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Effect of Soil Loading and Soil Sequestration on Dermal Bioavailability of Polynuclear Aromatic Hydrocarbons

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An estimation of polynuclear aromatic hydrocarbon (PAH) dermal bioavailability from PAH-contaminated soils is critical in assessing human health risks associated with dermal exposure. *In vitro* percutaneous absorption studies using intact human skin is one way to estimate bioavailability in dermal exposures (Roy et al., 1992; Wester et al., 1990; Kardy et al., 1995; Bronaugh 1998). *In vivo*, chemicals permeate the skin's diffusional barriers and enter the systemic circulation via capillaries at the dermo-epidermal junction (re: Figure 1). Percutaneous absorption begins with diffusion through the non-viable stratum corneum (SC). Diffusion through the SC is the rate-limiting step in the percutaneous absorption process for the vast majority of chemicals. Therefore, *in vitro* methods using non-viable skin can provide data which adequately reflect those from *in vivo* experiments. Fick's first law of diffusion is used to relate the flux (J) of a chemical through the skin under "infinite dose" (i.e., concentration differential across the membrane equals zero over time), steady-state conditions. Flux is directly proportional to chemical concentration and the skin/vehicle partition coefficient. For the special case of contaminated soils, the general term K_p becomes $K_{s/soil}$, the skin/soil partition coefficient (see equation in Figure 1). *In vitro* dermal absorption studies often use diffusion cells such as the one shown in Figure 2. Dermal flux is determined by calculating the slope of cumulative absorption of chemicals in the solution chamber over time.

In a recent study (Roy et al., 1998a), *in vitro* dermal penetration experiments were carried out to determine the dermal penetration properties of PAH in manufactured gas plant (MGP) tar contaminated soils and compare the measured dermal flux rates with those for the same PAH in the soil extracts. Results showed that sorption on soil retarded the dermal penetration rate of PAH by a factor of 160-900. The study further showed that skin penetration rate reductions of nearly 30-fold could be attributed to soil binding effects.

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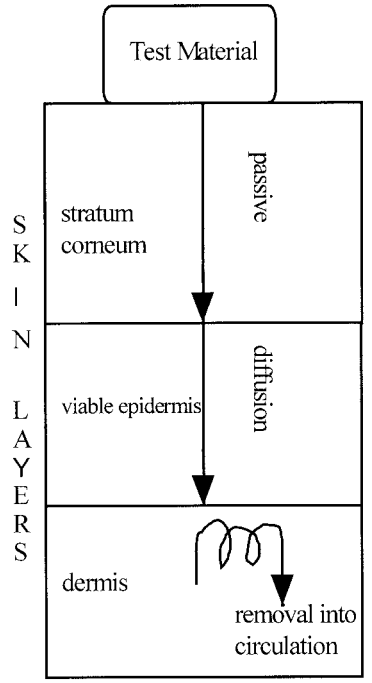


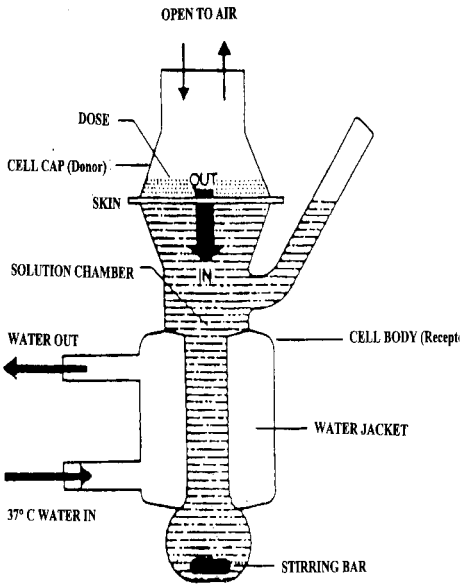


Figure 2. Diffusion cell schematic.

Dermal Flux = J = $DK_p C/h$

Where:

D = effective diffusion coefficient of chemical in SC

K_p = partition coefficient of chemical between skin & vehicle

C = concentration of chemical in vehicle

h = effective diffusion path length through the skin barrier

Figure 1. Skin schematic.

Two criticisms of the *in vitro* procedure used by Roy et al. (1998a) are 1) the "infinite dose" soil loading used (10 mg/cm^2) was well above what some consider the actual soil adherence factor (AF) of 0.2 mg/cm^2 and, 2) the procedure did not account for the effect of contaminant aging or soil sequestration phenomena (Chiou, 1989; Alexander, 1995; Alexander and Kelsey, 1997). To address these concerns, *in vitro* experiments have been carried out using the Roy et al. (1998a) procedure with human skin sections and PAH-spiked soil at 10, 5, 2.5 and 1 mg/cm^2 to evaluate the effect of soil loading on dermal flux and percent of applied dose absorbed (PADA). Additionally, dermal experiments were conducted with the freshly spiked (PAH) soil (day 1) and with aliquots of the same soil following 45 and 110 days of laboratory aging. Dermal flux of benzo[a]pyrene (BaP) and radiolabeled BAP, which provides an estimate of total PAH flux (Roy et al., 1998a,b), was measured using liquid scintillation counting and a newly developed HPLC-fluorescence assay for monitoring PAH in aged soil.

MATERIALS AND METHODS

Standard Reference Material (SRM) 1597 is a complex mixture of PAH derived from a medium crude coke oven tar and is available from the National Institute of Standards and Technology (NIST - Gaithersburg, MD). ^{3}H-BaP (65 Ci/m*M*) and ^{3}H-H_2O (1mCi/mL) were purchased from Amersham Life Science (Arlington Heights, IL) and NEN research products, (Wilmington, DE), respectively.

A field soil was sieved to <150 μm and extracted with methylene chloride using EPA SW-846 Method 3540B (Soxhlet extraction). The total organic carbon content of the extracted soil was 0.43%. Soil was spiked with the coal tar (SRM 1597) to achieve a final soil BaP concentration of 65 ppm. Aliquots of the soil were further spiked with trace levels of ^{3}H-BaP on days 1, 45 and 110 of the study. Several one gram aliquots of the coal tar spiked- and coal tar/^{3}H-BaP-spiked soil were brought to 80% field capacity moisture with filtered/deionized water in Teflon-lined screw top vials and stored at 20°C in the dark to allow for aging.

In vitro dermal penetration experiments were performed using abdominal skin from human cadavers procured from the National Disease Research Interchange (NDRI - Philadelphia, PA,). Skin sections were sliced to approximately 350 μm and mounted over 15 mm diameter Franz diffusion cells. The diffusion cells were contained in a Franz diffusion cell unit. An aqueous solution of 6% polyethylene glycol 20 oleyl ether (Volpo-20™) was used as receptor fluid. Mounted skin sections were dosed at 10, 5.0, 2.5 and 1.0 mg of soil/cm^{2} for the soil adherence study and at 10 soil/cm^{2} for the soil aging study. Receptor fluid was sampled at 1, 3, 5, 7, 24, 48, 72 and 96 hr post-dose.

Radioactivity (^{3}H-BaP) in the receptor fluid was quantitated using a Beckman LS 5000 liquid scintillation counter. Non-labeled, coal tar-derived BaP in the receptor fluid was analyzed by HPLC-fluorescence using a Hewlett Packard 1050 HPLC equipped with a Shimadzu fluorescence detector. The unit operating conditions were as follows: Column: VYDAC C_{18} 250 x 4.6 mm i.d., 5μm; Mobil phase: gradient – acetonitrile/water; Flow rate: 1.5 mL/min; Injection volume: 100 μL; Emissionλ: 427 nm; Excitationλ: 385 nm

RESULTS AND DISCUSSION

The impact of soil loading on PADA can be seen in Figure 3. Visually, we estimate monolayer coverage for human abdominal tissue to be approximately 3 mg/cm^{2} with (dry) <150 μm soil. Both the 10 and 5 mg/cm^{2} coverage can be considered "infinite dose" situations – halving the dose (10 → 5 mg) doubles the PADA (2 → 4 mg) which also suggests that all the BAP partitioning from the soil to the skin is contained in the monolayer of soil in intimate contact with the

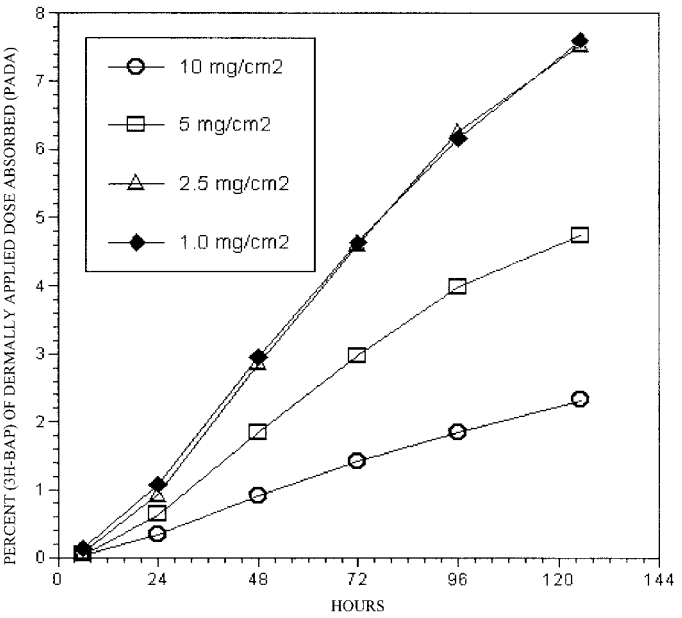


Figure 3. Impact of soil loading on percent dose of absorbed (PADA).

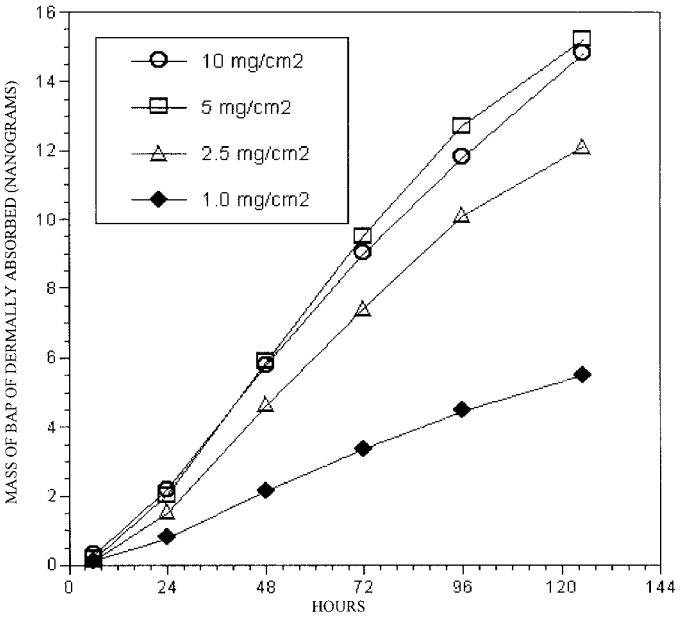
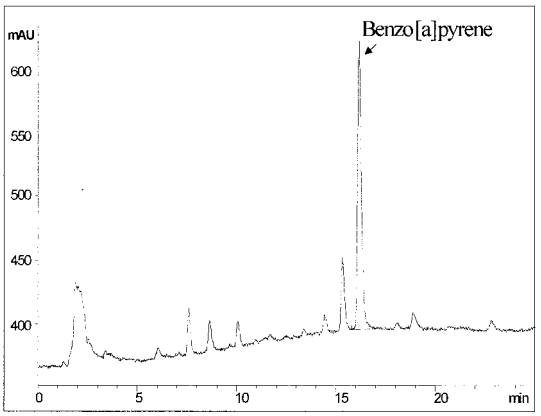


Figure 4. Impact of soil loading on dermal flux.



TIME (MINUTES)

Figure 5. Chromatogram BaP in receptor fluid (6% aqueous PEG 20 oleyl ether).

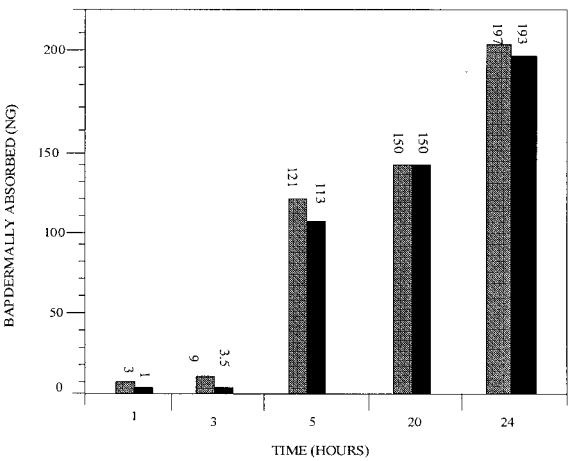
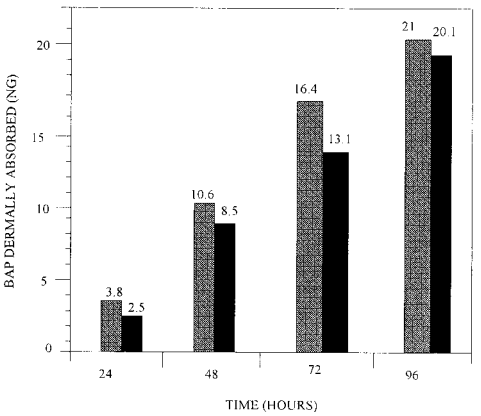
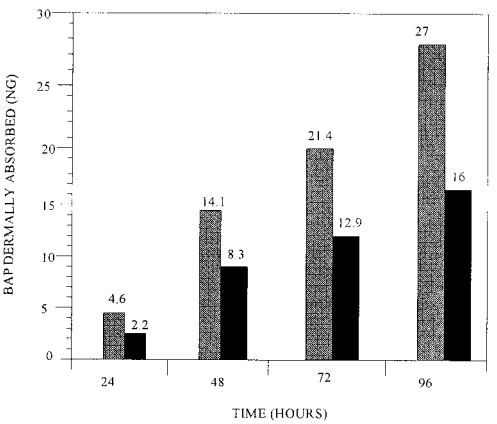


Figure 6. *In Vitro* percutaneous absorption of NIST coal tar through human skin: comparison of liquid scintillation (gray) & HPLC/Fluorescence (black) endpoints monitoring ^{3}H-BAP & BAP.

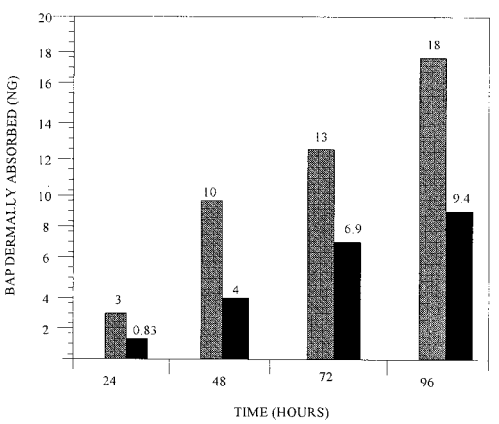
(7a) DAY 1



(7b) DAY 45



(7c) DAY 110



Figures 7a - 7c. Coal tar-contaminated soil aging experiment. Gray = freshly spiked ^{3}H-BaP. Black = HPLC/FL "cold" or "endogenous" BaP.

skin surface. The data clearly show that PADA has to be adjusted to soil coverage. Both the 2.5 and 1.0 mg/cm^2 soil loadings are less than monolayer coverage. The data support the predictions of Kissel and McAvoy (1988; 1989) and earlier key experimental work by Duff and Kissel (1996) that PADA remains constant at sub-monolayer soil coverage, i.e., the total mass of contaminant absorbed decreases proportionately with decreasing soil loading. The impact of soil loading on dermal flux (J) can be seen in Figure 4. Flux is not affected by soil loading above monolayer (5 & 10 mg/cm^2) and decreases in proportion to soil loading below monolayer (1 & 2.5 mg/cm^2).

Figure 5 is an HPLC/fluorscence chromatogram of receptor fluid from the *in vitro* dermal coal tar experiment. BaP is easily detected in the presence of the other coal tar components; the BaP peak in the figure represents ~100 picograms. The method detection limit is 10 pg/100 µL. The bar chart in Figure 6 compares the mass of BaP in neat NIST coal tar penetrating through the skin, overtime, as measured by the two analytical techniques – liquid scintillation counting of tracer ^{3}H-BaP (gray) and HPLC-fluorescence (black).

Figures 7a, 7b and 7c compare the mass absorbed/time values for BaP from soil aged for 1, 45 and 110 days based on: 1) the freshly spiked ^{3}H-BaP tracer (gray) and 2) HPLC-fluorescence analysis of the unlabeled BaP originally spiked (as NIST coal tar extract) in the soil on day 1 (black). The data show that sorption on soil decreases the dermal flux of coal tar BaP by a factor of 10. Most of the reduction is a consequence of concentration (re: Fick's Law - Figure 1) but, approximately 30% of the reduction is due to the initial rapid or "labile" (Linz and Nakles, 1997) sorption of coal tar on soil. The data also show that the labile sorbed ^{3}H-BaP accurately reflects the flux of labile sorbed coal tar BaP at day 1 in the neat coal tar and coal tar-contaminated dermal experiments (Figures 6 & 7a). However, as a result of aging, the coal tar BaP becomes more sequestered (non-labile) and less dermally bioavailable. By day 110, the bioavailability of the coal tar-derived BaP is only half of that for the freshly spiked (labile) ^{3}H-BaP (re: Figures 7a, 7b, and 7c).

REFERENCES

Alexander M (1995) How toxic are toxic chemicals in soil? Environ Sci Technol 29: 2713-2717

Alexander M, Kelsey JW (1997) Declining bioavailability and inappropriate estimation of risk of persistent compounds. Environ Toxicol Chem 16:582-585

Bronaugh RL (1998) Current issues in the *in vitro* measurement of percutaneous absorption. In: Roberts MS, Walters KA (ed) Dermal Absorption and Toxicity Assessment. Marcel Dekker, New York, p 155

Chiou CT (1989) Reactions and movement of organic chemicals in soils. Soil Sci Soc America J: 1-29

Duff RM, Kissel JC (1996) Effect of soil loading on dermal absorption efficiency from contaminated soils. J Toxicol Environ Health 48:83-106

between oral and dermal bioavailability of soil-sorbed phenanthrene in female rats. Toxicol Lett 78:153-163

Kissel JC, MacAvoy DR (1988) Estimating dermal exposure from soil using the fugacity approach. Proc. 9th National Conf Hazardous Materials Control Research Institute: pp 142-144. Washington, DC: HMCRI

Kissel JC, MacAvoy DR (1989) Reevaluation of the dermal bioavailability of 2,3,7,8-TCDD in soil. Haz Waste Haz Material 6:231-240

Roy TA, Yang JJ, Krueger AJ, Mackerer CR (1992) *In vitro* percutaneous absorption of benzo[a]pyrene (BaP) from crude oil sorbed on soil using rat and human skin (abstract). The Toxicologist 12:114

Roy TA, Blackburn GR, Mackerer CR (1996) Evaluation of physicochemical factors affecting dermal penetration and carcinogenic potential of mineral oils containing polycyclic aromatic compounds. Polycyclic Aromatic Compounds 10:333-342

Roy TA, Krueger AJ, Taylor BB, Mauro DM, Goldstein LS (1998a) Studies Estimating the dermal bioavailability of polynuclear aromatic hydrocarbons from manufactured gas plant tar-contaminated soils. Environ Sci Technol 32: 3113-3117

Roy TA, Neil W, Yang JJ, Krueger AJ, Arroyo AM, Mackerer CR (1998b) SAR models for estimating the percutaneous absorption of polynuclear aromatic hydrocarbons. SAR /QSAR Environ Res 9:171-185

Wester RC, Maibach HI, Bucks DA, Sedik L, Mellendres J, Liao C, Dizio S (1990) Percutaneous absorption of [14C]DDT and [14C]benzo[a]pyrene from soil. Fundam Appl Toxicol 15:510-516 9