field soil 0.39 mg/kg BaP and 3.36 mg/kg tPAHs	<250 µm	0.7% to 3.2% Also results for pH, CEC, soil texture	2.5 1 hour HCl, pepsin, various organic acids	7.0 4 hours Bile salts (1.8 g/L), pancreatin	NA	Tenax resin	Centrifuged (5 minutes at 3,000 rpm); Tenax harvested by filtration and extracted by sonication using acetone	For pyrene in spiked soils: −Without Tenax 8.3% to 20.8% −With Tenax 55.7% to 65.9% For BaP in field soil: −Without Tenax 3.7% −With Tenax 16.3%
Meyer et al. 2015 *(49)*	Spiked geosorbents: Quartz sand Montmorillonite clay Peat Charcoal	Suite of 10 deuterated PAHs	10 mg/kg of each PAH	<60 µm	NR	2.0 2 hours HCl, mucin, pepsin, NaCl	7.5 6 hours Bile (3 g/L), pancreatin, trypsin	NA	Whole milk powder	Centrifuged (10 minutes at 1,500 x g); filtered at 0.45 µm	For tPAH: From 0.1% (charcoal) to 26.9% (quartz sand), measured relative to total PAH spiked
Zhang et al. 2015 (*50*)	Soot sample (commercial fuel oil boilers)	Suite of 11 PAHs	3.8 mg/kg BaP 73 mg/kg tPAHs	<75 µm	14.2% Also results for pH, total carbon, hydrogen, nitrogen, oxygen, and ash	1.0 2 hours HCl, pepsin, KCl (included salivary phase)	7.8 4 hours Porcine bile extract (14 g/L), pancreatin, lipase	NA	Silicone sheet	Centrifuged (20 minutes at 3,000 rpm); PAHs extracted from digestive fluid and silicone sheet using hexane and acetone, respectively	For BaP (2 g sheet, 50 mg soot, 4-hour small intestinal extraction): −Without silicone sheet ~25% −With silicone sheet ~50% Presence of silicone sheet significantly increased bioaccessibility
Zhang et al. 2015 (*51*)	Soot sample (commercial fuel oil boilers)	Suite of 11 PAHs	3.8 mg/kg BaP 73 mg/kg tPAHs	<75 µm	14.2% Also results for pH, total carbon, hydrogen, nitrogen, oxygen, and ash	Variable (1.2-4.3) 2 hours HCl, pepsin, KCl (included salivary phase)	Variable (5.0 to 7.4) 4 hours Variable porcine bile extract (2.0 to 10 g/L), pancreatin, lipase	NA	Soybean oil (4.0 to 19.5 g/L lipid) Silicone sheet	Centrifuged (20 minutes at 3,000 rpm); PAHs extracted from digestive fluid and silicone sheet using hexane and acetone, respectively	For BaP: −Increased from ~20% to 30% across bile concentration range −Increased from ~10% to 40% across small intestinal pH range −Increased from ~40% to 70% across lipid content range −Gastric pH had no effect
James et al. (2016) *(22)*	Fourteen soils from contaminated sites: MGP sites (12 soils) Wood-treating site (1 soil) Unspecified, United Kingdom (1 soil)	Suite of 5 PAHs	2.5 to 290 mg/kg BaP	Not specified	1.3% to 33% Also results of pH and soil texture	Determined aqueous PAH concentrations by centrifugation and filtration to determine PAH partitioning and soil fugacity capacity					Did not measure bioaccessibility; compared soil fugacity capacity against % bioavailability
Juhasz et al. 2016 *(52)*	Eighteen soils: MGP sites (10 soils) Stockpiled material (4 soils) Industrial sites (2 soils) Petrogenic PAH source (2 soils)	EPA 16 priority pollutant PAHs	1.5 to 69.2 mg/kg BaP 18.4 to 871 mg/kg tPAH	<250 µm	2.0% to 13.2%	1.5 1 hour HCl, pepsin, BSA, mucin, NaCl, various organic acids	7.2 16 hours Bovine bile (4.0 g/L), porcine pancreatin	NA	Silicone cord	PAHs extracted from silicone cord using methanol	For BaP: ~ 2% to 38% For tPAH: ~ 4% to 50%

Notes:
BaP = benzo[*a*]pyrene
BSA = bovine serum albumin
CEC = cation exchange capacity
EPA = U.S. Environmental Protection Agency
EVA = ethylene vinyl acetate
MGP = manufactured gas plant
NA = not applicable
NR = not reported
NOM = natural organic matter
PAH = polycyclic aromatic hydrocarbon; tPAH = total PAHs
PBET = physiologically based extraction test
PN = phenanthrene
TOC = total organic carbon
TPH = total petroleum hydrocarbons

[a]Reference numbers correspond with those presented in the manuscript.

Table S3. Summary of Key Parameters and Ranges Used in Dermal Absorption Studies

PAH Type/Concentrations in Soil	PAH Source	Analytical Method	Quantity Measured
BaP, occasionally phenanthrene (range of 0.1 to 2,000 mg/kg; 10 to 200 mg/kg most common)	Application to soil in volatile solvent that was removed by evaporation was most common; petroleum crude, coal tar, and MGP soils sometimes used	Liquid scintillation counting of ^{3}H- or ^{14}C-radiolabeled compounds used in most studies; HPLC with fluorescence detection of cold BaP added to or naturally present in soil used occasionally	Penetration through skin reported most often; i.e., into diffusion cell receptor fluid *(in vitro)* or collected in excreta *(in vivo)* and tissues (if animal sacrificed); occasionally, absorption into skin measured
Soil Parameters Measured	**Soil Particle Size Tested**	**Aging/Weathering**	**Soil Load**
Total organic carbon (range of 0.4% to 20%; 1% to 3% most common); occasionally particle size distribution, black carbon, or C:H ratio measured	Range of <150 to >500 µm; <150 µm most common	Soils aged up to 110 days; Stroo et al. 2005 studied soils collected from MGP sites closed for ~50 years	Range of 1 to 40 mg/cm^2; >10 mg/cm^2 most common; most experiments contained multiple soil layers; mass required for single layer varies with particle size of applied soil
Species Skin Source	**Skin Location**	**Type of Experiment**	**Exposure Time**
Human, pig, rat, guinea pig, rhesus monkey	Back or abdomen most common	*In vitro* diffusion cell experiments most common; *in vivo* experiments in rats and rhesus monkey have been conducted	16 to 126 hours; 24 hours most common

Notes:

BaP = benzo[*a*]pyrene
HPLC = high-performance liquid chromatography
MGP = manufactured gas plant
PAH = polycyclic aromatic hydrocarbon

Table S4. Soils Used in Dermal Absorption Studies

Reference[a]	Soil Name and Source (if available)	TOC (%)	Particle Size (μm)
Yang et al. 1989 (*53,54*); Roy et al. 1992 (*56*)	Low OC, loam soil	1.64[b]	<150
Wester et al. 1990 (*55*)	Yolo County, California, soil	0.9[c]	180–320
Roy et al. 1992 (*56*)	High OC	19.4[b]	<150
Kadry et al. 1995 (*2*); Abdel-Rahman et al. 1998 (*58*); Abdel-Rahman et al. 1999 (*60*); Abdel-Rahman et al. 2002 (*62*); Turkall et al. 2010 (*65*)	Atsion (sandy) soil from Cohansey Formation near Chatsworth, New Jersey	2.6[d]	98.5% 50–250 1.5% >250
	Keyport (clay) soil from Woodbury Formation near Moorestown, New Jersey	0.93[d]	95.9% 50–500 4.1% >500
Moody and Chu 1995 (*57*)	Sludge sediment from St. Mary's River, Ontario	NR	NR
Roy et al. 1998 (*59*)	Nine soils; three from each of three manufactured gas plant sites containing BaP and PAHs	NR	< 150
Stroo et al. 2000 (*11*)	Source soil	2.4	< 150
	Lampblack soil	4.5	< 150
	Spiked soil	0.17	< 150
	Treated soil	2.1	< 150
	Aged soil	0.082	< 150
Roy and Singh 2001 (*61*)	Field soil	0.43	< 150
Stroo et al. 2005 (*63*)	CA-2: mainly lampblack (C:H ratio = 4.9)	76.9	< 150
	CA-5: soil + lampblack (C:H ratio = 2.1)	12.9	< 150
	CA-10: mainly lampblack (C:H ratio = 1.6)	82.9	< 150
	CA-13: soil + lampblack (C:H ratio = 1.0)	6.2	< 150
	CA-14: soil + lampblack (C:H ratio = 1.2)	6.2	< 150
	CA-17: mainly lampblack (C:H ratio = 5.0)	62.4	< 150
	CA-18: soil + lampblack (C:H ratio = 1.8)	24.2	< 150
Moody et al. 2007 (*64*)	Commercial gardening soil	NR	<710
Moody et al. 2011 (*66*)	Reference soil from coal-tar-contaminated site in Canada, stored “several years” in dark at 4°C	NR	<100 before storage

Notes:

BaP = benzo[*a*]pyrene
CA-X = sample identification number
NR = not reported
OC = organic content
PAH = polycyclic aromatic hydrocarbon
TOC = total organic carbon

[a] Reference numbers correspond with those presented in the manuscript.

[b] Roy et al. (*56*) and Yang et al. (*53,54*) reported organic content and not organic carbon content, which could mean organic matter. The numbers from these studies are reported here as organic carbon content.

[c] Wester et al. (*55*) did not report TOC. The value listed here is from descriptions of the same soil in other papers, although it is unclear if the reported value was for the original soil or for the particle size fraction used in their dermal absorption studies.

[d] These papers reported 1.6% and 4.4% organic matter (f_{om}) content for the clay (Keyport) and sandy (Atsion) soils, respectively. The TOC values listed here were estimated from $f_{oc} \div f_{om} = 0.58$.

Table S5. Experimental Conditions in Dermal Absorption Studies of PAHs

Reference[a]	PAH Source Information	Species/ Skin Source	Study Type	Number of Soils	Soil Aging Time (days)	T_{exp} (hours)	PAH Concentration (mg/kg unless noted)	Soil Load (mg/cm^2)	Soil Load Compared to Monolayer[b]
Benzo[*a*]pyrene (BaP) Studies									
Yang et al. 1989 (*53*)	Soil mixed with [^{3}H]BaP alone or with petroleum crude spiked with [^{3}H]BaP (petroleum crude results are the same as reported by Yang et al. 1989b and are not repeated here)	Rat	*In vitro*	1	0	96	0.1, 10, 1,000	9	Similar
Yang et al. 1989 (*54*)	Petroleum crude containing 1 mg/kg of native BaP spiked with [^{3}H]BaP and mixed with soil	Rat	*In vitro*	1	0–3	96	1	9, 56	Similar, greater
		Rat	*In vivo*	1	0–3	24, 48, 72, 96	1	9	Similar
Wester et al. 1990 (*55*)	[^{14}C]BaP in 7:3 (v:v) hexane:methylene chloride (1.2 mL) mixed with soil (9.6 g)	Human cadaver	*In vitro*	1	0	24	10	40	Similar
		Rhesus monkey	*In vivo*	1	0	24	10	40	Similar
Roy et al. 1992 (*56*)	Petroleum crude containing 1 mg/kg of native BaP spiked with [^{3}H]BaP and mixed with soil	Rat	*In vitro*	2	0–3	96	1	9	Similar
		Human cadaver	*In vitro*	2	0–3	96	1	9	Similar
[Moody and Chu 1995] (*57*)[c]	Sediment spiked with [^{14}C]BaP	Guinea pig	*In vitro*	1	NR	24	1 μg/mL (aqueous slurry)	NR	Unclear
[Roy et al. 1998] (*59*)[d]	Three soils from each of three manufactured gas plant (MGP) sites containing native PAH spiked with [^{3}H]BaP	Human cadaver	*In vitro*	9	NR	144	NR; Cold BaP reported but labeled BaP measured	25	Greater
[Stroo et al. 2000] (*11*)[e]	Five soil samples from a MGP site spiked with [^{3}H]BaP; four soils contained native PAH; soil without native PAH was spiked with BaP and three other PAHs	Human cadaver	*In vitro*	5	0	96 or >96 in paper; 126 in supplemental materials	NR; Cold BaP reported but labeled BaP measured	25	Greater
Roy and Singh 2001 (*61*)	Extracted field soil spiked with coal tar containing unlabeled BaP and also [^{3}H]BaP	Human cadaver	*In vitro*	1	0	24, 48, 72, 96, 125	65	1.12, 2.5, 5, 10	Less to greater
		Human cadaver	*In vitro*	1	0, 1, 45, 110	24, 48, 72, 96	65	10	Greater
[Abdel-Rahman et al. 2002] (*62*); [Turkall et al. 2010] (*65*)[f]	Keysport and Atsion soils spiked with [^{3}H]BaP and unlabeled BaP	Pig	*In vitro*	2	0, 90	16	1,670	10	Greater
Stroo et al. 2005 (*63*)	Samples of lampblack and lampblack/soil mixtures from MGP sites in California containing native PAH	Human cadaver	*In vitro*	7	Env. soil, many years	96	38–1,702	10	Greater
[Moody et al. 2007] (*64*)[c]	Gardening soil spiked with [^{14}C]BaP	Human cadaver	*In vitro*	1	0	24; 18 after cleaning	737 (dilute aqueous suspension)	5	Unclear
Phenanthrene (PN) Studies									
[Kadry et al. 1995] (*2*)[f]	Soils spiked with [^{14}C]phenanthrene	Rat	*In vivo*	2	0	96	185 in solvent	58	Greater
[Moody and Chu 1995] (*57*)[c]	Sediment spiked with [^{14}C]phenanthrene	Guinea pig and human surgical waste	*In vitro*	1	0	24	1 μg/mL (aqueous slurry)	NR	Unclear
Abdel-Rahman et al. 1998 (*58*)	Keyport and Atsion soils spiked with [^{14}C]phenanthrene	Pig	*In vitro*	2	90	24	110	47	Greater
[Abdel-Rahman et al. 1999] (*60*)[f]	Keyport and Atsion soils spiked with [^{14}C]phenanthrene (90-day aging data are the same as reported by Abdel-Rahman et al. 1998 and are not reported here)	Pig	*In vitro*	2	0	NR Either 24 or 16	110 in solvent	47	Greater
[Moody et al. 2011] (*66*)[c]	Reference soil from coal-tar-contaminated site in Canada, stored "several years" in dark at 4^{o}C	Human surgical waste	*In vitro*	1	Env. soil, many years	24	65.9 as dilute aqueous suspension	50	Greater

Notes:

NR = not reported
PAH = polycyclic aromatic hydrocarbon
T_{exp} = exposure time

[a] Reference numbers correspond with those presented in the manuscript.
[Brackets] around a study reference indicate that some or all of the study results are judged to be unsuitable due to inadequate experimental descriptions or critical flaws in the experimental protocol, as specified in the designated footnote.

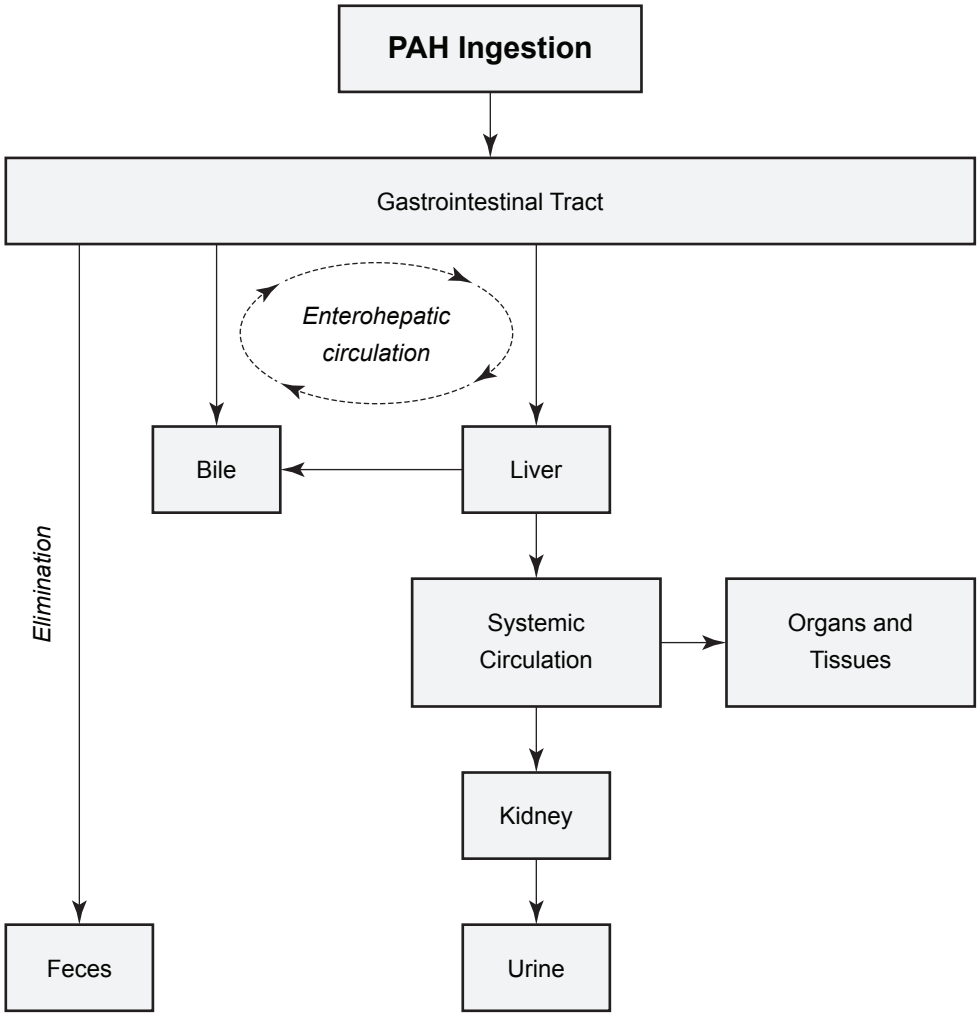
[b] Soil load is designated as greater than monolayer, less than monolayer, or similar to monolayer. Yang et al. (*53,54*) reported that 9 mg/cm^2 was the amount left on the skin when the excess was shaken off; they referred to this soil load as monolayer coverage.

[c] Soil was in an aqueous slurry; experimental description was inadequate.

[d] Flux of target PAHs was reported rather than flux of BaP.

[e] BaP flux data cannot be unambiguously ascertained due to contradictory descriptions of experiment procedures and flux calculations presented in the paper and the supplementary materials provided with the paper.

[f] Data from soils without aging appear to have contained solvent during application to skin. Solvent most probably had evaporated from aged soils.



PAH = polycyclic aromatic hydrocarbon

Source: Adapted from Ramesh et al. 2004 (*90*)

Figure S1. Absorption, distribution, and elimination of PAHs in mammals.

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Critical Review

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Oral Bioavailability, Bioaccessibility, and Dermal Absorption of PAHs from Soil—State of the Science

Michael V. Ruby,[†] Yvette W. Lowney,[*,‡] Annette L. Bunge,[§] Stephen M. Roberts,[∥] Jose L. Gomez-Eyles,[⊥,▽] Upal Ghosh,[⊥] John C. Kissel,[○] Priscilla Tomlinson,[¶] and Charles Menzie[□]

[†]Integral Consulting Inc., Louisville, Colorado 80027, United States

[‡]Alloy, LLC, Boulder, Colorado 80302, United States

[§]Colorado School of Mines, Golden, Colorado 80401, United States

[∥]University of Florida, Gainesville, Florida 32611, United States

[⊥]University of Maryland, Baltimore County, Maryland 20742, United States

[▽]Integral Consulting Inc., Seattle, Washington 98104, United States

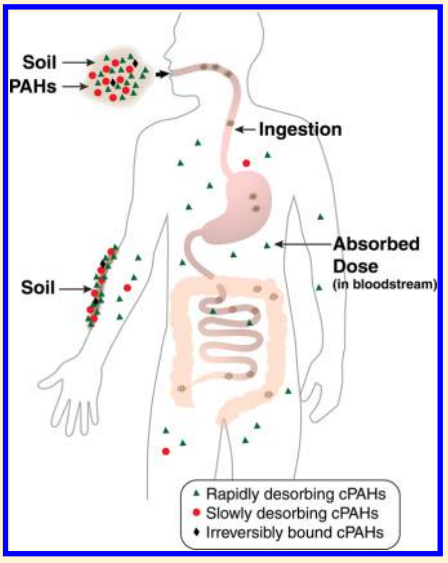
[○]University of Washington, Seattle, Washington 98195, United States

[¶]Washington Department of Ecology, Seattle, Washington 98503, United States

[□]Exponent, Alexandria, Virginia 22314, United States

Supporting Information

ABSTRACT: This article reviews the state of the science regarding oral bioavailability, bioaccessibility, and dermal absorption of carcinogenic polycyclic aromatic hydrocarbons (cPAHs) in soil by humans, and discusses how chemical interactions may control the extent of absorption. Derived from natural and anthropomorphic origins, PAHs occur in a limited number of solid and fluid matrices (i.e., PAH sources) with defined physical characteristics and PAH compositions. Existing studies provide a strong basis for establishing that oral bioavailability of cPAHs from soil is less than from diet, and an assumption of 100% relative bioavailability likely overestimates exposure to cPAHs upon ingestion of PAH-contaminated soil. For both the oral bioavailability and dermal absorption studies, the aggregate data do not provide a broad understanding of how different PAH source materials, PAH concentrations, or soil chemistries influence the absorption of cPAHs from soil. This article summarizes the existing studies, identifies data gaps, and provides recommendations for the direction of future research to support new default or site-specific bioavailability adjustments for use in human health risk assessment.



INTRODUCTION

This article reviews the state of the science regarding oral bioavailability, bioaccessibility, and dermal absorption of carcinogenic polycyclic aromatic hydrocarbons (cPAHs) in soil and discusses how chemical interactions may control the extent of absorption. The focus of this review is on the potential exposures that may be incurred by humans; the article does not attempt to characterize exposures by receptors of ecological interest, such as soil invertebrates. Of particular interest are the seven priority pollutant cPAHs (benzo[*a*]-anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]-fluoranthene, chrysene, dibenzo[*a*,*h*]anthracene, and indeno-[1,2,3-*cd*]pyrene) currently regulated by the U.S. Environmental Protection Agency (EPA) as carcinogens, as these cPAHs drive human health–based cleanup goals at PAH-contaminated sites. Attention is given to benzo[*a*]pyrene (BaP) because the toxicity of BaP has been better characterized than that of other cPAHs. Studies using naphthalene, considered to be a carcinogen by the inhalation route of exposure, are not reviewed because its high volatility and water solubility renders it chemically distinct from the cPAHs associated with human exposures via oral and dermal routes of exposure. Studies of noncarcinogenic PAHs such as pyrene and phenanthrene (PN) are reviewed to the extent that they provide information about important precedents, inform the discussion of research

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methods and study design, or elucidate the processes that control oral bioavailability, bioaccessibility, or dermal absorption of cPAHs from soil.

Publications regarding dermal absorption of PAHs from soil began to appear in the late 1980s and early 1990s. These were followed by studies of oral bioavailability in animal models and the development of physiologically based extraction tests (PBETs) to measure PAH bioaccessibility, with the latter topic yielding most of the publications in the last 10 years. To date, 67 publications or abstracts have been identified for review regarding the oral bioavailability,[1−22] bioaccessibility,[16,17,20,21,23−52] and dermal absorption[2,11,53−67] of PAHs in soil or PAH source materials (soot, char, coal, coke, coal tar, pitch, creosote, and petroleum products).

The bulk of this article discusses the sources and chemistry of PAHs in soil; in vivo and in vitro models that have been developed to assess the oral bioavailability and bioaccessibility, respectively, of cPAHs; and dermal absorption of cPAHs from soil. This article concludes with recommendations for future research that would fill data gaps and yield studies that are readily applicable to human health risk assessment.

Definitions. Because the terms "bioavailability" and "bioaccessibility" are sometimes defined in different ways by different authors, the following definitions, which are standard in the fields of mammalian toxicology and environmental exposure assessment for humans, are used throughout this article.

Oral Bioavailability. "Oral bioavailability is defined as the fraction of an ingested dose that crosses the gastrointestinal epithelium and becomes available for distribution to internal target tissues and organs."[68] This is commonly referred to as absolute oral bioavailability.

Relative Oral Bioavailability. Relative oral bioavailability refers to comparative bioavailabilities of different forms of a substance or for different exposure media containing the substance; it is expressed herein as relative bioavailability (RBA).[68,69] For the studies reviewed in this article, the exposure medium of interest is soil and the appropriate reference medium is BaP in rodent chow, because this is the medium that was used for dosing in the critical toxicity studies.

Bioaccessibility. Bioaccessibility is a measure of the physiological solubility of a chemical at the portal of entry into the body.[68−70] In this article, bioaccessibility refers to the solubility of PAHs in benchtop extraction tests (or "in vitro" extraction tests) conducted to estimate the relative oral bioavailability that might be measured in an animal study. Bioaccessibility is an operationally defined measure, dependent on parameters such as extraction fluid pH and chemical composition, extraction time, and temperature.

Dermal Absorption. Dermal (or percutaneous) absorption of a chemical in soil describes the transport from soil through the skin to subcutaneous circulation. In this discussion, it is assumed to include cPAHs remaining in or on the skin after washing, because lipophilic chemicals in the skin could eventually be systemically absorbed.[71]

SOURCES OF PAHs TO SOIL AND CHEMICAL INTERACTIONS

PAHs are deposited in soil from different source materials (e.g., soot, char, coke, coal, pitch, coal tar, creosote, oil tar, crude oil, or petroleum products) and subsequently interact with soil components, which could affect their oral bioavailability and dermal absorption. Because research on this topic specific to human exposures is limited, the concepts presented here have been formulated from research on chemical interactions of PAHs with carbonaceous materials and the published literature on how these interactions control uptake in benthic invertebrates and earthworms.

PAH Source Materials. Carcinogenic PAHs are emitted into the environment either as byproducts of incomplete combustion and pyrolysis processes (pyrogenic PAHs), or when released from petroleum products or coal (petrogenic PAHs). Petrogenic PAHs are generally released within nonaqueous-phase liquid (NAPL) matrices such as crude oil or petroleum distillates, while pyrogenic PAHs are generally emitted within and sorbed to the surface of a matrix of tar, pitch, or black carbons such as soot and char. Table 1 summarizes the PAH sources of natural, industrial, and nonindustrial origins and the primary PAH-bearing materials produced by these sources. Black carbon from both natural and industrial origins is ubiquitous in soil and is particularly elevated at specific types of industrial sites, such as manufactured gas plants (MGPs) and coking operations.

Table 1. Sources of PAHs to Soils[a]

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 oil
	sedimentary rock	kerogen
industrial	manufactured gas plants	coal tar, oil tar, pitch, coal, char, soot
	coking operations	coal tar, coal, coke, soot
	aluminum production	coal tar or petroleum pitch (making and disposing of anodes)
	foundries	coal tar pitch, creosote, fuel oil (used in making sand casts), soot
	wood treating facilities	creosote
	refineries	soot, various NAPLs (crude oil, fuel oil, diesel)
	carbon black manufacture	soot, oil tar
	fuel spills and/or disposal	various NAPLs (crude oil, fuel oil, waste oil, diesel)
nonindustrial	skeet	coal tar pitch or bitumen (used as binder in targets)
	asphalt sealants	coal tar
	landfills	creosote (treated wood), soot, char
	incinerators (industrial, municipal, hospital)	soot
	open burning	soot, char
	fire training	soot, char
	auto/truck emissions	soot

[a]Notes: NAPL = nonaqueous-phase liquid; PAH = polycyclic aromatic hydrocarbon.

PAH Sorption and Desorption from Different Forms of Organic Carbon. Within the soil environment, sorption of PAHs can be broadly described as a combination of sorption to natural organic matter (NOM) and black carbon domains.[72] While NOM typically displays linear and noncompetitive *ab*sorption or partitioning,[73] black carbon typically displays

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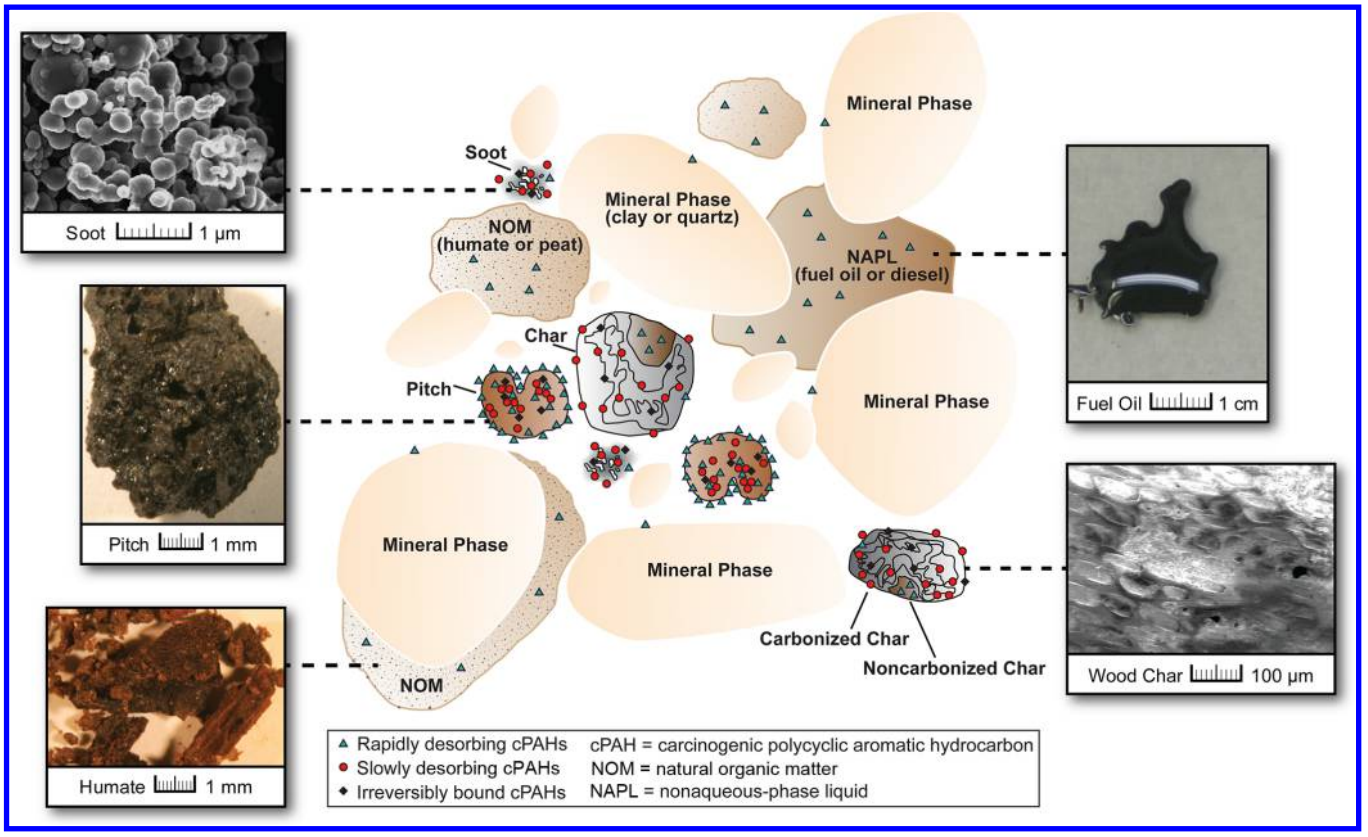

Figure 1. PAH availability for oral or dermal absorption as a function of PAH source materials and soil chemistry.

nonlinear and competitive surface *ad*sorption.[74,75] The PAH fraction weakly absorbed within NOM or petroleum, or on mineral surfaces, can be defined as the *rapidly desorbing* fraction[76] and is widely regarded as the PAH fraction potentially available for uptake by organisms living in soil or sediment. PAHs strongly adsorbed to the surface or residing within narrow nanopores of more carbonized materials have enhanced sorption to the carbon phase, diminishing 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.[76] This fraction includes *strongly bound/recalcitrant* PAHs that are regarded as unavailable for degradation by soil organisms and are only extractable from the soil matrix using harsh solvents.[77] Some PAHs can be so tightly bound or entrapped within these carbonized materials[78,79] that they cannot be removed by vigorous solvent extractions; these are considered to be in the *irreversibly bound* or *nonextractable* fraction.[77] These distinctions are depicted conceptually in Figure 1, in which certain types of organic carbon, like NOM and NAPL (e.g., crude oil or petroleum products), contain only rapidly desorbing PAHs. Black carbon materials contain primarily slowly desorbing and irreversibly bound PAHs, and some types of organic carbon, such as pitch, contain a mixture of rapidly desorbing, slowly desorbing, and irreversibly bound PAHs (depending on the production process and extent of weathering).

Black carbons such as soot and char have been shown to provide strong sorption domains for PAHs.[72,74,80−82] In the presence of these strong binding domains, the sorption of organic contaminants to soils and sediments can be up to 2 orders of magnitude higher than that predicted for NOM.[74,83] A number of studies have shown how this enhanced sorption can reduce the fraction of PAHs available for uptake by earthworms and benthic invertebrates.[84−86] While the oral bioavailability of PAHs ingested by a human is complex, it is likely that strong sorption, especially to black carbon domains, may limit the release of PAHs in the gastrointestinal tract environment. For dermal absorption to occur, the sorbed PAHs must be released at the skin surface. Thus, it is likely that the presence of slowly desorbing PAHs reduces the dermal absorption of PAHs from soil, although this has yet to be demonstrated. This chemical model suggests that the PAH source material or the organic carbon form into which the PAHs have predominantly partitioned will act as controlling factors in determining the relative oral bioavailability and dermal absorption of PAHs.

Competition and Saturation Effects. Another important issue to consider when examining the interaction between PAHs and soils is that adsorption to black carbons is competitive and nonlinear, so the lower the concentration of PAHs in soil, the more likely that black carbons will dominate sorption.[74] However, at higher organic compound concentrations, which include not only PAHs but also other organic contaminants and native organic compounds in soils (e.g., natural aromatic acids), competition effects can saturate or block the available surface adsorption sites.[74,87] Absorption into NOM may therefore gain increasing importance at high PAH concentrations. Whether PAH partitioning is governed by adsorption to black carbon or by absorption in NOM can result in as much as 2 orders of magnitude difference in aqueous equilibrium partitioning of PAHs.[74] These differences suggest that studies of oral bioavailability or dermal absorption conducted at elevated PAH concentrations (tens to thousands of milligrams per kilogram of BaP) may overestimate oral

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Table 2. Summary of Key Parameters and Ranges Used in Oral Bioavailability Studies[a]

PAH Sources	PAH Concentration in Soil	Soil Particle Size Tested	Number of Soils in Study
Coal tar, pitch, soot (lampblack), MGP wastes, creosote, petroleum products, industrial sites, soils spiked with PAHs	Range of 0.2 to 270 mg/kg BaP; most studies in the range of 20 to 120 mg/kg BaP Range of <1 to >4,000 mg/kg total PAHs, with few <10 mg/kg	Range of <45 to <6,400 μm; some studies conducted on bulk or pulverized soils	1 to 10; most studies included ≤ 4 soils, generally all from the same site
Test Species Used	**PAHs Assessed**	**Dose (μg/kg-day)**	**Dosing Medium**
Mouse Rat Mini pig Juvenile swine Goat	BaP Dibenzo[*a*,*h*]anthracene Benzo[*a*]anthracene Pyrene Phenanthrene Anthracene cPAHs EPA priority pollutant PAHs	Expressed as individual PAHs, cPAHs, or total PAHs; doses not always reported; generally in the range of 0.011 to 27 μg/kg-day BaP, 0.2 to 20,000 μg/kg-day total PAHs	Soil in mouse, rat, mini pig, juvenile swine, or goat feed; soil by gavage; soil on floor of cage
Reference Dose Medium[b]	**Endpoints Measured**		
PAHs in feed PAH source material in feed PAHs in aqueous solution by gavage PAHs in oil by gavage Clean soil spiked with PAHs in feed Clean soil in feed Clean soil or sand on floor of cage	AUC of BaP and metabolites in blood AUC of BaP in blood or plasma Urinary excretion of PAH metabolites Fecal excretion of parent PAH DNA adduct formation in tissues Parent PAHs in various tissues Liver enzyme induction		

[a]AUC = area under the curve; MGP = manufactured gas plant; BaP = benzo[*a*]pyrene; PAH = polycyclic aromatic hydrocarbon; cPAH = carcinogenic PAH; EPA = U.S. Environmental Protection Agency. [b]Reference dose medium refers to the dosing medium against which relative bioavailability is reported.

bioavailability or dermal absorption compared to what would be seen at more environmentally relevant concentrations (e.g., in the range of soil cleanup goals of approximately 0.1 to 1 mg/kg as BaP equivalents).

Effects of Aging or Weathering on PAH−Soil Interactions. It is also important to consider the processes that occur during weathering of PAHs in soils over many decades in the natural environment. In this context, weathering is associated with losses by biodegradation, leaching, or volatilization of the rapidly desorbing fraction of PAHs, and the continuous diffusion and retention of PAH molecules into remote and inaccessible regions within the soil matrix.[88] The diffusion of PAHs into less accessible regions over time—from less strongly sorbing NOM or NAPL into more strongly sorbing black carbon phases, or into even more inaccessible nanopores within the black carbon particles—is likely to reduce the oral bioavailability and dermal absorption of PAHs from soil. Thus, studies that rely on soils that have been spiked with PAHs, including those in which the spiked PAHs have been artificially weathered in the laboratory, may lead to oral bioavailability or dermal absorption measurements that are biased higher than would be seen with PAHs weathered into soils in the environment. Experiments utilizing spiked soils may be appropriate for providing initial insights into bioavailability and bioaccessibility processes, making preliminary comparisons across soil types or concentrations, or evaluating the effects of mixtures. However, the limitations of utilizing spiked soils should be acknowledged in any interpretation of the data resulting from such studies.

■ ORAL BIOAVAILABILITY OF PAHs FROM SOIL

Several approaches can be used for estimating the oral bioavailability of a chemical in soil to laboratory animals, including measurement of the parent chemical and/or metabolite(s) in blood, tissue, or excreta (urine or feces). These approaches have been used in a number of studies to assess the relative oral bioavailability of PAHs from soil. Table 2 summarizes the key experimental parameters of these studies, and Supporting Information (SI) Table S1 describes the soils, experimental conditions, and results for studies on the oral bioavailability of PAHs from soil that have been conducted to date. All of these approaches have theoretical rationales, and if their underlying assumptions are met, can yield reasonable estimates of bioavailability. However, many of the assumptions are difficult to satisfy, especially when evaluating the bioavailability of PAHs in vivo. Because of this, there are

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substantial practical limitations in the choice of methods, and these limitations must be considered carefully when designing or interpreting results from studies of the bioavailability of PAHs from soil. The following sections describe the different fundamental approaches to assessment of the oral bioavailability of PAHs from soil; SI Figure S1 provides a schematic of the absorption, distribution, metabolism, and excretion pathways for PAHs in mammals to illustrate the concepts described below.

Measurement in Blood. In classical pharmacological terminology, the bioavailability of a chemical is the fraction of an administered dose that is absorbed into systemic circulation.[89] Bioavailability is calculated from the concentration of the chemical in blood (whole blood, serum, or plasma) over time, and reported as the *area under the curve* (AUC) from the blood concentration versus time profile. The AUC captures the rise and subsequent decline in concentration following dosing and is assumed to be proportional to the amount of chemical absorbed systemically. *Absolute* oral bioavailability is derived from the ratio of the AUCs following matched doses administered orally versus intravenously.[90] *Relative* oral bioavailability (expressed as the RBA) of a chemical, for use in risk assessment, is derived from the ratio of the AUC following an oral dose of the chemical in the medium of interest (e.g., soil) versus the AUC from an equivalent oral dose of the chemical in the medium used in the critical toxicity study[69] (e.g., rodent chow for BaP). Note that doses do not have to be equivalent as long as they are in the linear pharmacokinetic range and the AUCs are corrected by the ratio of the doses administered. In the case of PAHs, the critical studies that currently form the basis of EPA's cancer potency estimate used BaP provided to animals in their diet (rodent chow),[91,92] and EPA has recently proposed a potency estimate based on BaP dietary exposure from a different rodent study.[93] Therefore, absorption of PAHs from soil relative to absorption from diet is the appropriate metric for determining RBA for use in human health risk assessment. (As noted in Table 2 and SI Table S1, absorption from the diet is not always selected as the basis for calculating RBA values reported in the literature.)

The AUC can be determined for blood concentrations of a parent chemical, one or more metabolites, or the parent chemical plus metabolites. Among the published studies evaluating RBA of PAHs using blood measurements, most have measured the parent chemical (usually BaP),[2,6,21] while one has measured the parent chemical plus metabolites by measuring radioactivity in blood following a dose of radiolabeled BaP (Goon et al.,[1] as described in Magee et al.[3]). The AUC of parent PAH after an oral dose is dependent on not only the amount of PAH that is absorbed and enters the systemic circulation, but also the rate of removal of the PAH from the blood, either through metabolism, excretion (biliary and urinary), or deposition into tissues. In order for the ratio of AUCs of parent PAH from soil versus diet to reflect RBA, rates of removal from the blood must be the same under both dosing conditions. This is a difficult condition to meet when bioavailability is measured subsequent to multiple PAH doses.

Carcinogenic PAHs, such as BaP, are potent inducers of cytochrome P450 (CYP) enzymes, including enzymes that mediate PAH metabolism.[94] Unless the extent of enzyme induction is equivalent following both soil and diet doses, measurement of RBA is confounded by differential extents of PAH metabolism. In theory, administered doses from soil and food could be adjusted so that the internal dose of BaP to the liver is the same and the induction state is equivalent. This can be accomplished by bracketing the estimated internal dose from test soils with multiple doses of reference material and monitoring hepatic enzyme activities.[95] We note that this approach can be challenging, in that substantial variability in induction can occur among animals in the same treatment group, making comparisons difficult. This problem can be avoided by assessing RBA in naïve animals after a single dose. As long as the animals for each of the treatment groups have been housed under the same conditions with the same diet, interference with RBA measurement from differences in CYP activity and BaP clearance should be minimal. Although the dosing regimen does not mimic environmental exposures, in that it is not repeated over time, it is well suited for measuring the extent to which PAHs in a soil matrix have diminished gastrointestinal absorption relative to PAHs in diet.

As noted above, the fundamental assumption underlying blood measurements to establish RBA values is that the AUC is directly proportional to the amount of chemical that has been absorbed from the gastrointestinal tract and that reaches systemic circulation. While this assumption is generally valid over a limited oral dose range, chemical-specific saturable processes affecting absorption or metabolism can cause the relationship between absorbed dose and AUC to be nonlinear. Ideally, when assessing the RBA of a chemical from soil, it should be demonstrated that the doses of PAH administered are in the range of linear pharmacokinetics, although most studies simply select similar doses to administer from the media being compared and assume that the basic pharmacokinetics (other than fraction absorbed) will be the same.

To measure an AUC with reasonable accuracy, blood concentrations at several time points are needed. At progressively lower PAH concentrations in soil and diet, blood concentrations can decrease until most are below analytical detection limits and the error in estimating the AUC becomes unacceptably high. Based on studies published to date (e.g., van Schooten et al.[6] and Duan et al.[21]), soil BaP concentrations need to be minimally in the tens of milligrams per kilogram to produce blood concentrations sufficient to determine BaP bioavailability (SI Table S1). In comparison, EPA's current screening levels for BaP in residential and commercial soils are 0.015 and 0.21 mg/kg, respectively;[96] in practice, site-specific cleanup goals tend to be in the range of 0.1 to 1 mg/kg BaP equivalents. Thus, there is a wide range of BaP concentrations in soil for which bioavailability information might be useful but cannot be quantified by direct measurement of BaP in blood. At present, it is unclear whether RBA measurements obtained for highly contaminated soil (i.e., soil with BaP concentrations in the range of 50 to 200 mg/kg) can be assumed to apply to soil with lower, more environmentally relevant concentrations (e.g., 0.1 to 1 mg/kg). As discussed above, some of the processes that control the binding of PAHs to soil are concentration dependent, so bioavailability may also be concentration dependent, and the extrapolation of RBA values from soil with high PAH concentrations may overestimate RBA for soils with lower PAH concentrations.

Rather than measuring an AUC, some bioavailability studies have measured the blood concentration of BaP at a single time point as an indicator of absorbed dose. This approach is valid only if the time course of increasing and decreasing blood concentrations is identical following administration in soil and diet, so that the ratio of blood concentrations at any single time point reflects the comparative fraction of dose that is absorbed

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from these two dosing media. Any shift in the blood concentration versus time profile (e.g., if absorption from soil occurs more slowly than from diet) can cause blood concentrations at a given time point to be different even if the total absorbed dose is the same. If blood concentrations are measured at only one time point, there is no way to determine whether a shift has occurred, making this approach unreliable under most circumstances.

Measurement in Urine. PAH metabolites, and to some extent parent PAHs, are excreted in urine.[97−99] If the amount excreted is proportional to the absorbed dose, then urinary excretion can be used as a quantitative measure of absorption. Previous attempts to use urinary excretion to measure bioavailability have focused on metabolite excretion, for example, 3-hydroxybenzo[*a*]pyrene following exposure to BaP in soil[9] or 1-hydroxypyrene following exposure to pyrene in soils.[5,7] A principal problem caused by using urinary metabolites as an indicator of PAH absorption stems from the fact that urinary excretion is a minor pathway of elimination. For example, Jacob et al.[100] observed that only 0.4% of an oral dose of pyrene in rats was excreted in urine as pyrene plus 1-hydroxypyrene, and Jongeneelen et al.[101] observed that only 0.22% to 0.35% of an oral dose of BaP in rats was excreted as 3-hydroxybenzo[*a*]pyrene (parent BaP was not detectable). In studies by Ounnas et al.[19] and Costera et al.,[102] goats were given daily oral doses of soil spiked with 100 mg/kg of PN, pyrene, and BaP (each) for 10 days, and the predominant hydroxylated metabolite for each was measured in urine. Goats excreted 20% to 32% of the pyrene dose as 1-hydroxypyrene and 5% to 7% of the PN dose as 3-hydroxyphenanthrene, but 3-hydroxybenzo[*a*]pyrene concentrations in urine were too low to quantify. Finally, in rats dosed with a mixture of PAHs, including 35 mg/kg pyrene and 9.2 mg/kg BaP, only 0.2% of the pyrene in soil was excreted in urine as 1-hydroxypyrene, and 3-hydroxybenzo[*a*]pyrene was not detected.[6]

The very low urinary excretion rates for larger PAHs like BaP, particularly after doses in soil, create three limitations that are related to (1) analytical detection limits, (2) contamination by fecal matter, and (3) signal-to-noise ratio. Because the fraction excreted in urine is low, doses of PAH administered must be high (relative to environmental doses) to be able to detect and reliably measure the metabolite in urine. For example, as noted above, in studies involving goats, rats, and mice, excretion of 3-hydroxybenzo[*a*]pyrene in urine following doses of BaP in soil with concentrations ranging from 10 to 100 mg/kg was vanishingly small, if quantifiable at all. Analytical data close to the practical detection limit is prone to high uncertainty. The second limitation is the potential for contamination from feces. Specialized metabolism cages for rodents allow for separation of urine and feces, but none are completely effective in this regard. Extensive biliary excretion of PAHs and metabolites means that both absorbed and unabsorbed PAHs are eliminated predominantly in feces. As an example, Grimmer et al.[103] found that the hydroxylated metabolite profile for chrysene administered orally to rats was very similar between urine and feces, but that feces contained 100-fold higher concentrations. Because of the much higher concentrations of PAHs and metabolites in feces relative to urine, even transient contact of urine with fecal matter as they are separated in the metabolism cage can confound measurements of urinary concentration and result in overestimates of bioavailability due to PAHs and metabolites detected in urine but actually excreted in feces. Lastly, estimates regarding the extent of absorption based on measurement of urinary metabolites must be made on changes in very small numbers, which is inherently prone to error—a classic signal-to-noise problem.

Measurement in Feces. Ingested PAHs that are not absorbed from the gastrointestinal tract are eliminated in feces, either as parent compounds or as metabolites formed by gut microflora. PAHs absorbed from the gastrointestinal tract are largely metabolized and returned to the gut through biliary excretion. For PAHs with four to six rings (i.e., the cPAHs), biliary excretion is the predominant route of elimination of metabolites.[97−99] For example, Foth et al.[97] found that approximately 40% of an intravenous dose of BaP in rats was excreted in bile as metabolites within 4 hours of dosing. As a result, fecal contents reflect both absorbed and unabsorbed PAHs, and distinguishing between the two for the purpose of estimating bioavailability is difficult. Both hepatic metabolism of absorbed PAHs and microbial metabolism of unabsorbed PAHs produce hydroxylated metabolites.[90,104] While it might be possible to identify distinctive metabolite profiles from the two sources so that they can be individually quantified from fecal measurements, this has never been demonstrated. Studies of gut microbial metabolism of PAHs are limited, but it is reasonable to speculate that PAH metabolism patterns are dependent on the specific microflora present, which in turn would be expected to vary with host species (e.g., rat, mouse, or human), diet, and other factors.

An additional confounding factor is the enterohepatic recirculation of PAHs. Glucuronide and sulfate conjugates of PAHs excreted in bile can be cleaved by intestinal microbial flora, facilitating reabsorption of the PAH or metabolite from the intestine. Subsequent conjugation and biliary excretion followed by microbial deconjugation and reabsorption continues the cycle, delaying elimination from the body. Enterohepatic recirculation has been demonstrated for a variety of PAHs, including pyrene and BaP.[6,90,105,106] Enterohepatic recirculation (SI Figure S1) affects not only the time course over which PAHs appear in feces but the form in which they appear. Conceivably, the complicated nature of these processes would be immaterial in determining RBA if microbial metabolism and enterohepatic recirculation apply equally to doses from any medium (i.e., if the amount of PAH or metabolite excreted in feces is directly proportional to absorbed dose), but this has not been demonstrated.

Collection of bile directly (rather than feces) would capture the primary route of excretion for PAHs while avoiding confounding effects from microbial metabolism in the gut and enterohepatic recirculation, and the amount of PAH metabolite eliminated in the bile should be proportional to the absorbed dose. However, the presence of bile salts in the intestinal lumen is very important for the absorption of PAHs, particularly four- and five-ring PAHs.[107] As a consequence, interruption of bile flow by cannulation of the bile duct and collection of bile samples creates a model in which PAH absorption is artificially diminished and its reliability in determining RBA is untested (and could result in a low bias for estimates of absorbed dose).

Measurement in Tissue. With repeated doses, and once a steady state has been achieved between blood and tissues, the concentration of a PAH or metabolite in tissues will be proportional to the absorbed dose. Thus, tissue concentrations could be used to estimate RBA by comparing results from animals given the same dose (e.g., from soil versus food). The

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relationship between tissue concentration and absorbed dose is more tenuous when not at steady state. To achieve steady state, multiple doses must be given over time. As discussed above, self-induction of metabolism that occurs with repeated doses can produce differences in metabolic clearance among animals ingesting PAHs in soil versus diet, and the direct proportionality between tissue concentrations and absorbed dose needed for bioavailability determination may be lost. Although difficult to address experimentally, this problem can be approached in the same manner as described above ("Measurement in Blood") if differences in metabolic clearance from multiple doses are likely.

Measurement of Bioavailability Using Biomarkers. The use of biomarkers as end points for bioavailability measurements has appeal because it can potentially provide highly relevant indicators of the internal dose of a chemical. Although biomarkers as end points may not fit the classical definition of bioavailability, they offer an alternative and potentially informative view of differential absorption of environmental chemicals. There is particular interest in biomarkers related to critical toxic effect(s). For PAHs, limited studies have attempted to assess bioavailability using CYP induction[8,15,108] and PAH−DNA adducts[5,7,10,12] as end points. CYP metabolism of PAHs produces reactive, genotoxic metabolites;[90] DNA adducts are biomarkers because they are considered precursor events leading to PAH carcinogenesis.[109] As long as a biomarker is a better quantitative indicator of toxicity than simply measuring a chemical or its metabolite(s) in the body, there is a logical basis to use it for bioavailability assessment. Establishing a quantitative relationship between a biomarker and toxicity is difficult, particularly for cancer risk in the case of PAHs. For example, while CYP activity is clearly an important factor in PAH carcinogenesis, there is currently no established quantitative relationship between CYP activity and cancer risk. Similarly, while the presence of DNA adducts is considered necessary for PAH carcinogenesis and is associated with increased cancer risk in humans, DNA adducts do not always correlate well with tumor formation. In mice treated with BaP, DNA adducts are found in tissues that do not develop tumors as well as those that do.[110] Therefore, unless the relationship between the selected biomarker and the risk or incidence of the toxic effect is well established, this approach may be unreliable. Further, because biomarkers usually result from the culmination of a number of biological processes, the likelihood that they are linearly related to dose over the entire exposure range of interest is small. As noted by Godschalk et al.,[109] DNA adduct formation from PAHs does not display a strong proportional relationship to exposure in humans. Hence, an RBA value generated using biomarkers may be dose dependent. In other words, RBA will depend on the PAH concentration in soil, along with other variables, making it not only site-specific but concentration-specific. This greatly complicates its use for RBA assessment.

Finally, there is the issue of using an RBA based on internal dose metrics (i.e., biomarkers) but with a toxicity value based on external doses, such as a cancer slope factor. If the RBA is based on something other than a difference in absorption from the exposure medium and incorporates other biological processes, then it is addressing a fundamentally different form of "dose" than the one used to derive the toxicity value. An exposure estimate from a biomarker-based RBA would be incompatible with a standard cancer slope factor or reference dose for risk estimation.

Review of Existing in Vivo Studies. SI Table S1 provides a summary of some of the key parameters reported in various in vivo models/studies that have been conducted to evaluate the oral bioavailability of PAHs, including BaP. The table shows (1) studies that have appeared in peer-reviewed publications and for which enough information is provided that the quality of the study can be evaluated, (2) studies that have appeared in peer-reviewed publications but with insufficient information to fully evaluate their quality, and (3) studies that are available only as abstracts or are referred to in other publications. Studies published only in non-English languages are excluded from this review.

The 22 studies summarized in SI Table S1 used a variety of animal models to evaluate the RBA of various PAHs, including mice, rats, mini pigs, juvenile swine, and goats, and have attempted to evaluate the RBAs of BaP, dibenzo[*a*,*h*]-anthracene, benzo[*a*]anthracene, pyrene, PN, anthracene, cPAHs, and total PAHs. Measurement end points have included the AUC of BaP and metabolites in blood (based on radiolabel), the AUC of BaP in blood and plasma, excretion of hydroxylated PAH metabolites in urine, excretion of parent PAHs in feces, DNA adduct formation in lung and liver tissue, concentrations of parent PAHs in various tissues, and liver enzyme induction. Most of the studies (17 of 22) report the soil particle size dosed, but most of them dosed soil particles much larger than those that adhere to human hands and may be incidentally ingested (<150 to <250 μm).[111,112] Given this array of animal models, PAHs evaluated, measurement end points, and soil particle sizes dosed, it is difficult to compare results directly across studies. Of the 22 studies reviewed in SI Table S1, RBA values that could be used in human health risk assessment were either not reported, or could not be calculated from the data presented in the publication, for ten of them.

Together, these studies provide a general basis for establishing that the bioavailability of PAHs from soil is reduced relative to absorption from diet, and that the default assumption of 100% RBA likely overestimates actual exposure to cPAHs from soil. The studies do not, however, provide a strong basis to support conclusions regarding the specific reduction in bioavailability or allow for further understanding beyond the individual samples tested. Because of the limited scope of each individual study and the large variability in animal models and study designs utilized, the aggregate data do not indicate what soil chemical conditions will yield a particular RBA estimate, nor do they provide a broad understanding of how different PAH source materials (e.g., soot, char, coal, coke, coal tar, pitch, creosote, petroleum products) influence the RBA of cPAHs from soil.

In general, the studies used test materials with BaP concentrations in the 20 to 120 mg/kg range, well above the soil cleanup goals utilized by regulatory agencies (0.1 to 1 mg/kg, as BaP equivalents). As discussed in the Competition and Saturation Effects section, it appears that studies conducted in the higher BaP concentration range may tend to overestimate RBA for soils in the 1 mg/kg concentration range.

In the four studies that evaluated the effect of aging on RBA, one showed a slight reduction in RBA values (14% to 27% reduction after 6 to 12 months of aging)[1] and three showed no reduction in RBA values after weathering for 3 to 12 months.[10,13,21] Only nine of the studies reported the total organic carbon (TOC) of the soils they tested, and none of them characterized the types of organic carbon in the test soils. These data are needed to fully understand the effects of PAH

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Table 3. Key Parameters and Ranges Used in PBETs[a]

PAH Concentrations in Soil	Soil Parameters Measured	Soil Particle Size Tested	Number of Compartments
Highly variable; range of 2 to 300 mg/kg BaP and <1 to 5,000 mg/kg total PAHs	pH Total organic carbon Black carbon Particle size distribution Surface area	Range of <45 μm to <4 mm; <250 μm is most common	1 to 3; gastric, small intestine, large intestine
Soil:Solution Ratio	**Fed versus Fasted**	**Gastric pH and Extraction Time**	**Gastric Components**
1:100 is most common; ratios of 1:10 to 1:250 have been used	Both fasted and fed test systems in use; food sources in use are highly variable	pH 1 to 2, generally unbuffered; 1 to 2 hours	Highly variable: • Pepsin • Mucine • Bovine serum albumin • Lipid sources • Various salts
Small Intestinal pH and Extraction Time	**Small Intestinal Composition**	**Colon Phase**	**Additional Sink in Small-Intestinal Phase**
pH 6.5 to 8.5, occasionally phosphate buffered; 2 to 6 hours	Variable: • Bile salts • Pancreatin • Lipase • Bovine serum albumen • Various salts	Uncommon (pH 6 to 7; 8 to 18 hours)	Used in more recent studies: caco-2 cells, EVA thin films, C18 disks, silicone rods, and silicone sheets

[a]EVA = ethylene vinyl acetate; PAH = polycyclic aromatic hydrocarbon; PBET = physiologically based extraction test.

source materials and different forms of organic carbon on cPAH RBA values. There is some indication that soil TOC is inversely related to the RBA of BaP,[1,21] but the data are very limited. If true, this would be consistent with results from PBET studies, as described below.

BIOACCESSIBILITY OF PAHs FROM SOIL

In vitro extraction tests are defined herein as any benchtop chemical extraction test that is designed to measure the bioaccessibility of PAHs. The focus of our analysis has been on studies that use bioaccessibility as a surrogate to understand oral bioavailability to human receptors. This is to be distinguished from studies related to bioaccessibility to ecological receptors, such as earthworms or benthic communities, for which a separate body of literature exists. To date, these tests have been almost entirely PBETs, developed for two general reasons: (1) to obtain a simple, inexpensive tool to predict the RBA of PAHs from soil; or (2) as a tool to study the chemistry of PAHs in a simulated gastrointestinal system. Whether development of a PBET that correlates to in vivo RBA measurements across a wide range of PAH sources and soil types is feasible has not yet been resolved. Although this has been achieved for arsenic and lead in soil using relatively simple in vitro tests,[113,114] PAHs present a more complex system in which solubilization in the small intestine, absorption mechanisms, metabolism, and elimination are likely to come into play. However, based on the success of in vitro systems in evaluating the absorption of lipophilic pharmaceuticals,[115] the development of a reliable in vitro test for PAHs in soil should be feasible.

From the time that the first article regarding a PBET for measuring PAH bioaccessibility from soil appeared in the peer-reviewed literature,[23] there have been 33 additional articles addressing some aspect of PBET test development and/or application for PAHs. This has caused a proliferation of methods, most of which are permutations of earlier PBET methods for PAHs and other contaminants.[23,116,117] PBETs originating in Europe have tended to be more complex because of an attempt to mimic gastrointestinal-tract chemistry as closely as possible, based on the assumption that this will yield an extraction system that more accurately predicts uptake in humans. In contrast, tests originating in Canada, the U.S., and Australia have tended to rely on correlation with in vivo data, to try to establish that they are accurately predicting the bioavailable fraction as measured in animal models. As a result, these tests can be less complex because they are focused on capturing the critical test components that allow for a correlation with animal data. Most recently, simple extractions using chemical solvents and PAH partitioning approaches have also been evaluated for their ability to predict the oral bioavailability of BaP[21] and other PAHs.[22]

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Only the key findings from available in vitro studies and the attempts to validate an in vitro test against in vivo RBA data are discussed below. Table 3 summarizes the key PBET parameters addressed in the published literature on the bioaccessibility of PAHs from soil, and the ranges of values that have been used to date. SI Table S2 describes the soils, experimental conditions, and results in detail for the PBET studies conducted to date.

Key Findings of Bioaccessibility Research. Reported values for the bioaccessibility of PAHs from soil are highly variable, depending on the PBET method used and the substrates evaluated; however, in general, reported bioaccessibility values are <50% for BaP and other cPAHs. Overall, the available studies we identified indicate that the TOC content of soils is inversely related to bioaccessibility.[16,25,26,32,37,39,40]

Bile salts in the small intestine act as the primary agent for solubilization of PAHs from soil and greatly increase the bioaccessibility of PAHs.[23,26,27,40,118] Consistent with this observation, Rahman et al.[107] demonstrate that in rats, the absence of bile in the small intestine results in a 77% decrease in BaP absorption. Bile salts, in the presence of lipids and cholesterol, or dietary lipids, form mixed micelles into which the PAHs can partition.[26,119] These mixed micelles can then deliver the PAHs to the intestinal epithelium, where they can be absorbed. Factors affecting the formation of micelles, such as bile concentration,[23,26,27,40,118] lipid concentration,[23,51] and pH of the small intestine[51] are therefore important components in the development of PBET methods for PAHs. The literature also identifies kinetic and PAH solubility constraints that can reduce the amount of PAHs extracted by PBETs, and demonstrates how the addition of an infinite sink to the small-intestinal phase (Table 3) enhances PAH dissolution from soil.[43,45,46,50] The addition of an infinite sink could better mimic conditions of the gastrointestinal tract, where passive diffusion of PAHs across the intestinal epithelium and binding to other components within the intestinal lumen are likely to maintain steep diffusion gradients, enhancing PAH dissolution from soil. A study by James et al.[20] reports an improvement in the in vitro to in vivo correlation (IVIVC) for PAHs from soil with the addition of a C18 membrane to the extraction fluid. However, this is in comparison to results from a simple, buffered, acidic extraction system developed for metals; it specifically excludes additions to mimic the intestinal environment and the reported IVIVC is still poor. Therefore, whether the addition of an infinite sink improves the correlation of in vitro data with in vivo models is yet to be determined.

Validation of in Vitro Tests. Validation of an in vitro test against RBA results from an animal model (i.e., an IVIVC) has been attempted in five of the existing studies on the bioaccessibility of PAHs from soil.[16,17,20,21,31] Of these five studies, Gron et al.[31] observed the best IVIVC (r^2 = 0.81), based on RBA values for BaP in seven test soils. However, Gron et al. combined two sets of in vivo data, which were based on different animal models (mouse [three soils] and mini pig [four soils]) and two different biological end points (urinary excretion of 3-hydroxybenzo[*a*]pyrene in mouse urine and unabsorbed BaP in mini-pig feces). Neither of these in vivo models or data sets was published in the peer-reviewed literature; thus it is impossible to confirm the quality of the in vivo data that serve as the basis of this IVIVC. Of the remaining four papers, only Pu et al.[16] provides sufficient detail on the in vivo methods used (measurement of the AUC for PN in blood for eight spiked soils) to allow for a critical assessment of the IVIVC. However, this in vivo study yielded RBA values in excess of 100% for three of the eight soils dosed, most likely because of the low absolute bioavailability measured for the corn-oil gavage reference dose (24%). The research by Duan et al.[21] evaluated in vivo results against two in vitro methods that used simple chemical extractions (butanol and cyclodextrin); however, the authors did not present RBA values or the data from which they could be calculated, so an IVIVC cannot be developed from that study. Finally, neither Stroo et al.[17] nor James et al.[20] observed strong correlations between their in vivo and in vitro results. As a result, the development of a reliable IVIVC for cPAHs in soil is an outstanding goal, and one that will require a set of RBA values from a range of soils that are derived from a competent in vivo model.

DERMAL ABSORPTION OF PAHs FROM SOIL

Assessment of the dermal absorption of PAHs from soils is important to ensuring the accurate evaluation of total systemic exposures from soil. Therefore, dermal absorption from soil should be addressed with rigor comparable to that applied to gastrointestinal absorption. This is especially important when skin is the target organ of concern or when considering occupational scenarios not impacted by child soil ingestion rates. Many of the soil−chemical interactions that affect the absorption of PAHs from ingested soil are likely to also influence the partitioning of chemicals from soil to skin, and hence affect dermal absorption. To date, 12 studies have addressed the dermal absorption of BaP[11,53−57,59,61−65] and five have addressed the dermal absorption of PN.[2,57,58,60,66] The key experimental parameters, and their ranges, for these dermal absorption studies are summarized in SI Table S3, and the test soils and the specific experimental conditions utilized in each study are detailed in SI Tables S4 and S5, respectively. Due to space limitations, only a few of the important findings from review of the dermal absorption studies are presented herein.

Given the wide variety of experimental conditions (e.g., PAH sources, species/skin sources, in vivo versus in vitro studies, particle sizes used, soil aging times, exposure times, PAH concentrations in test soils, and soil loadings; see SI Tables S4 and S5), it is difficult to compare across studies and draw conclusions from this body of literature. The effects of PAH source material, soil particle size, and aging or weathering of PAHs in soil are discussed below.

Effect of PAH Source Material. Soils in five of the dermal absorption studies contained PAHs in a source material added to the soil (petroleum crude or coal tar) or present in the contaminated soil sample as lampblack.[54,56,61,63,66] The TOC varied among the soils and lampblack samples from less than 0.5% to more than 80%, although the proportion of TOC that is black carbon is not reported. Soils in all of the other studies were spiked with BaP or PN in a solvent solution assumed to be subsequently removed by evaporation. Given this limited data set and the variability in study designs, the effect of PAH source material on dermal absorption is difficult to evaluate.

Effect of Soil Particle Size. Soil particle size affects skin adherence and chemical transfer to skin, as well as the soil's capacity to sorb contaminants. Thus, experiments meant to provide dermal absorption measurements for risk assessment should include only fine particles, on the order of <150 μm or smaller, that would preferentially adhere to human skin.[71,120] For the studies included in this review, soil particle sizes ranged from <100 to <710 μm (SI Table S4), with the majority of studies focused on soil particles <150 μm. Of particular note is the study of Wester et al.,[55] which forms the basis for the

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recommendation from EPA to assume a dermal absorption fraction of 13% for PAHs in soil,[121] and which used a particle size fraction of 180 to 320 μm (fine to medium sand).

Effect of Aging or Weathering of PAHs in Soil. It is generally expected that aging or weathering of laboratory-contaminated soils would reduce PAH absorption. While there is extensive literature on the effects of aging on uptake of PAHs from soil and benthic organisms,[69] experimental evidence related to dermal uptake in mammals is very limited in the studies to date. For soils contaminated with coal tar, Roy and Singh[61] observed no difference in BaP absorption from coal tar added to soils and aged 1 day compared with soils aged for 45 days, and an approximately two-fold reduction from soils aged 110 days. Notably, the flux through skin after 110 days of aging was the same as that observed by Stroo et al.[63] for two samples with similar BaP levels from an MGP site that had been closed for approximately 50 years. In both of these studies, BaP would have been incorporated into the PAH source material (either coal tar or lampblack), which may explain the minimal effects of laboratory aging and environmental weathering in these studies. In the studies of Abdel-Rahman et al.,[58,62] the effect of aging cannot be assessed because the freshly contaminated soil experiments used as comparison samples appear to have contained residual solvent.

RESEARCH NEEDS

This detailed review of the literature indicates that much effort has been expended in assessing the oral bioavailability and dermal absorption of PAHs from soil. An extensive body of literature on the effect of PAH source materials and soil−PAH interactions is also available and facilitates a theoretical understanding of the factors likely to control oral bioavailability and dermal absorption of PAHs in humans. However, significant limitations still exist that hamper broad application of bioavailability adjustments for cPAHs in risk assessment. Among these is a lack of (a) validated animal and in vitro models, and (b) studies that demonstrate the influence of PAH source material and soil chemistry, particularly in concentration ranges that are of significance for remediation of contaminated sites.

Future research into the oral bioavailability of cPAHs should include a variety of soils that reflect a range of PAH source materials and soil chemistries. It is particularly important to gain an understanding of the role of different types of organic carbon, particularly black carbon, in sequestering cPAHs and limiting their oral bioavailability. Measuring oral bioavailability requires a validated measurement end point that reflects absorbed dose. It is possible that a viable in vivo model based on urinary or fecal excretion, tissue concentration, or biomarkers could be created; however, it should be demonstrated that the end point reflects absorbed dose as indicated by the AUC. For risk assessment at contaminated sites, it is also important that the RBA values are based on a comparison to absorption of PAHs from soil versus the diet, because dietary exposures form the basis of the current regulatory toxicology of PAHs (i.e., the carcinogenic potency of BaP). Ideally, the method will provide adequate sensitivity to allow for characterization of absorption at environmentally relevant doses (in the range of 0.1 to 10 mg/kg BaP in soil) so that PAH−soil interactions accurately reflect factors operating in the PAH concentration range of relevance to remediation of contaminated sites. Finally, if a broad-scale research effort were undertaken that included an adequate range of soils, an in vitro method could be validated for a wide range of PAH source materials and types of contaminated sites.

With respect to dermal absorption of BaP, data gaps and limitations are apparent and should be addressed in future research. Of particular concern are issues of soil particle size, effects of PAH source materials and soil chemical characteristics, and chemical concentration in soil. Important factors requiring further study are the use of environmentally relevant concentrations (i.e., $\leq$ 10 mg/kg BaP) and the fine-particle-size fraction of soils.[122,123] To avoid the complications of animal skin and in vivo adjustment factors that cannot be independently tested, in vitro studies using human skin are recommended. Investigations into the effect of BaP in different PAH source materials compared with BaP in a solvent vehicle are especially needed. Nevertheless, given that EPA's default value for PAH absorption is derived from the Wester et al.[55] in vivo values, studies that are designed to critically evaluate the validity of the Wester et al. results would also be useful.

Such studies would form a basis to support broader application of bioavailability adjustments at PAH-contaminated sites, either by updating current default assumptions regarding oral or dermal absorption from soils, or by identifying key considerations and tools for assessing bioavailability on a site-specific basis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b04110.

Tables that provide details on the soils, experimental conditions, and results for all of the oral bioavailability, PBET, and dermal absorption studies of PAHs in soils that have been published to date, along with a figure detailing the absorption, distribution, and elimination of PAHs in mammals (PDF).

AUTHOR INFORMATION

Corresponding Author

*Phone: 303-589-9955; email: YLowney@Alloy-llc.com.

Notes

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