Dermal absorption of benzo[a]pyrene from soil: Assessment of flux and application to risk assessment of contaminated sites

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ABSTRACT

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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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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

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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

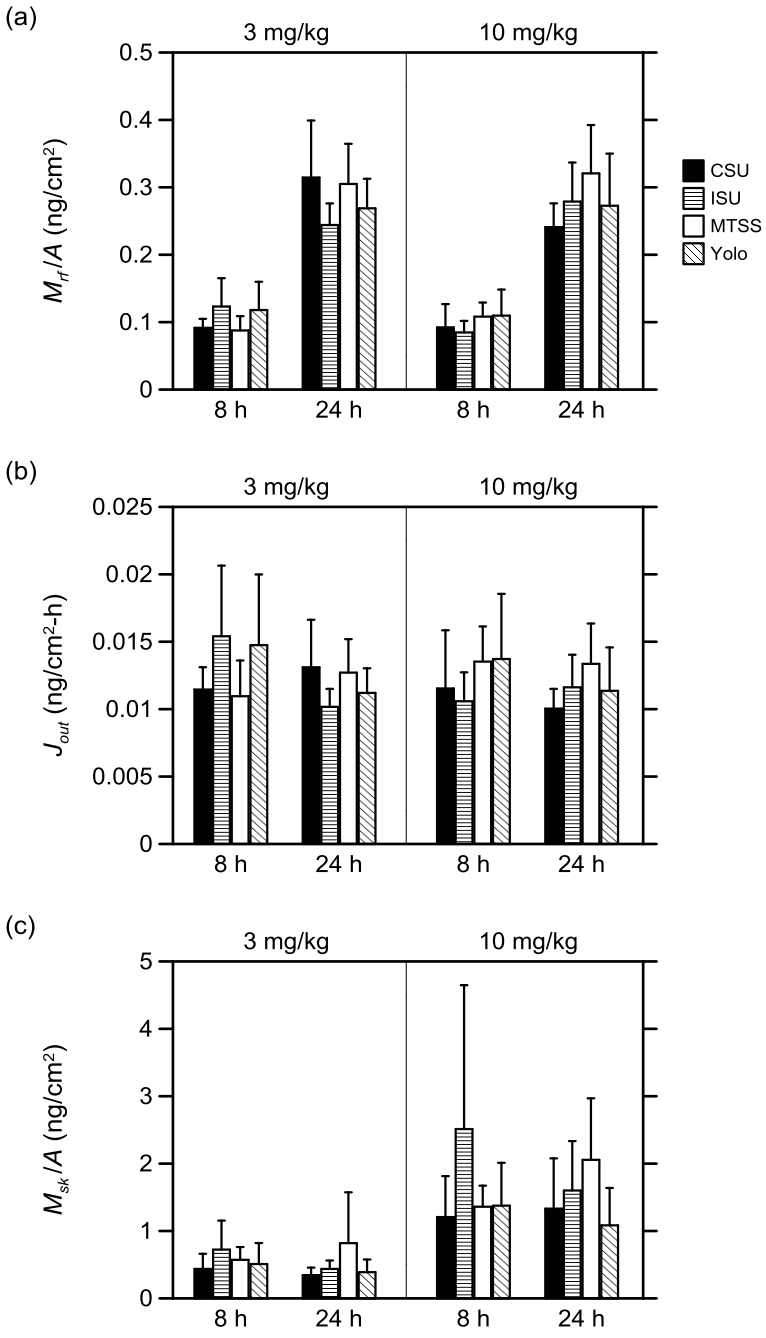

Figure 1. Results for four weathered soils at 3 and 10 mg/kg BaP concentration after 8- and 24-h exposures: (a) M_{rf}/A, (b) J_{out}, and (c) M_{sk}/A. Error bars represent 95% confidence intervals.

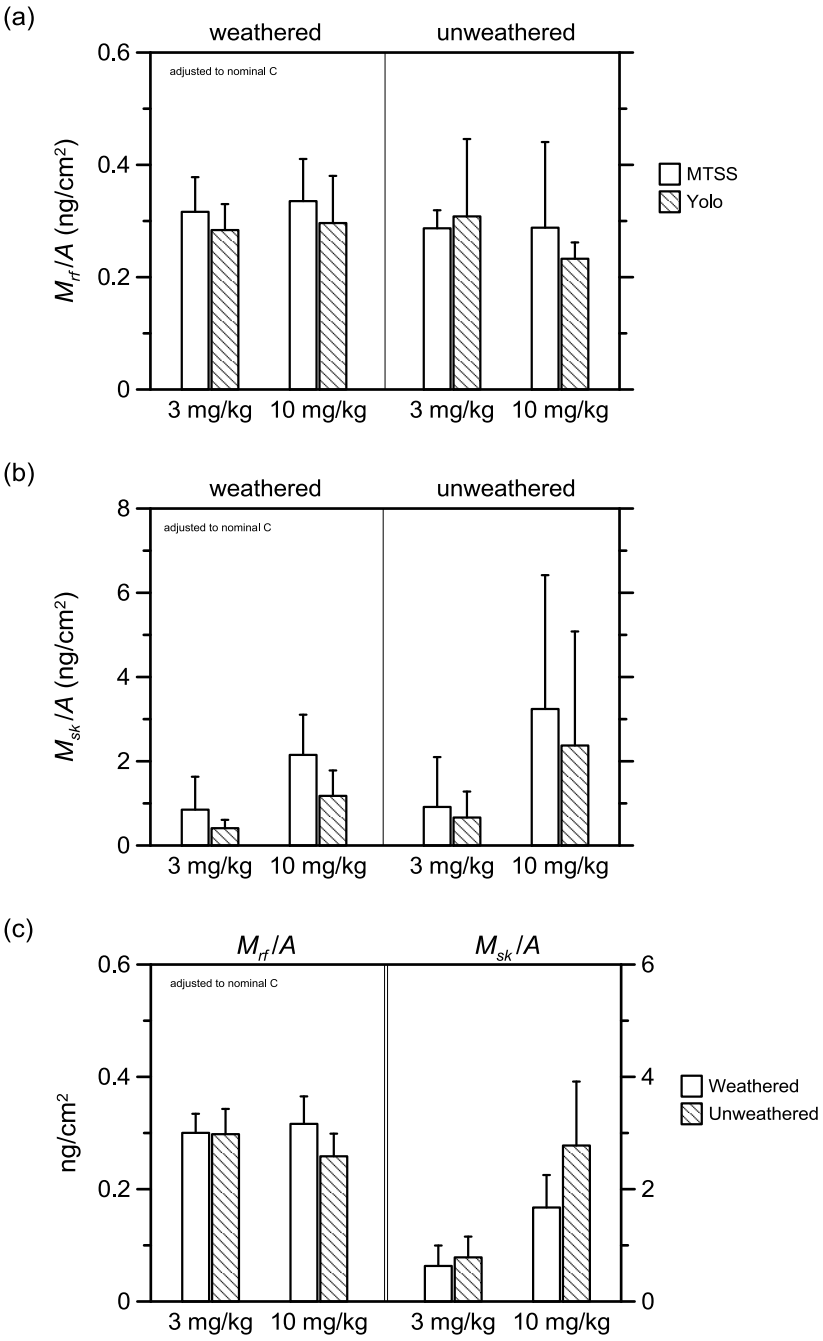

Figure 2. Results for weathered and unweathered Yolo and MTSS soils adjusted to the nominal BaP concentrations of 3 and 10 mg/kg after a 24-h exposure: (a) M_{rf}/A for each soil, (b) M_{sk}/A for each soil, and (c) average of Yolo and MTSS soils combined for M_{rf}/A (left axis) and M_{sk}/A (right axis); n = 3 and 6 for each unweathered and weathered soil, respectively. Error bars represent 95% confidence intervals.

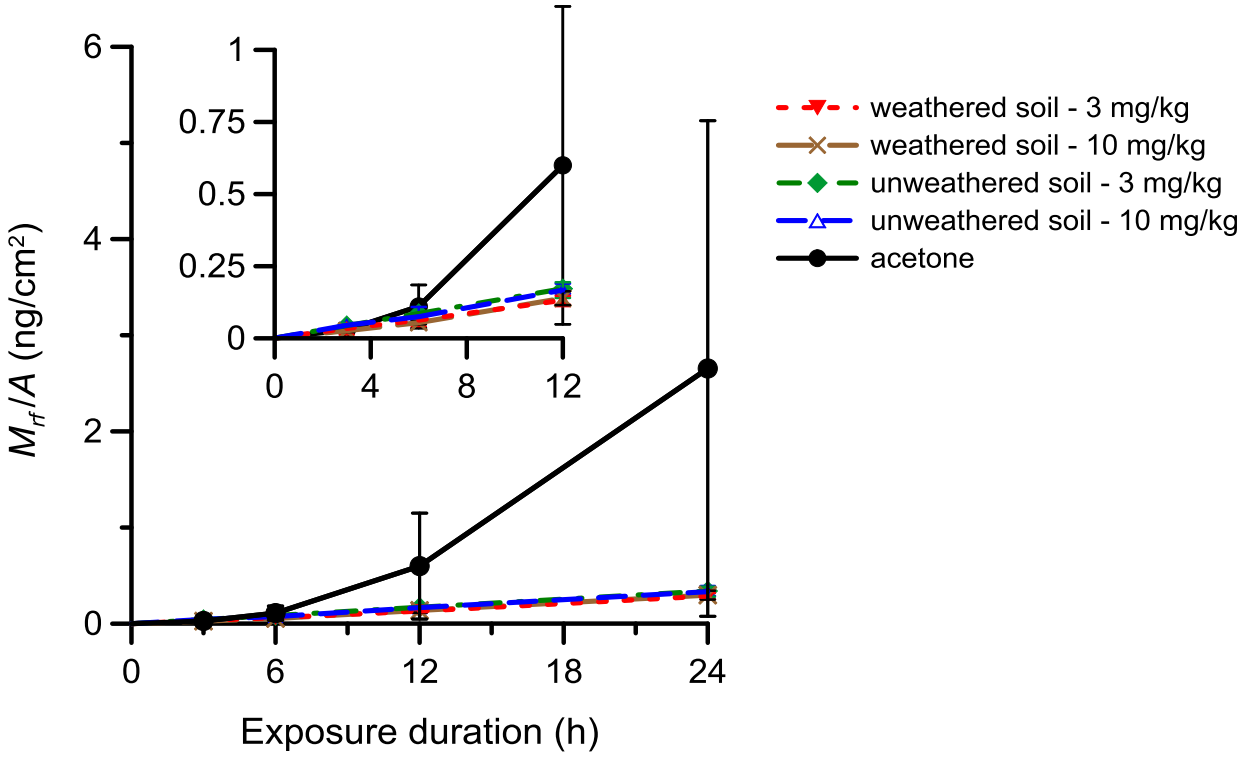

Figure 3. M_{rf}/A from BaP deposited in acetone and from BaP in the weathered and unweathered MTSS and Yolo soils at 3 and 10 mg/kg BaP concentrations. Lines connecting the results are drawn to guide the eye. Inset is an enlargement of the data to 12 h. Error bars represent 95% confidence intervals.

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.

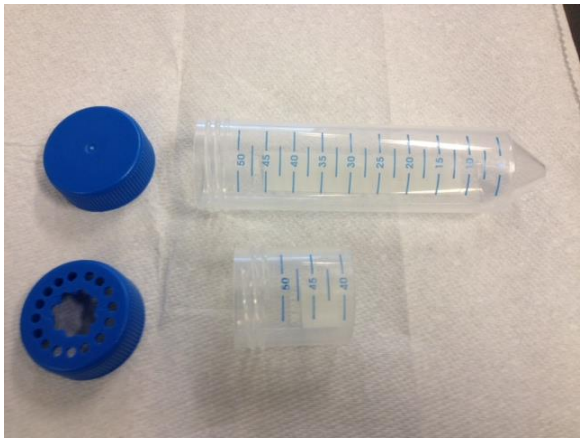

Figure S-1

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.

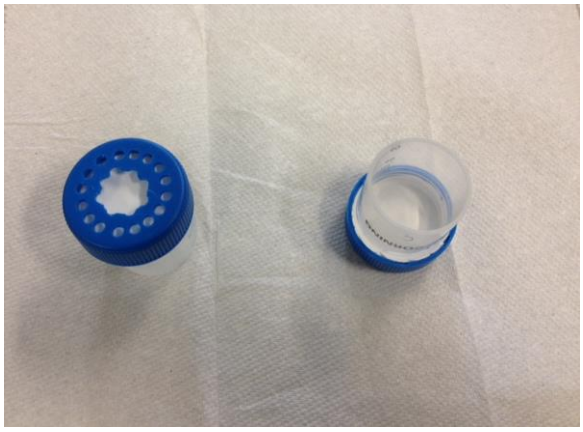

Figure S-2

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).

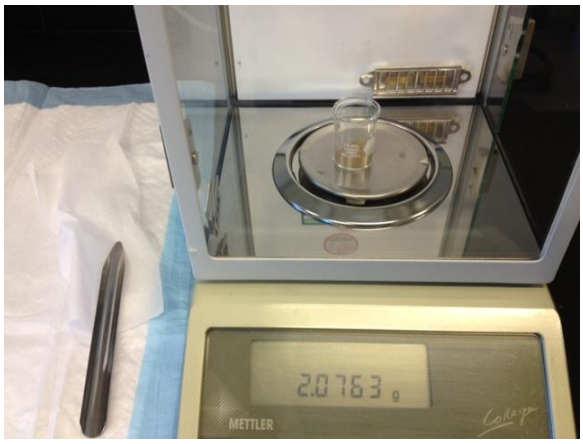

Figure S-3

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.

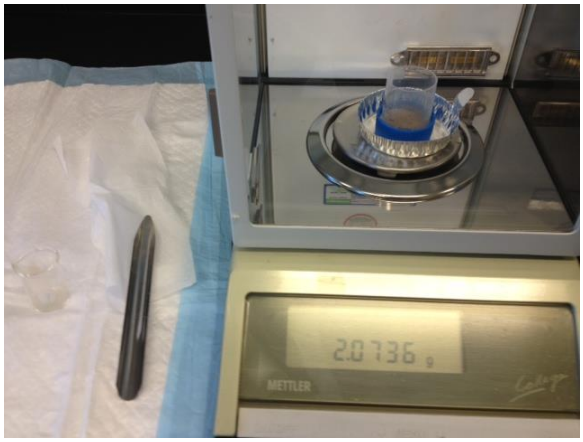

Figure S-4

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).

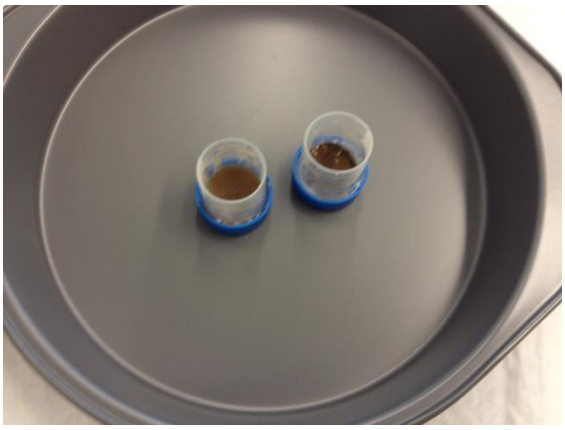

Figure S-5

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).

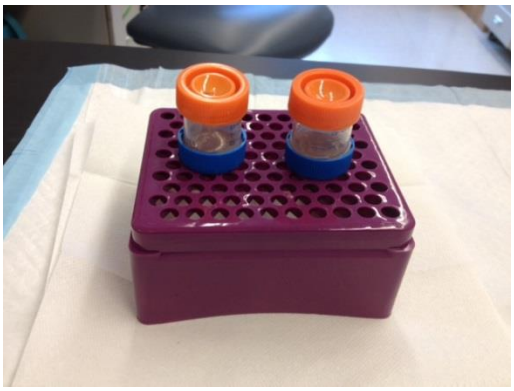

Figure S-8

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).

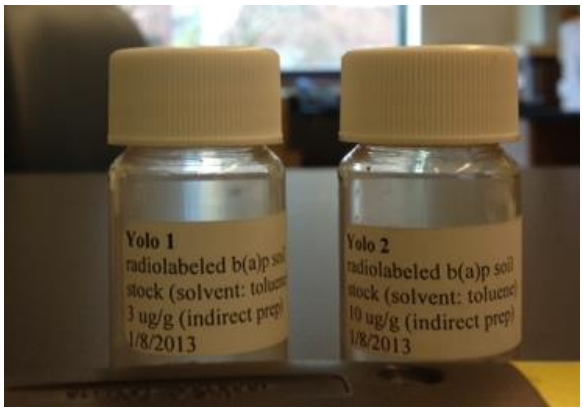

Figure S-9

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).

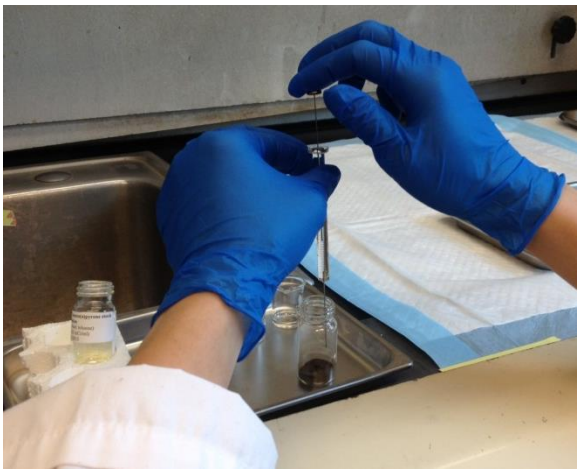

Figure S-10

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.

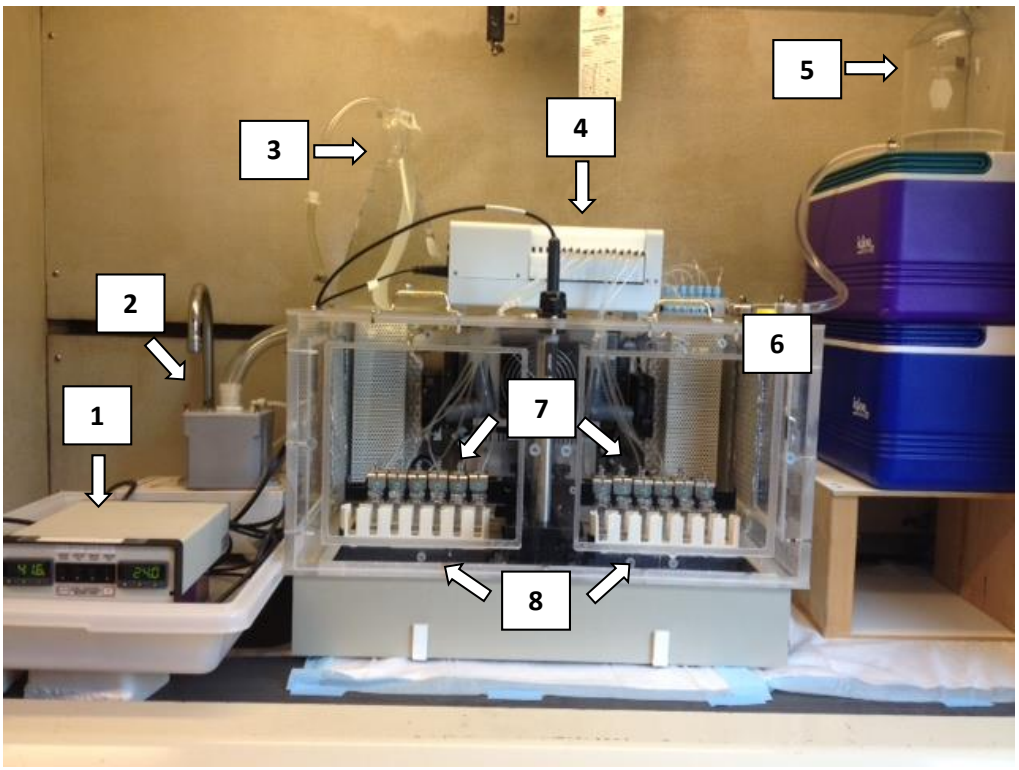

Figure S-13. Experimental apparatus for evaluating percutaneous absorption of chemical from soil. (1) Controller (heat, humidity, etc.); (2) humidifier; (3) humidifier reservoir; (4) peristaltic pump; (5) receptor fluid reservoir; (6) dermal chamber; (7) diffusion cell racks with diffusion cells connected 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).

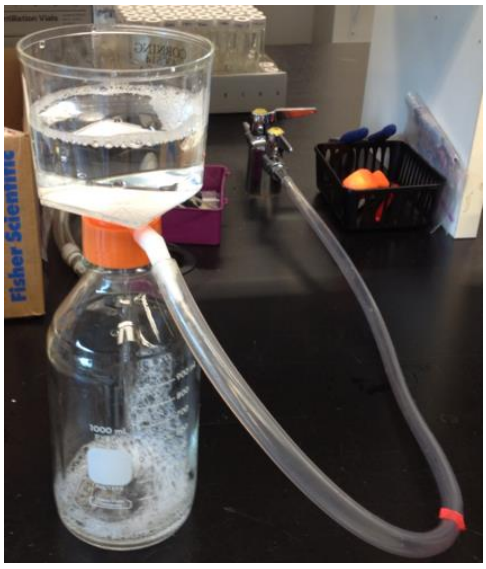

Figure S-14

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).

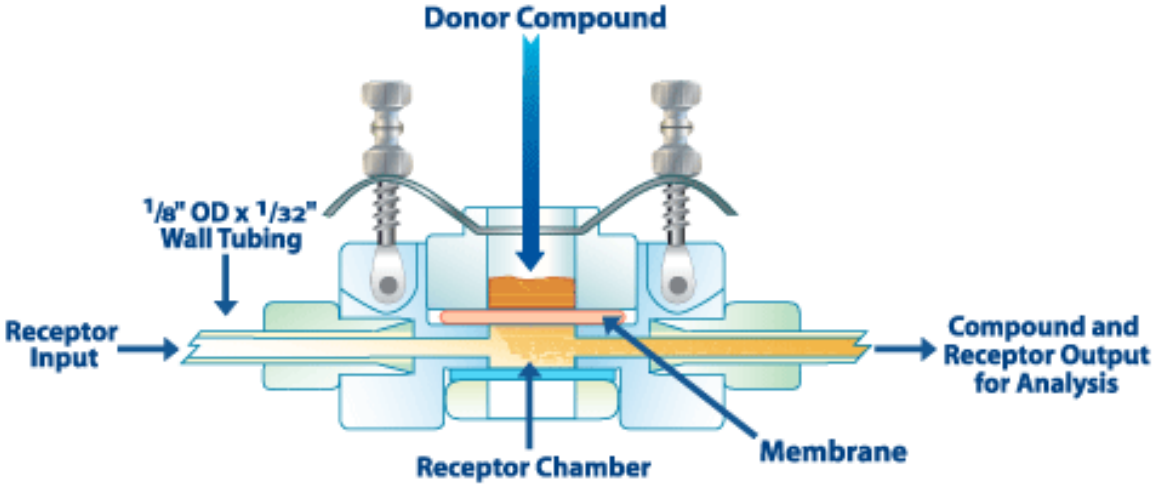

Figure S-15 **(http://www.permegear.com/inline.htm)**

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).

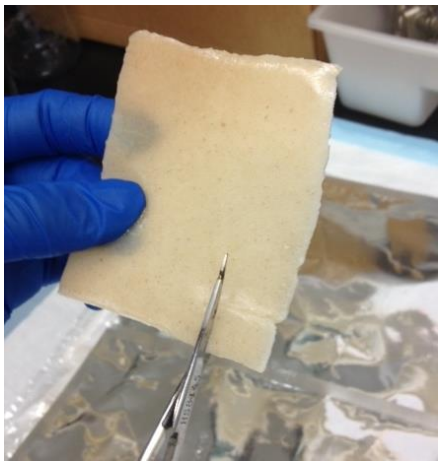

Figure S-16

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).

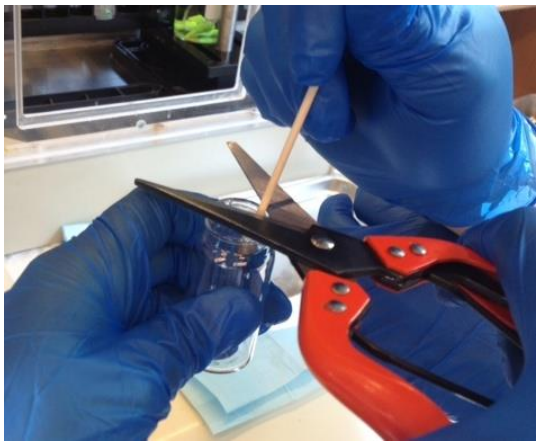

Figure S-19

   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).

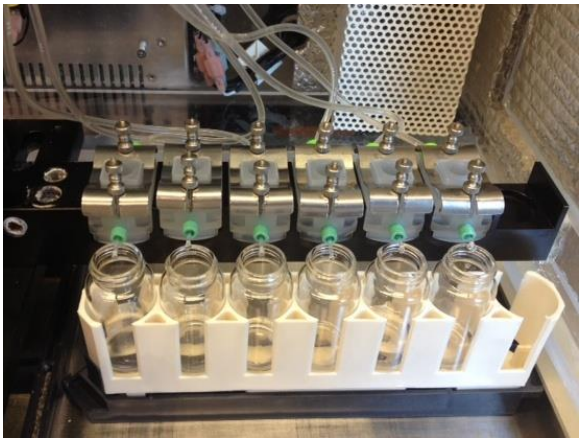

Figure S-20

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#).

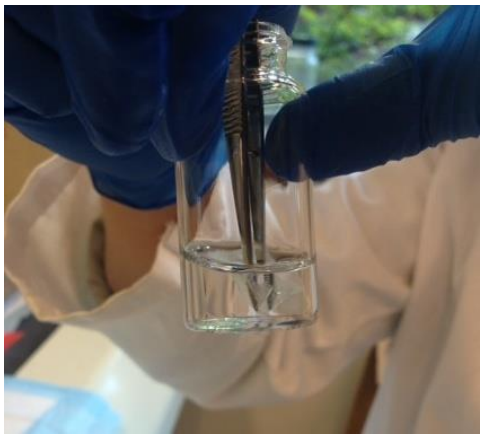

Figure S-21

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.

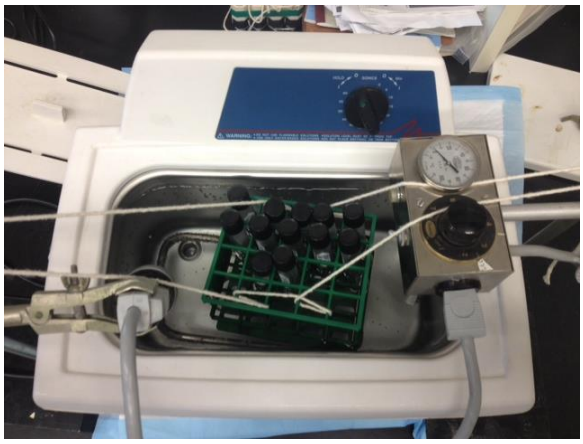

Figure S-23

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%.