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Evaluation of a Rat Model versus a Physiologically Based Extraction Test for Assessing Phenanthrene Bioavailability from Soils

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The soil matrix can impact the bioavailability of soil-bound organic chemicals, and this impact is governed in part by soil properties such as organic carbon (OC) content, clay minerals, and pH. Recently, a physiologically based extraction test (PBET) was developed to predict the bioavailability of soil-bound organic chemicals. In the current study, the bioavailability of phenanthrene (PA) from laboratory-treated soils varying in OC content, clay, and pH was investigated using an *in vivo* rat model and an *in vitro* PBET. The relationship between these two approaches was also examined. In the *in vivo* assay, soils and corn oil containing equivalent levels of PA were administered to Sprague-Dawley rats by gavage at two dose levels: 400 and 800 mg/kg body weight. Equivalent doses were given via intravenous injection (iv). The areas under the blood concentration-versus-time curves (AUC) were measured, and the absolute and relative bioavailabilities of PA were determined for each soil. In the PBET tests, one g of each soil was extracted by artificial saliva, gastric juice, duodenum juice, and bile. The fraction of PA mobilized from each soil was quantified. The AUCs of PA in all soils were significantly lower than those following iv injection ($p < 0.05$), indicating that the soil matrix could reduce the bioavailability of PA from soil. There were obvious trends of soils with higher OC content and clay content, resulting in the lower bioavailability of PA from soil. A significant correlation ($p < 0.05$) was observed between the fraction of PA mobilized from soil in the PBET and its *in vivo* bioavailability. The data also showed that the absolute bioavailability of PA from corn oil was low: approximately 25%. These results suggest that PBET assay might be a useful alternative in predicting bioavailability of soil-bound organic chemicals. However, due to the limited soil types and use of one chemical vs. a variety of contaminants and soil properties in the environment, further efforts involving more chemicals and soil types are needed to validate this surrogate method.

Key Words: **phenanthrene; bioavailability; soil; physiologically based extraction test.**

Oral intake of soil contaminants may present a serious health risk for children, especially for those with soil-pica behavior. (Ingestion of soil in amounts for exceeding those observed in the average child.) An epidemiological study demonstrated that the median soil intake by children ranged from 9 to 96 mg/day (Calabrese *et al.*, 1989). It also reported that the daily intake of soil by a 3.5-year-old female with soil-pica behavior ranged from 10 to 13 g/day (Calabrese *et al.*, 1991). Soil ingestion can also occur in adults. An average intake of up to 50 mg/day has been estimated for adults (Calabrese *et al.*, 1990).

Although there is little debate about the need to avoid adverse impacts to human health and the environment from soil contamination, there are questions about the extent and magnitude of the risk posed by organic chemicals in soil, as well as the cleanup goals required (Loehr, 1996). Evidence has accumulated during the last two decades that only a fraction of any chemical in soil is available to organisms, including humans (Alexander, 1995 and 2000; NEPI, 2000). A number of studies have demonstrated that the soil matrix can reduce the bioavailability of organic chemicals such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), some polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs), etc. (Fries and Marrow, 1992; Poiger and Schlatter, 1980; Schooten *et al.*, 1997; Shu *et al.*, 1988). To accurately analyze the risk to human health and determine the cleanup levels necessary in soil remediation, soil-related effects on the bioavailability of chemicals must be considered. Due to the lack of data regarding the impact of the soil matrix on the bioavailability of many organic contaminants, regulatory agencies often assume 100% bioavailability, i.e., the assumption is made that the absorption of those chemicals from soil is equal to that from the carrier used in the critical study. Studies noted above suggest that such assumption may be incorrect, which might adversely impact prioritization in cleaning up waste sites.

Soil properties such as organic carbon content (OC), clay content, particle size, pH, and aging impact soil sorption and desorption processes, and thus may have significant impacts on the bioavailability of chemicals from soils (NEPI, 2000). Organic matter in soil is thought to be the most significant factor dominating organic compound interactions within soil, and thus the bioavailability of these compounds (Calvet, 1989).

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Sorption of organic chemicals by soils rises with increasing amounts of organic matter, and the greater the hydrophobicity of a chemical, the greater potential it has to be sorbed onto organic matter (Schwarzenbach *et al.*, 2003). Mass-transfer rates of organic chemicals from soil particles are inversely proportional to the chemical's soil-water distribution coefficient and are thus indirectly related to organic matter content (Pignatello, 2000). Clays, which typically have high surface areas, can enhance sorption through weak physical interactions and can impede chemical mass transfer due to clay aggregation and clay interlayers (Ake *et al.*, 2001). As a result, with increasing organic matter content and sometimes clay content, retention of an organic chemical increases and the rate of release decreases, potentially decreasing overall chemical availability.

In vivo assessment of bioavailability of soil-bound organic contaminants using animal models is straightforward, and results from these studies are relatively easy to interpret. The disadvantages of this approach are that it is expensive and time consuming, and needs to use animals. These disadvantages have led investigators to develop surrogate *in vitro* approaches to estimate the bioavailability of organic chemicals from soil, such as mild extraction with organic solvents and physiologically based extraction tests (PBET). These *in vitro* approaches are usually simple, cheap, fast, and reproducible without using animals. Among these approaches, PBET has promise. In the PBET assay, digestive juices based on human physiology are used to extract the soil contaminants, mimicking the varied physiological conditions in the digestive tract (Oomen *et al.*, 2003). The rationale behind this test is that, for a soil-bound chemical to be absorbed by organisms, it has to be released from the soil matrix and dissolve in water; i.e., becomes bioaccessible. Although some studies have demonstrated that PBET could be a useful tool to predict the bioavailability of organic chemicals from soil (Hack and Selenka, 1996; Jin *et al.*, 1999; Oomen *et al.*, 2001), there are very limited data on the relationships between this test and the *in vivo* approach. However, such information is essential for understanding, interpreting, and eventually applying the data resulting from these *in vitro* tests in order to predict the bioavailability of soil-bound contaminants— the ultimate goal in the development of these surrogate tests.

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants and have been identified in a variety of soils, natural waters, and wastewaters. Phenanthrene (PA), a three-ring PAH, is one of the major PAHs emitted in coal and fossil fuel combustion and in coal combustion wastes such as coal tar, which has contaminated soils at manufactured-gas plants globally (Laor *et al.*, 1999). Because of its widespread existence in the environment and its structural similarity to carcinogenic PAHs, PA has been used as a model substrate in many environmental studies. Like other hydrophobic organic compounds, one of the primary fates of PA in terrestrial and aquatic environments is sorption to natural organic matter (Salloum *et al.*, 2002). Such association between PA and organic matter in soil could limit the bioavailability to some extent, depending upon the OC content of the soil. In a sequestration study, Nam *et al.* (1998) found that the organic matter in soil was a major determinant of sequestration, but that other properties of soil might also contribute to a decrease in the availability of PA.

In the current study, soils with varying characteristics were selected to investigate the effect of soil properties on PA bioavailability, using a rat model and a PBET test. We examined the relationship between the two approaches.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Harlan, Indianapolis, IN), 60 to 90 days old and weighing 275 to 350 g, were used. Animals were housed individually in a room maintained at 25°C, 50% humidity, and on a 12-h light/dark cycle. The animals received Purina Laboratory Chow (No. 5001, Purina Mills, St. Louis, MO) and tap water *ad libitum*. All animals were allowed at least one week to adapt to the animal facility and diet before being used in the experiments.

Chemicals. Phenanthrene at >96% purity, anthracene at 99% purity, bile, pepsin, and mucin were purchased from Sigma Chemical Company (St. Louis, MO). All other agents were analytical grade.

Soil-sample preparation. Three uncontaminated surface soils (Milford, Toronto and Bloomfield) were collected from different sites in the state of Indiana, and one uncontaminated subsurface soil (Heiden) was collected from western Texas. These soils were air-dried and sieved through a 2-mm sieve. Only the fraction of particles <2 mm was used. The reason for using this fraction of soil was that this research was part of a larger multidisciplinary project involving other physicochemical and ecological tests, and the same soil types and chemicals were required to be used in all the tests. Selected soil properties obtained from Zhao (2002) are presented in Table 1.

Uncontaminated soils were treated with phenanthrene in aqueous slurries, using the procedure outlined by Zhao (2002) to achieve concentrations of approximately 200 and 400 mg PA/kg soil. The amount of chemical (M_i, mg) needed to achieve a target soil concentration was estimated by assuming mass balance and using a soil-water distribution coefficient (K_d, l/kg) as follows: $M_i = (C_s\ m_s) + (C_w\ V_w)$ where C_s (mg/kg) and C_w (mg/l) are the mass of chemical sorbed by the soil and in the aqueous phase at equilibrium, respectively; V_w (l) is the volume of the aqueous phase; and m_s (kg) is the soil mass. The K_d values for PA previously determined by Zhao (2002) for each of the four soils was used to estimate M_i for an m_s/V_w ratio of 0.5 to achieve the target concentrations. The predetermined amounts of phenanthrene (M_i) dissolved in ethanol were added to empty vessels, and the ethanol was evaporated. A 5 mM $CaCl_2$ solution and soil were added ($m_s/V_w = 0.5$), and the aqueous soil slurry was shaken for three days, followed by centrifugation. Final concentrations of phenanthrene in soil samples were determined by two sequential extractions for one h each of one g (dry weight) of soil. After each extraction, samples were centrifuged at 700 × *g* for 10 min and the supernatant then

TABLE 1
Soil Properties

Properties	Milford	Bloomfield	Heiden	Toronto
Organic C (%)	0.70	0.52	1.74	1.39
Clay (%)	36	8	38	20
pH	7.4	6.4	7.4	4.6

removed. PA in the combined supernatants was analyzed using a Shimadzu HPLC system with a UV detector at 254 nm, a Discovery C-18 reverse phase analytical column (4.6 × 250 mm, Supelco, Bellefonte, PA), a guard column (4.6 × 50 mm), and a 30/70 v/v water/acetonitrile mobile phase at a flow rate of 1 ml/min.

Evaluation of bioavailability. Both absolute and relative bioavailabilities were determined in this study using a rat model. Absolute bioavailability refers to the fraction of an extravascular dose that reaches the systemic circulation compared to the same dose administered intravenously. Relative bioavailability refers to comparative bioavailability of a chemical from different exposure media (e.g., bioavailability of a chemical from soil relative to its bioavailability from water or other carrier) (NEPI, 2000).

Groups of 4 rats were used to evaluate the bioavailability of PA from each soil at two dose levels, which were used in consideration of the possible effect of concentration on bioavailability. Animals were fasted overnight, prior to the administration of any test material, to decrease the possible impact of food on the absorption of PA. Immediately before dosing, soil samples were suspended in water and administered, as slurry, by gavage. A soil suspension containing 0.25 g soil/ml was administered in a volume of 8 ml/kg body weight (bw), resulting in two dose levels of 400 and 800 μg/kg bw. The same doses of PA in corn oil were given by gavage to determine the relative bioavailability. Equivalent doses were also given via intravenous injection (iv) via the tail vein to determine the absolute bioavailability. Because the aqueous solubility of PA is very low, the iv formulation was prepared by dissolving PA into emulphor: ethanol:water (1:1:8). The volume of injection was 2 ml/kg bw.

Blood samples (250 μl) were collected from the orbital sinus at 18 min, 42 min, and 1, 2, 4, 6, 8, 10, and 12 h after administration. In the iv groups, additional blood samples were taken at 6 min after injection. Blood was stored at -20^0C until analysis.

The PA concentration in blood was analyzed by the following procedure. Ten μl of 50-μg/l anthracene was added into 200-μl blood as internal standard and incubated at 37^0C for 30 min. One ml acetonitrile was added, and then the sample was sonicated for 10 min and vortexed for 30 min. Supernatants were separated by centrifugation at 700 × *g*. The extracts were analyzed using a Shimadzu HPLC system with a fluorescence detector at an excitation wavelength of 261 nm and an emission wavelength of 381 nm, a Discovery C-18 reverse-phase analytical column (4.6 × 250 mm, Supelco, Bellefonte, PA), a guard column (4.6 × 50 mm), and a 30/70 v/v water/acetonitrile mobile phase at a flow rate of 0.9 ml/min. The retention times of PA and the internal standard anthracene were 13.5 and 12.4 min, respectively. The recovery at a blood concentration of 5 μg/l was 94.1 ± 7.5% and the relative standard deviation (RSD) was 6.0%. At a blood concentration of 100 μg/l, the recovery was 94.2 ± 3.5% and the RSD was 3.0%.

The blood concentrations of PA were plotted versus time. The pharmacokinetic parameters of interest were determined by standard model-independent methods (WINNonlin, version 4.0, Pharsight, Mountain View, CA). The terminal elimination rate constant was determined by linear regression. AUCs from time zero to infinity were determined by both linear and logarithmic trapezoidal methods, with extrapolation to infinity. The portion of extrapolation was less than 5%. The absolute bioavailability was equal to the average AUC of soil/average AUC of corn oil × 100%. AUC of soil/average AUC of iv injection × 100% and the relative bioavailability was equal to the average.

Physiologically based extraction test (PBET). The PBET assay was carried out using the procedure of Oomen *et al.* (2001). The extraction procedure is schematically presented in Figure 1. The artificial digestive juices were prepared using the method of Hamel *et al.* (1999). Saliva included mucin (4 g), urea (1 g), Na_2HPO_4 (0.6 g), $CaCl_2 \cdot 4\ H_2O$ (0.99 g), KCl (0.4 g), and NaCl (0.4 g) in a volume of 1 liter of deionized water, and the pH was adjusted to 6.5. To prepare gastric juice, 2 g NaCl were dissolved in a solution of 7 ml hydrochloric acid and approximately 250 ml of deionized water. Pepsin (3.2 g) was added, and the solution was brought to 1 liter. The pH was adjusted to 3.0 for immediate use. A 0.2 M, pH 7.0 $NaHCO_3$ solution prepared with deionized water was used as duodenal fluid.

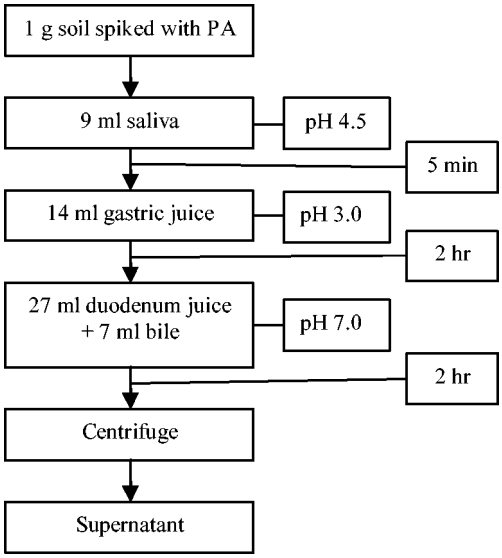

FIG. 1. Scheme of physiologically based extraction test (PBET) (Adapted from Oomen *et al.*, 2001).

At the end of the extraction, the resulting mixture was centrifuged. An aliquot of supernatant was diluted with acetonitrile, filtered, and analyzed by HPLC using the same conditions mentioned in the analysis of PA concentration in the laboratory-treated soils.

Tests of PA solubility in water and artificial digestive juices. An amount of PA exceeding its solubility was added to 20 ml water or artificial juices, shaken at room temperature for two days, and separated by centrifugation. The concentration of PA in supernatants was determined by HPLC, using the procedure described for the PBET assay.

Statistical analysis. Mean values and standard errors (SE) are presented. A one-way analysis of variance (ANOVA) was used to analyze the overall differences among groups, and the Newman-Keuls test was used to detect the differences between groups. The level of significance was set at $p < 0.05$.

RESULTS

Representative curves of PA blood concentration versus time, after a single iv injection or gavage dose, are presented in Figure 2. Following iv injection, concentrations declined in a biexponential manner. Pharmacokinetic parameters of interest are presented in Table 2. The bioavailability of PA in corn oil, after a single dose by gavage, was 24 and 25% after 400 and 800 μg/kg, respectively. No difference was observed in dose-normalized AUCs between the two dose levels (Tables 3 and 4).

At the 400-μg/kg doses, the absolute bioavailability across the different soils ranged from 14.9 to 48.6% (Table 3), and all values were significantly lower than the AUC of the iv injection ($p < 0.05$). Among the soils, Bloomfield had a significantly larger AUC ($p < 0.05$); i.e., the absolute bioavailability of PA was higher than those of the other three soils (Table 3). There were no significant differences detected between AUCs of the corn oil control and other soils, except for Bloomfield.

At the higher dose level, 800 μg/kg bw, absolute bioavail-

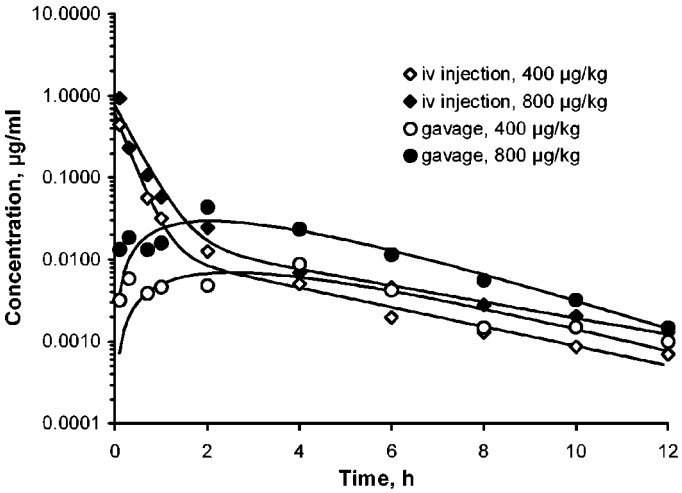

FIG. 2. Representative time-course of blood concentration of phenanthrene after administration by iv injection and gavage in corn coil at 400 μg/kg or 800 μg/kg body weight (bw).

ability ranged from 22.5 to 34.5% and followed a trend similar to what was observed at the lower dose, but there were no differences in absolute bioavailability among soil groups (Table 4). Regarding the dose effect, the absolute bioavailability at the 800-μg/kg bw appeared somewhat higher than that for 400 μg/kg bw, but the difference between the two doses was only significant for the Milford soil. For the Bloomfield soil, the absolute bioavailability was lower at the higher loading level, although not statistically significant. A much higher bioavailability from Bloomfield at the 400 mg/kg loading level had been expected, because at this higher level, PA soil concentrations were 30% higher than the measured sorption capacity of the soil (K_d* $C_{w,max,}$ where $C_{w,max}$ is the aqueous solubility limit). For all other soils, loadings were well below measured sorption capacities. No significant differences were observed in relative bioavailability.

TABLE 2
Pharmacokinetic Parameters for PA after Single iv Injection or Gavage Dose to Sprague-Dawley Rats

Group	400 μg/kg bw	800 μg/kg bw
Iv injection		
CL (ml/min/kg)	24.3 ± 1.7	24.0 ± 4.1
Vd (ml/kg)[a]	524.4 ± 99.3	348.5 ± 63.0[b]
$T_{1/2}$	6.5 ± 1.0	6.7 ± 1.5
Gavage		
CL/F(ml/min/kg)[a]	103.4 ± 13.1	96.7 ± 16.5
$T_{1/2}$	6.0 ± 1.0	6.9 ± 2.4
Bioavailability (%, F)	23.9	24.8

Note. bw, body weight
[a]Values represent mean ± SE.
[b]Significantly different from 400 μg/kg bw; $p < 0.05$.

TABLE 3
Bioavailability of Soil-sorbed Phenanthrene in Rats Treated with 400 μg/kg bw

Group	AUC (μg · h/ml)[a]	Absolute bioavailability (%)	Relative bioavailability (%)
iv injection	0.276 ± 0.022	-	-
Corn oil	0.066 ± 0.013[b]	23.9	-
Bloomfield	0.134 ± 0.047[b,c]	48.6	203.0
Milford	0.043 ± 0.009[b]	16.3	65.2
Toronto	0.055 ± 0.018[b]	20.8	83.3
Heiden	0.040 ± 0.012[b]	14.9	60.6

[a]Values represent mean ± SE of four rats.
[b]Significantly different when compared with iv injection; $p < 0.05$.
[c]Significantly different from other soil groups and corn oil; $p < 0.05$.

The fraction of PA mobilized from the four different soils in the PBET assay is summarized in Table 5. At an initial soil concentration of 200 mg/kg, the fraction of PA mobilized ranged from 17.7 to 69.8% and varied significantly among the four soils. At an initial soil concentration of 400 mg/kg, more PA was mobilized from all soils, ranging from 53.0 to 88.8%. The recovery of PA was 95%, indicating no significant amount of chemical adsorbed to glassware during the extraction process.

In determining which compartment of the digestive fluids contributes to the mobilization of PA from soil matrix, the solubility of PA in different artificial digestive fluids was measured (Table 6). The relative contribution of each digestive fluid to the PA solubility in the PBET supernatant was calculated, based on the relative volume (Fig. 1) of each fluid used in the PBET assay and PA solubility in each fluid (Table 6). The results show that the solubility of PA in the PBET supernatant was 8.5-fold greater than in water. Saliva and bile enhanced solubility of PA by 6.7- and 45-fold, respectively, and gastric and duodenum juice did not increase the solubility compared with water. Bile contributed 74% of the PA solubility in the PBET supernatant, indicating that bile has a signif-

TABLE 4
Bioavailability of Soil-sorbed Phenanthrene in Rats Treated with 800 μg/kg bw

Group	AUC (μg/h/ml)[a]	Absolute bioavailability (%)	Relative bioavailability (%)
Injection (iv)	0.574 ± 0.106	-	-
Corn oil	0.143 ± 0.028[b]	24.9	-
Bloomfield	0.198 ± 0.033[b]	34.5	138.5
Milford	0.129 ± 0.037[b]	22.5	90.2
Toronto	0.161 ± 0.025[b]	28.0	112.6
Heiden	0.135 ± 0.033[b]	23.5	94.4

[a]Values represent mean ± SE of four rats.
[b]Significantly different compared with iv injection, $p < 0.05$.

TABLE 5
Percent of PA Mobilized from Soils Using Physiologically Based Extraction Test

Group	200 mg/kg soil	400 mg/kg soil
Bloomfield	69.8 ± 1.5[c]	88.8 ± 1.0[b]
Milford	22.4 ± 0.7[c]	55.5 ± 0.8[c]
Toronto	58.6 ± 2.0[b]	76.2 ± 0.4[d]
Heiden	17.7 ± 0.7[c]	53.0 ± 1.1[c]

[a]Values represent mean ± SE of three measurements
[b–c]Within each column, values with different superscripts are significantly different from one another, $p < 0.05$.

icant impact on the mobilization and subsequently the bioavailability of PA from soil.

DISCUSSION

The soil matrix can influence the bioavailability of organic chemicals. In the current study, both the absolute and relative bioavailabilities of PA were examined. Absolute bioavailability, the fraction of an extravascular dose that reaches the systemic circulation, is an indicator of the fraction of chemical that can be delivered to the target tissues to exert adverse effects. Knowing the absolute bioavailability of a chemical is important in exposure assessment and the determination of soil remediation cleanup levels. PA is a major PAH in coal tar (CT). In a feeding study using DNA adducts as a biomarker, Bordelon *et al.* (2000) found that the bioavailability of CT from soil was significantly decreased compared to a CT/SiO_2:TiO_2 control. Kadry *et al.* (1995) found that the oral bioavailability of PA from a sandy soil and a clay soil with different organic carbon contents were 59.6 and 55.7%, respectively. No significant difference was observed between the two soils. In the current study, the absolute bioavailability of PA from all four types of soil was significantly decreased at both dose levels, indicating that the interactions between PA molecules and soil matrix made PA less available to organisms. Since the exposure level in the natural environment varies with the doses tested in this study, caution should be applied when extrapolating the current findings over the exposure level in other situations.

Among the soil properties that govern the bioavailability of soil-bound organic chemicals, it is believed that the OC content is the most important factor. In the current study, no clear numerical relationship was found between soil OC content and the fraction-of-mobilization and bioavailability. However, soils with higher OC content did tend to result in a lower PA fraction mobilized in the PBET assay and a lower PA *in vivo* bioavailability, except for Milford soil, suggesting that the OC content might play an important role in the oral absorption of soil-bound PA (Figs. 3a and 3b). Another important soil property that could impact the bioavailability of soil-bound chemicals is the type and amount of clay present. Clays typically have high surface areas, which can enhance sorption through weak physical interactions. Clays can also impede chemical mass transfer due to clay-organic matter aggregation and clay interlayers, which will differ with soil origin and genesis. In this study, an inverse relationship was observed between clay and lability, with the higher clay content resulting in lower bioavailability and mobilization of PA (Figs. 3a and 3b). The higher OC-normalized sorption of PA by the Milford soil may be partially attributed to interactions with the clay fraction; however, this alone may not be sufficient to explain the higher sorption. The Heiden soil also has similarly high clay content, but not correspondingly higher OC-normalized sorption of PA. The Heiden soil is a subsurface, western Texas soil, while the Milford soil is from an agricultural area in the Midwest. Organic matter characteristics such as polarity and aromaticity, which change with soil origin and genesis, and the presence of soot particles may also account for the differences in sorptive behavior (Gustafsson *et al.*, 1997; Salloum *et al.*, 2002).

In the current study, relative bioavailability was also examined by using corn oil as a control. A toxicological definition of the relative bioavailability refers to comparative bioavailability of a chemical from different exposure media containing the chemical (NEPI, 2000). The importance of the definition of relative bioavailability is evident, because most toxicity data used in risk assessments are based on laboratory animal studies in which the chemical is delivered to the test animal in certain media. What was surprising in this study was that the absolute bioavailability of PA from corn oil was only about 24% at both dose levels. The reason is unknown. The hepatic clearance of PA after iv injection was 24 ml/min/kg for both doses (Table 2). The reported hepatic blood flow in the rat ranges from 47 to 81 ml/min/kg (Kwon, 2001). The hepatic extraction ratio is relatively low, indicating the first-pass effect shouldn't be significant. The low oral bioavailability of PA in corn oil might be due to the poor water solubility of PA. Since diarrhea was observed in some corn oil-dosed animals during the study, it is also possible that some PA might be excreted in feces along with unabsorbed corn soil. Nonetheless, this finding emphasizes that the bioavailability of the test chemical from the

TABLE 6
PA Solubility in Water and Artificial Digestive Fluids and Relative Mass Contribution to the Solubility of PA

Media	Solubility (mg/l)	Vol. in PBET test (ml)	Mass (mg) contribution	% Relative mass contribution
Water	1.20	-	-	-
PBET supernatant	8.46	-	-	
Saliva	6.74	9	0.061	14
Gastric juice	1.17	14	0.016	4
Duodenum juice	1.22	27	0.033	8
Bile	44.96	7	0.315	74
Sum	-	57	0.425	100

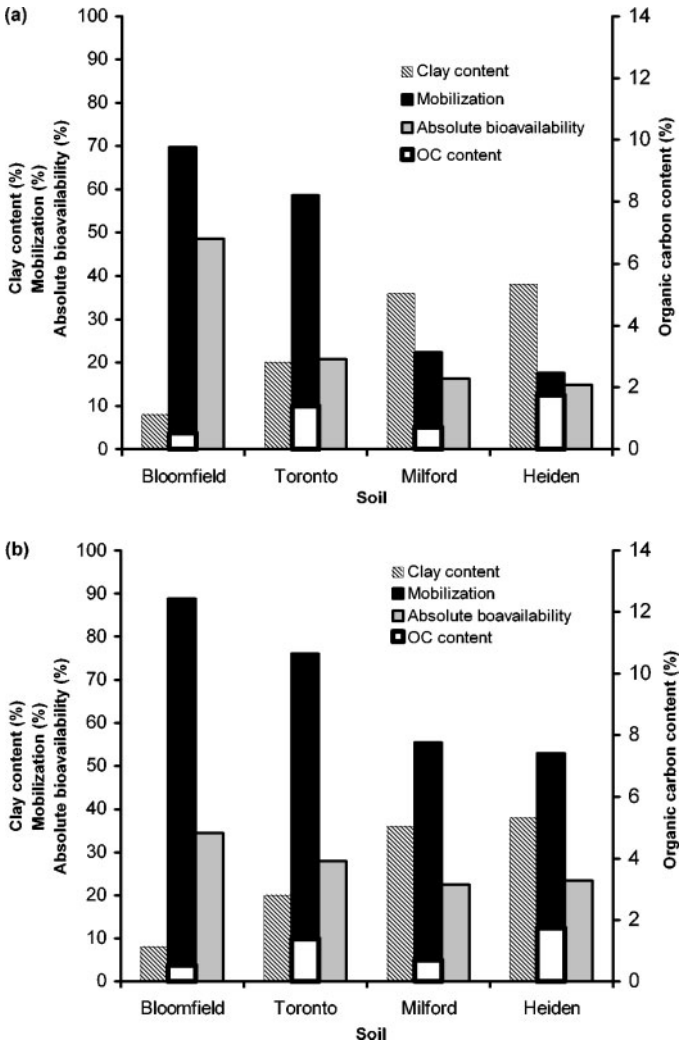

FIG. 3. Relationships among the soil organic carbon content, clay content, and PA mobilization from soils in the *in vitro* physiologically based extraction tests and the absolute bioavailability of PA from soils in rats *in vivo* at (a) 400-μg/kg bw and a soil concentration of 200 mg/kg, or (b) 800 μg/kg bw and a soil concentration of 400 mg/kg.

carrier must be considered in toxicological studies. Due to this low bioavailability, the relative bioavailability of PA from soils was high, especially for the sandy Bloomfield soil.

In vivo assessment of the bioavailability of soil-bound contaminants using animal models is generally easy to interpret and accurate, but it is also costly, time consuming, and likely to draw ethical concerns regarding the use of animals. Thus, some surrogate tests have been developed. The physiologically based extraction test (PBET) is one of these alternatives that chemically simulate human digestive processes with a bench top *in vitro* assay to estimate the bioavailability of chemicals from the soil matrix (Hamel *et al.* 1999; Ruby *et al.* 1996). Hack and Selenka (1996) reported that the mobilization of PAHs and PCBs from soils by artificial gastric fluid ranged from 3% up to 22%. The mobilization under gastric, and subsequently intestinal, conditions with bile concentrations of 3 g/l increased the mobilization to from 5 to 40%. Certain food materials, such as lyophilized milk, significantly increased the mobilization of contaminants from soil. In the current study, the mobilization of PA from different soils ranged from 17.7 to 69.8% at a concentration of 200 mg/kg and 53.0 to 88.8% at a concentration of 400 mg/kg. The comparison of the solubility of PA in different artificial digestive juices showed that bile enhanced the solubility of PA by 45-fold compared to its water solubility. These results indicate that bile plays an essential role in the release of PA from the soil matrix after oral administration.

Due to the complexity of the composition of digestive fluids, the dynamic processes of digestion and absorption, and the physiological variability among individuals, there is no single *in vitro* assay that can completely simulate the absorption process in organisms. Thus, caution should be applied when extrapolating the data from *in vitro* tests. Thus far, there is very limited information regarding the relationship between the *in vivo* and *in vitro* tests of bioavailability of soil-bound organic chemicals. In a bioavailability study of sediment-bound benzo[*a*]pyrene and phenanthrene, Weston *et al.* (1998) compared the *in vitro* digestive fluid extraction with other approaches: uptake clearance, absorption efficiency, and bioaccumulation factors. Uptake clearance represents the amount of sediment that would have to be completely cleared of PAHs to provide the measured body burden. The absorption efficiency is measured by the comparison of PAH concentrations in the foregut and rectal sediments of worms. There was generally good agreement among the four methods on the relative bioavailability of benzo[*a*]pyrene from six sandy sediments. Due to both a more limited data set and perhaps greater importance of uptake from the dissolved phase, no strong correlation among these methods was observed in the PA bioavailability from sediments. In the current study, the relationship between the *in vitro* extraction test and the *in vivo* bioavailability assay of PA from soil was evaluated. The results showed good agreement between these two methods; i.e., when more PA was mobilized by the digestive fluids and made accessible to the gut wall, more PA was absorbed by rats. A significant correlation was observed between the PBET results and *in vivo* bioavailability of PA from soils (Fig. 4, $r = 0.73$, $p < 0.05$). These results suggest that the PBET assay may be a useful tool in the estimation of soil-bound PA bioavailability.

Some studies indicate that the concentration of a contaminant in soil may influence its bioavailability. The bioavailability of pentachlorophenol from some soils increased with increasing dose levels (Pu *et al.*, 2003). Wendling *et al.* (1989) reported increased TCDD bioavailability with higher doses, whereas Shu *et al.* (1988) found that TCDD bioavailability from soil was independent of dose over a 500-fold dose range. In this study, bioavailability of PA from only one of four soils significantly increased at the higher concentration. In the PBET test, the mobilization PA from all soils increased at the higher

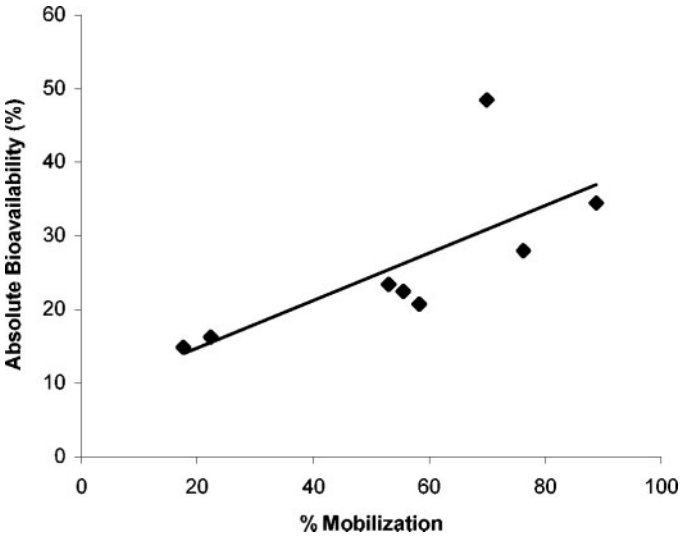

FIG. 4. Correlation between the mobilization of PA from soils in the *in vitro* physiologically based extraction tests and the bioavailability of phenanthrene from soils in rats *in vivo* ($r = 0.73$, $p < 0.05$).

soil concentration. Since no difference was observed in dose-normalized AUCs between the two dose levels after iv injection, linear pharmacokinetics of PA after iv injection or oral dosing in the dose range used can be assumed, which means that PA bioavailability is independent from dose. Thus, this increase in bioavailability and solubilization at the high concentration might be due to the nonlinear sorption of PA to the soot-like component of soil OC (Ran *et al.*, 2002), resulting in more desorption of chemical from soil at higher concentration.

In conclusion, the results of this study suggest that the soil matrix can significantly decrease the oral absorption of PA from soil, and this decrease in bioavailability may be related to soil properties: specifically OC content and clay content. The good agreement between the *in vivo* bioavailability data and results from the *in vitro* approach suggest that PBET assay is a promising tool in predicting the bioavailability of soil-bound organic contaminants. However, due to limited soil types and the use of one chemical versus the variety of contaminants and soil properties in the environment, further efforts involving more chemicals and soil types are needed to validate this surrogate method.

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