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In vitro and *In vivo* Percutaneous Absorption of Benzo[a]pyrene from Petroleum Crude–Fortified Soil in the Rat

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Concern regarding dermal exposure to soils contaminated with potentially toxic materials such as dioxins (Poiger and Schlatter 1980; Shu et al. 1988), pesticides, heavy metals, polynuclear aromatics (PNA) and petroleum products containing PNA, has prompted government and private sectors to examine and formulate dermal risk assessment methodologies for contaminated soils (Kimbrough et al. 1984; Schaum 1984; Hawley 1985; Calabrese et al. 1986; Paustenbach et al. 1986; Ryan et al. 1987). In the absence of experimentally determined percutaneous absorption values, many risk assessment schemes substitute available animal toxicology data on the pure contaminant, estimate dermal penetration of pure contaminant based on physicochemical models or, in the extreme, assume 100% bioavailability of the contaminant from soil (U.S. EPA 1986). Risk assessment based on these approaches is likely to result in an overestimate of dermal bioavailability of contaminants from soil, particularly for the water insoluble lipophilic compounds found in petroleum products.

Our laboratory has performed dermal bioavailability studies on a variety of lipophilic materials (Yang et al. 1987, 1988) and special emphasis has been placed on the development and implementation of a modified *in vitro* technique (Bronaugh and Stewart 1984) for evaluating the percutaneous absorption of PNA and PNA mixtures (Yang et al. 1986a,b; Roy et al. 1987). In the present paper we apply the modified *in vitro* technique to evaluate the percutaneous absorption of benzo[a]pyrene (BaP) in petroleum crude oil sorbed on soil and compare the results with those for the percutaneous absorption of BaP from crude oil alone. Results of the *in vitro* experiments are compared with findings from parallel *in vivo* experiments.

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MATERIALS AND METHODS

[1,3,6-^{3}H]-BaP and [G-^{3}H]-BaP, with specific activities of 30-70 mCi/mmol, were purchased from New England Nuclear (Wilmington, DE) and Amersham (Arlington Heights, IL), respectively. SRM 1582, a petroleum crude oil containing 1.1 ppm of native BaP, was obtained from the National Bureau of Standards (Gaithersburg, MD) and spiked with ^{3}H-BaP at 100 ppm. Reagent grade acetone and dichloromethane were purchased from Baker Chemical (Phillpsburg, NJ), carbon disulfide and thimerosal from Aldrich Chemical (Milwaukee, WI) and Volpo-20, a nonionic surfactant, from Croda (New York, NY). An air-dried loam soil was kindly provided by Dr. Harry L. Motto of the Soil Testing Laboratory, New Jersey Agricultural Experimental Station at Rutgers University, New Brunswick, NJ. The soil property data is presented below:

Table 1. Soil Properties

Cation Exchange Capacity	9.97 meq/100 g
Organic Content	1.64%
Sand	46%
Silt	36%
Clay	18%
Texture	Loam
pH	6.0

Soil was sieved and the fraction with particle size of <150 μm were used to prepare the crude-fortified soil for percutaneous absorption experiments. Typically, the soil preparation involved adding the crude, in 10 mL dichloromethane, per gram of soil followed by removal of solvents on a rotary evaporator. Radioactivity in the crude was counted directly in a Beckman LS 9000 or LS 5801 liquid scintillation counter after addition of scintillation cocktail (Beckman HP/b). Radioactivity in 1% (w/w) crude-fortified soil was determined by either a combustion method using an oxidizer (Packard Instruments, Downers Grove, IL) or an extraction method using dichloromethane. Radioactivity in tritiated water, which was trapped in the cocktail after sample combustion, was counted directly. The dichloromethane extract or its concentrate was mixed with the cocktail prior to counting. The results obtained from these two methods were comparable. All prepared soils were consumed within 72 hr or discarded.

Female Sprague-Dawley rats (Taconic Farms, Germantown, NY), 3-6 months old, were housed individually in wire mesh cages prior to treatment. Animal rooms were set to maintain 20°C and 50% relative humidity with a 12-hr light-dark cycle. Food (Lab Chows, Ralston Purina, St. Louis, MO) and water were provided *ad libitum*.

In the *in vitro* experiments, the rats were sacrificed by exposure to 100% carbon dioxide. Dorsal skin was lightly shaved with an electric clipper before excision. Skin sections (~350 μm) were prepared from full-thickness skin with a Padgett dermatome (Kansas City, MO) and covered in saline-treated gauze before use within 2 hr. The thickness of the sections was measured with a pressure sensitive micrometer.

An aqueous solution of 6% Volpo-20 and 0.01% thimerosal antibacterial agent was used as the receptor fluid. Consoles containing 15 mm diameter Franz diffusion cells (Crown Glass, Somerville, NJ) were used in the experiments. The crude was administered in 70-145 μL acetone-carbon disulfide (1:1, v/v) to skin on the donor side of the diffusion cell, followed by evaporation of the vehicle with nitrogen or air. The 1% crude-fortified soil was weighed and applied evenly on the skin surface. In the experiment with a minimal amount ("monolayer") of soil coverage on the skin surface (9 mg/cm^2), the excess soil was removed by gentle tapping of the inverted cell and weighed to obtain the exact amount applied. The temperature of the diffusion cells was maintained at 37°C by attaching the water-jacketed cells to a circulating water bath. After dosing, the receptor fluid in each cell was sampled (100 μL duplicates) once every 24 hr for four days. Radioactivity in the receptor fluid was counted directly after addition of cocktail.

In the in vivo experiments, the dorsal area of each rat was lightly shaved after mild anesthesia. Appropriate amounts of crude alone or 1% crude-fortified soil were applied over a 7 cm^2 skin surface area. The amounts administered per sq cm of skin surface in the parallel in vivo and in vitro experiments were equivalent. The dosed area was covered with a non-occlusive glass cell specifically designed for this study (Krueger and Yang, in preparation). The cell was attached to the skin with an epoxy adhesive and further secured with Elastoplast® tape (Beiersdorf, Norwalk, CT). The dosed animals were individually housed in Nalgene metabolism cages and offered food and water ad libitum. Urine and feces were collected once daily for four days. At the termination of the experiment, samples of liver, kidney, small and large intestine, stomach, bladder and blood were collected from each animal. Radioactivity in the urine, feces, blood and tissue samples was determined by methods reported previously (Yang et al. 1986b, 1987).

A Student's t-test ($p<0.05$) was used to compare the various percutaneous absorption data. Linear regression analysis of the absorption data was carried out using SAS (Cary, NC).

RESULTS AND DISCUSSION

Preliminary experiments to determine the minimum amount of soil adhering to the skin were conducted using the in vitro method. Close examination of the skin sections after removal of excess soil indicated that it was largely the silt and clay fractions (<50 μm particle size) that remained on the skin surface. It is in these fine fractions that the bulk of the soil organic carbon content resides (Brady 1984). Karickhoff et al. (1979) have shown that the soil organic carbon is the dominant sorbent for lipophilic compounds and that the silt and clay fractions are considerably more effective sorbents than the sand fraction (>50 μm particles). Indeed, we observed similar results when attempting to uniformly sorb crude on the native loam soil. Subsequent sieving of the fortified soil revealed that a majority of the BaP radioactivity resided in the soil fraction with particle size of <150 μm (data not shown).

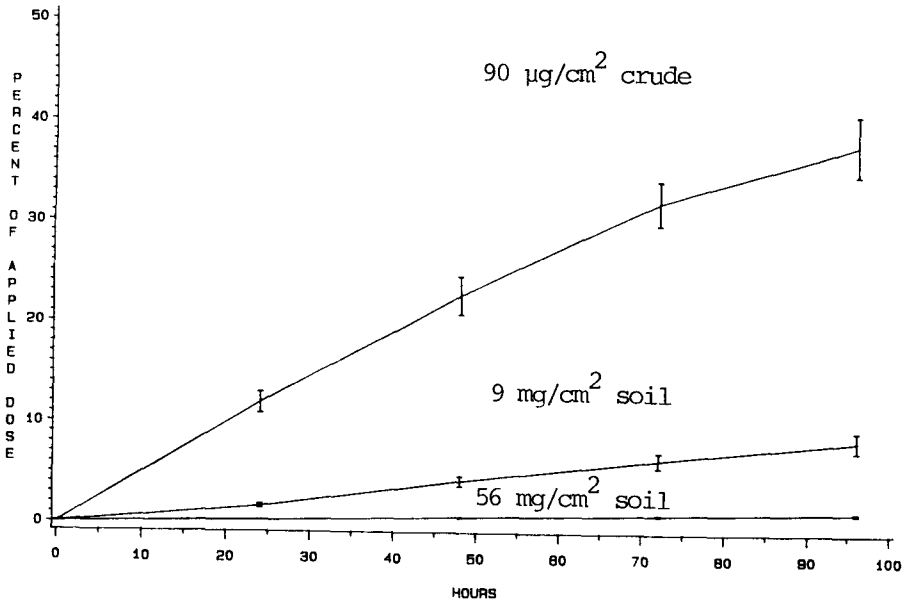

Figure 1. In vitro percutaneous absorption of BaP from crude alone and 1% crude-fortified soil (mean ± SE for n=5 per time point)

Based on the results above, percutaneous absorption experiments with the crude-sorbed soil were carried out exclusively with soil particles of <150 µm to represent the composition of the soil adhering to the skin surface. Approximately 9 mg/cm^2 of soil was found to be the minimum amount required for a "monolayer" coverage of the skin surface in both in vitro and in vivo experiments. This value is larger than the <1 mg/cm^2 of soil (dust) reported for human skin (Lepow et al. 1975; Roels et al. 1980; Que Hee et al. 1985). The differences between the rat and human soil adhesion findings may result from differences in rat and human skin texture, the types of soils used, soil moisture content or possibly the methods of measuring soil adhesion.

A comparison of the two in vitro percutaneous absorption experiments with 1% crude-fortified soil is shown in Figure 1. The percentage of BaP absorbed (96 hr) was 8.4 and 1.3% at exposure levels of 9 ("monolayer") and 56 mg/cm^2, respectively. These experiments were designed to evaluate the effect of excess soil (i.e., more than a "monolayer") on the dermal bioavailability of crude-sorbed soil. The results show that a much lower percentage of BaP was absorbed from the larger dose even though both doses contain the same concentration of BaP (1 ppm). The actual amount of BaP absorbed from 9 and 56 mg/cm^2 of the fortified soil was nearly identical (1.3 ng). A parallel in vitro experiment was conducted with 90 µg/cm^2 of crude alone which is the same amount of crude contained in the 9 mg/cm^2 crude-fortified soil dose. In the absence of the soil matrix, approximately 38.1% of the BaP was dermally absorbed in 96 hr.

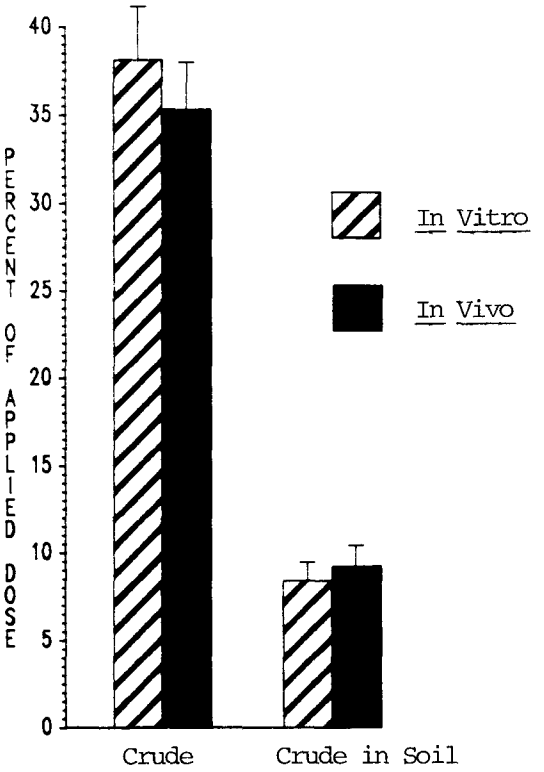

Figure 2. A comparison of 96-hr in vitro and in vivo percutaneous absorption of BaP from crude alone and 1% crude-fortified soil (mean ± SE for n=5 per experiment)

Very good correlation was observed between the in vivo (Tables 2 and 3) and in vitro data (Figure 1) for dermal bioavailability of BaP. A comparison of the results from percutaneous absorption experiments with crude alone (90 $\mu g/cm^2$) and 1% crude-fortified soil (9 mg/cm^2) is shown in Figure 2. No statistical difference between the in vitro and in vivo results was observed. The correlation supports the earlier results of Yang et al. (1986b) which showed that in vitro techniques using nonviable skin sections closely mimic the in vivo percutaneous absorption of neat BaP. The present results indicate that the in vitro method will be useful for evaluating dermal bioavailability of PNA and other lipophilic compounds in a soil matrix.

The in vivo and in vitro experiments with the "monolayer" of 9 mg/cm^2 of 1% soil-sorbed crude and 90 $\mu g/cm^2$ of crude alone were intended to compare the absorption of equal amounts of BaP from either the oil matrix or the 1% oil-in-soil matrix. The results (re: Figure 2) show that the absorption of BaP from the crude alone is 4-5 times higher than from the soil-sorbed crude.

Table 2. In vivo percutaneous absorption of BaP from petroleum crude[a] in female Sprague-Dawley rats at a dose of 90 $\mu g/cm^2$ of skin surface

Hours after dosing	% Applied dose recovered[b]			
	Urine	Feces	Tissues	Total
24	1.6 (0.3)	3.9 (1.2)	-	5.5 (1.4)
48	3.2 (0.5)	16.9 (1.7)	-	20.1 (2.1)
72	4.3 (0.6)	23.3 (1.8)	-	27.6 (2.1)
96	5.3 (0.6)	27.5 (2.0)	2.5 (0.4)	35.3 (2.6)

[a] The petroleum crude (NBS SRM 1582) was fortified with 3H-BaP at 100 ppm.

[b] Values shown for 48, 72 and 96 hr are cumulative. Results are expressed as the mean (SE) for five rats.

Table 3. In vivo percutaneous absorption of BaP from petroleum crude-fortified soil[a] in female Sprague-Dawley rats at a dose of 9 mg/cm^2 of skin surface

Hours after dosing	% Applied dose recovered[b]			
	Urine	Feces	Tissues	Total
24	0.4 (0.1)	0.7 (0.2)	-	1.1 (0.3)
48	0.8 (0.2)	2.8 (0.6)	-	3.7 (0.8)
72	1.4 (0.2)	4.4 (0.9)	-	5.8 (1.0)
96	1.9 (0.2)	5.8 (1.0)	1.5 (0.2)	9.2 (1.2)

[a] The petroleum crude (NBS SRM 1582) was fortified with ^{3}H-BaP at 100 ppm. The final concentration of BaP in soil was 1 ppm.

[b] Values shown for 48, 72 and 96 hr are cumulative. Results are expressed as the mean (SE) for five rats.

Based on the in vitro and in vivo findings from the fortified soil experiments (re: Figure 1 and Table 3), the rate of dermal uptake for BaP (at 1 ppm) from a 1% crude-fortified soil matrix was estimated to be 0.2 ng/cm^2/day. In a review of limited comparative in vivo data from human and rat, Wester and Maibach (1987) noted that percutaneous absorption of lipohilic compounds is generally greater in the rat. Thus, it is reasonable to expect that the absorption rate of BaP in crude-fortified soil through human skin will be significantly lower under similar conditions.

The soil-sorbed crude experimental results showed a significant reduction in the rate of BaP skin penetration when compared with the crude-only experiments. The rate of BaP absorption observed from soil was so low that it remained constant (0.2 ng/cm^2/day) over the 96-hr experiment. The constant slope for both the 9 and 56 mg/cm^2 doses indicates that the small amount of BaP absorbed was entirely derived from the "monolayer" of soil in direct contact with the skin. The amount of BaP absorbed represents less than 10% of the BaP contained in the "monolayer". Under the conditions of the present study, the degree of soil binding of BaP impedes the movement of BaP into skin to the extent that the migration of BaP from layers of soil above the "monolayer' is negligible.

The results indicate that the percutaneous absorption of BaP and therefore, other PNA from petroleum crude-contaminated soil will be significantly less than the percutaneous absorption of these compounds from crude alone. The results suggest that only the "monolayer" of soil in intimate contact with the skin surface (rather than the total amount of soil on the skin) is important in the determination of risk associated with dermal exposure to soils contaminated with petroleum components.

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