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Environmental Toxicology and Chemistry, Vol. 26, No. 3, pp. 387–393, 2007

Printed in the USA
0730-7268/07 $12.00 + .00

BENZO[*a*]PYRENE BIOAVAILABILITY FROM PRISTINE SOIL AND CONTAMINATED SEDIMENT ASSESSED USING TWO IN VITRO MODELS

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(*Received* 6 *July* 2006; *Accepted* 19 *September* 2006)

Abstract—A major route of exposure to hydrophobic organic contaminants (HOCs), such as benzo[*a*]pyrene (B*a*P), is ingestion. Matrix-bound HOCs may become bioavailable after mobilization by the gastrointestinal fluids followed by sorption to the intestinal epithelium. The purpose of this research was to measure the bioavailability of [^{14}C]-B*a*P bound to pristine soils or field-contaminated sediment using an in vitro model of gastrointestinal digestion followed by sorption to human enterocytes (Caco-2 cells) or to a surrogate membrane, ethylene vinyl acetate (EVA) thin film. Although Caco-2 cells had a twofold higher lipid-normalized fugacity capacity than EVA, [^{14}C]-B*a*P uptake by Caco-2 lipids and EVA thin film demonstrated a linear relationship within the range of B*a*P concentrations tested. These results suggest that EVA thin film is a good membrane surrogate for passive uptake of B*a*P. The in vitro system provided enough sensitivity to detect matrix effects on bioavailability; after 5 h, significantly lower concentrations of [^{14}C]-B*a*P were sorbed into Caco-2 cells from soil containing a higher percentage of organic matter compared to soil with a lower percentage of organic matter. The [^{14}C]-B*a*P desorption rate from Caco-2 lipids consistently was twofold higher than from EVA thin film for all matrices tested. The more rapid kinetics observed with Caco-2 cells probably were due to the greater surface area available for absorption/desorption in the cells. After 5 h, the uptake of B*a*P into Caco-2 lipid was similar in live and metabolically inert Caco-2 cells, suggesting that the primary route of B*a*P uptake is by passive diffusion. Moreover, the driving force for uptake is the fugacity gradient that exists between the gastrointestinal fluid and the membrane.

Keywords—Benzo[*a*]pyrene Bioavailability Caco-2 cells Soil In vitro tests

INTRODUCTION

Environmental toxicologists define bioavailability as the ability of a chemical to cross a cell membrane and enter a cell [1]. Currently, risk assessment procedures define the bioavailability of sorbed contaminants as the amount extractable by organic solvents [2]; however, the total amount of contaminant extracted often is much greater than the amount of contaminant that actually is bioaccumulated [1,3,4]. It has been shown repeatedly that many hydrophobic soil contaminants are less than 100% bioavailable, and that bioavailability decreases over time, a process called aging [5,6]. The mechanism of aging has been much investigated [1], particularly with polycyclic aromatic hydrocarbons (PAHs). As a result, it is known that interactions between soil and PAH are affected by the nature and amount of soil organic matter [7], by inorganic components with particular reference to pore size and structure [8], by microbial populations, and by the amount of contamination.

Ingestion of contaminated soil is the major route of exposure to many hydrophobic organic contaminants. The potential for exposure to contaminants via this route is greater for children because they are likely to ingest more soil as a result of inadvertent soil ingestion through the mouthing of objects or hands [9]. For the purpose of estimating risk, the U.S. Environmental Protection Agency assumes that most children ingest relatively small quantities of soil (e.g., $<$100 mg/d), and the upper 95th percentile is estimated to ingest 200 mg/d on average [10,11]. For that reason, oral bioavailability values for ingested soil contaminants are critical for an accurate assessment of human health risk. Absorption of soil particles in the gastrointestinal tract of higher animals is thought to be negligible [12] and, in many cases, the sorbed fraction of contaminant is considered to be unavailable for absorption. Thus, a molecule must be desorbed to become bioavailable. It has been proposed that intestinal secretions and digestive enzymes could mobilize a proportion of the sorbed fraction during digestion, which ultimately could be absorbed by the tissue [13–15].

Animal testing has been a standard in determining the bioavailability of environmental pollutants; however, it is expensive and unethical for large-scale screening. The hydrophobic partitioning coefficient (octanol-water partition coefficient [log K_{OW}]) is used for predicting the bioavailability of hydrophobic organic chemicals (HOCs) because these chemicals partition into tissues to a much greater extent than more polar compounds [1]. However, this does not take into consideration metabolic processes nor account for the strong binding of high log K_{OW} compounds to soil/sediment matrices [16]. Thus, in vitro methods urgently are needed to screen compounds for their potential bioavailability. Several reports have focused on the development of in vitro methods designed to mimic gastrointestinal digestion as a means of estimating relative bioavailability. These models, based on human physiology, generally are cheaper, faster, and more reproducible than animal tests, and differ in composition of synthetic digestive fluids, the duration of the elution procedure, the concentration of the contaminants or contaminated material, as well as the methodology applied for the separation of the mobilized fractions [4,17]. In vitro models have been used successfully for some time in the evaluation of the bioavailability of polar compounds, such as pharmaceuticals and metals. For example, Ruby [18] evaluated the relative bioavailability of lead in a weanling rat model and demonstrated that the stomach phase

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Table 1. Properties of soil and sediment samples used in this study

	Topsoil	Subsoil	Sydney Harbor (NS, Canada) sediment
Depth	0–15 cm	15–20 cm	0–10 cm
Organic matter (%, loss on ignition)	28.9 ± 0.1	11.0 ± 0.2	8.8 ± 0.0
pH in water	3.4	3.8	3.0
Particle size	125–250 μm	125–250 μm	66.4% < 63 μm (by mass)

of the in vitro test correlated with the bioavailability values from the in vivo model. However, similar success with hydrophobic compounds has not yet been achieved and, at present, there are few reports that evaluate the in vitro bioavailability of HOCs. To date, most digestive models are static gastrointestinal models that simulate mouth, gastric, and small intestinal fluids, but do not employ an intestinal epithelium to determine uptake.

A few researchers have measured intestinal transport of HOCs through cultured human enterocytes (Caco-2). Using a Caco-2 cell monolayer, Oomen et al. [13] showed that up to 54% of polychlorinated biphenyls (PCBs) were mobilized from spiked artificial standard soil after in vitro gastrointestinal digestion, but that <10% of these organic contaminants were transported across the Caco-2 monolayers to the basolateral compartment. A more recent study showed that Caco-2 cells formed a biochemical barrier for benzo[*a*]pyrene (B*a*P) because of extensive metabolism and subsequent luminally directed active transport of polar metabolites [19].

The specific aims of this study were to measure the effect of aging and soil organic matter content on the extent of mobilization and uptake of [^{14}C]-B*a*P bound to pristine soil or field-contaminated sediment. Benzo[*a*]pyrene, a 5-ring PAH (log K_{OW} of 6.04), is a procarcinogen of great concern, because it is dispersed widely in the environment and has been shown to bind strongly to soils and sediments [20]. To achieve these aims, we employed an in vitro digestion protocol including exposure to either Caco-2 cells or an ethylene vinyl acetate (EVA) thin film. Caco-2 cells are a human enterocyte cell line derived from a human colon adenocarcinoma [21]. This cell line is well characterized with regard to transport properties of drugs and metals, and it has been used extensively to predict intestinal absorption of pharmaceuticals [22] and, more recently, xenobiotics [13,23,24]. Wilcockson and Gobas [25] developed the EVA thin film solid phase extraction technique with which they investigated the bioavailable fraction of semivolatile and poorly volatile organic chemicals in air [26], biota [25], sediments, and soils.

MATERIALS AND METHODS

*Experimental approach to measure mobilization of B*a*P from a solid matrix during digestion*

The digestion process was based on physiological constituents and transit times for fasting conditions in children, beginning with stomach digestion [4,17,27]. The B*a*P concentrations bound to soil were based on the concentrations found in contaminated sites [28]. Incubation of substrate ([^{14}C]-labeled B*a*P bound to soil) with gastric fluid was followed by pH neutralization and addition of bile salts, pancreatin, and bovine serum albumin to mimic digestion of the contaminant. The neutralized digestive mixture then was applied to either a thin film of ethylene vinyl acetate or to fixed Caco-2 cells on glass cover slips in 2-ml beakers. In some experiments, the intestinal fluid was added to live Caco-2 cells on the apical side of Transwell inserts. The amount of [^{14}C] in the aqueous compartment and in the Caco-2 cells or EVA film was monitored over time. To account for nonspecific binding of the chemical, we performed a complete mass balance of the chemical movement in each experiment by determining the amount of [^{14}C] in each compartment of the system, including the tissue culture supports. A small percentage of [^{14}C], 10 to 20%, was associated with tissue culture supports and other plastic ware.

Soil characteristics

The model soil was collected between 0 and 15 cm (topsoil) and between 15 and 20 cm (subsoil) in depth from Burnaby Mountain (BC, Canada). The soil was fractionated into various particles sizes in a mechanical shaker. The 125 to 250 μm soil fraction was employed because it is more likely to adhere to the skin and be transferred by hand-to-mouth activity of children [10] (Table 1).

Field-contaminated sediment was collected from Sydney Harbor, Nova Scotia, Canada ([29]; http://www.hc-sc.gc.ca/sr-sr/pubs/funding-finance/tsri-irst/sum-som/summary-contaminants-sommaire_e.html) in September of 2002 by Environment Canada, stored in a sterilized glass jar, and refrigerated until use. Prior to the experiments, soils and sediment were air-dried and autoclaved three times with a 24-h interval between autoclaving to eliminate microbial populations.

Measurement of soil/sediment organic matter (loss-on-ignition method)

The loss-on-ignition (LOI) method [30] was modified from a method described by Ben-Dor and Banin [31]. Briefly, air-dried soil and sediment samples were added to tared crucibles and heated at 105°C for 24 h. The samples were ignited in a muffle furnace at 400°C for 16 h and cooled in a desiccator under vacuum; the weight of ignited samples then was determined. The LOI content of the sample was calculated as LOI % = ([Sample_{105} − Sample_{400}]/Sample_{105})·100, where Sample_{105} is weight of soil sample after heating at 105°C and Sample_{400} is weight of soil sample after ignition at 400°C. The LOI was corrected for dehydroxylation of inorganic constituents through a regression analysis model: Soil organic matter = (b·LOI) + a, (g/100 soil); where a and b are 0.972 and −0.37, respectively, as determined by Nelson and Sommers [30] and Ben-Dor and Banin [31].

*Spiking soil with [^{14}C]-B*a*P*

The [7,10-^{14}C]-B*a*P was obtained from GE Healthcare (formerly Amersham, Oakville, ON, Canada) (specific activity of 60 mCi/mmol). To prepare spiked soil, 10 mg of soil was put into 20 ml borosilicate scintillation vials to which 10 μl of toluene containing [^{14}C]-labeled B*a*P was added. The toluene was allowed to evaporate overnight at room temperature. After spiking, soils were kept in closed containers at room temper-

ature in the dark for a minimum of 7 d prior to use. In experiments with Sydney Harbour sediments, after spiking with [^{14}C]-B*a*P as described above, samples were kept in sealed vials for four months under the same conditions. To ensure consistency of spiking procedures, the amount of [^{14}C] in three soil samples were determined at the beginning of each experiment.

Model gastrointestinal fluids

The composition of synthetic gastric and intestinal fluids was based on in vitro digestion models designed by Hack and Selenka [4], Ruby et al. [17], and Rodriguez et al. [27], and modified as described previously [23] with a further addition of 10 mg/ml bovine serum albumin, added to intestinal fluid. The soil-to-fluid ratio was 10 mg of spiked soil/sediment to 1 ml of gastric fluid (1:100) for the first 1.5 h, and 10 mg of soil to 1.1 ml of intestinal fluid (1:110) subsequently. A soil-to-fluid ratio in the range of 1:100 to 1:5000 (w/v) is assumed to cover all physiological conditions in vivo [4,32].

Cell culture

The human colorectal carcinoma cell line (Caco-2) was obtained from the American Type Culture Collection (catalogue HTB-37). Cells were maintained in Dulbecco's modified Eagle's medium (GIBCO BRL, Burlington, ON, Canada) with high glucose (4.5 g/L) supplemented with 1% nonessential amino acids, penicillin (25 International units/ml), and streptomycin (100 μg/ml), with 10% fetal bovine serum, at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity. Caco-2 cells were used between passages 30 to 70. For transport experiments, cells ($1–2 \times 10^5$ cells/insert) were seeded on Transwell polycarbonate cell culture inserts (Becton Dickinson, Franklin Lakes, NJ, USA) with high pore density (0.4 μm pore size). Cells were incubated for three weeks and used between 21 and 26 d after seeding. Lipid content of Caco-2 cells was determined as described previously [23].

Sorptive surfaces

In some experiments, a thin film of EVA [25] was used to determine the amount of freely available chemical. Glass cover slips (12-mm diameter, Fisher Scientific, Ottawa, ON, Canada) were rinsed with 40 μl of dichloromethane and then coated on one side with 40 μl of EVA solution (0.6% EVA and 0.04% silane). For absorption/partitioning experiments, cells ($1–2 \times 10^5$ cells/well) were seeded on glass cover slips in a 24-well plate and used 21 d after seeding. After incubation with 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS) and pH 7.4 for 30 min at room temperature, Caco-2 cells were washed twice with PBS and covered with PBS until use.

The fixed Caco-2 or EVA-coated cover slips were added to 2-ml glass beakers with intestinal digestion material and agitated on an orbital shaker at 70 rpm at 37°C. At specific time intervals, triplicate samples were removed. The EVA or Caco-2 cell-coated cover slips were rinsed in PBS, extracted into Biodegradable Counting Scintillant (Amersham Biosciences Oakville, ON, Canada, a division of GE Healthcare) and [^{14}C]-bound to Caco-2 cells, or EVA was determined by liquid scintillation counting using a Beckmann 6500 Liquid Scintillation Counter with automatic quench correction (Beckman Coulter Canada, Mississauga ON, Canada).

Transport experiments

Caco-2 cells were grown to confluence on cell culture inserts as described above and the formation of a sealed monolayer was confirmed by the measurement of transepithelial electrical resistance as described in Vasiluk et al. [33]. Cells were washed with a buffered solution (pH 7.4) to remove culture medium and neutralized intestinal fluid from gastrointestinal digestion was added to the apical chamber. Cells were incubated at 37°C with gentle shaking and the basal media was changed every hour to mimic blood flow. Aliquots of 20 μl from apical and 0.5 ml from basal medium were sampled over the next 6 h. Apical samples were cleared of soil particles by centrifugation at 2,000 *g* for 1 min. Uptake into the Caco-2 cells also was measured at the end of the experiment by addition of biodegradable counting scintillant and liquid scintillation counting.

Data and statistical analysis

The raw data (sampling time and concentrations) were fitted to a one-compartment model assuming first-order kinetics. The one compartment was fit to desorption data using nonlinear regression technique with software designed to minimize the sum of squared residuals (SPSS 13.0, Chicago, IL, USA).

$$C = C_p(1 - e^{-kt}) \quad (1)$$

where C is the concentration in Caco-2 or EVA (mol/m^3) at time t (h), C_p is concentration in the sorptive epithelia at equilibrium (mol/m^3), and k is the apparent elimination rate constant (h^{-1}).

Data are presented as means ± standard error of at least three independent experiments, each performed in triplicate. Data were analyzed using one-way analysis of variance test, followed by the Wilcoxon rank-sum test.

RESULTS AND DISCUSSION

*Effect of varying percent of organic matter on bioavailability of [^{14}C]-B*a*P bound to soil*

To measure the mobilization and sorption of soil-bound [^{14}C]-B*a*P from soil, soil containing 1 μg of [^{14}C]-B*a*P was incubated in simulated gastric and intestinal fluids, and the intestinal fluid was applied to the apical side of Caco-2 cells. Two soils were compared that differed primarily in their proportion of organic matter: High (29%) and low (11%) organic matter (Table 1). We found that the B*a*P concentrations in the gastrointestinal fluid increased rapidly over the 5-h incubation period (Fig. 1A). This [^{14}C], present in the 2,000 *g* supernatant of the digests, was considered as free or bound to soluble organic matter in the aqueous fraction. Significantly lower concentrations of B*a*P mobilized into aqueous solution from the high organic matter soil (Fig. 1A). Similar results were obtained when soils were spiked with 0.5 μg and 1.7 μg B*a*P (data not shown), i.e., the lower organic matter soil released approximately twice as much radioactivity into the gastrointestinal fluids compared to soil with higher organic matter content.

After 5 h, the levels of [^{14}C] (representing B*a*P and B*a*P metabolites, if any) associated with Caco-2 cells was measured. The amount of B*a*P sorbed to Caco-2 cells was significantly greater with low organic matter soil for all three B*a*P concentrations measured (0.5 μg, 1 μg, and 1.7 μg of B*a*P; Fig. 1B), which corresponded with the relative concentrations in the aqueous phase. These data clearly show the utility of such in vitro models in detecting differences in bioavailability of hydrophobic organic contaminants bound to different matrices.

Due to its high log K_{OW} value (6.04), B*a*P would be ex-

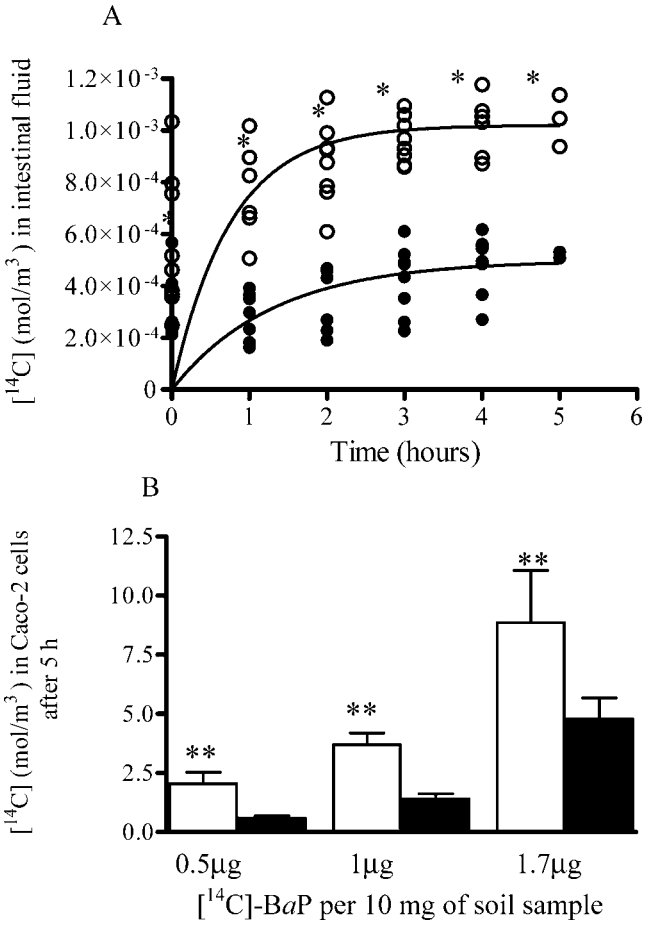

Fig. 1. Partitioning of soil-bound [^{14}C]-benzo[*a*]pyrene ([^{14}C]-B*a*P) to gastrointestinal fluid and to Caco-2 cells: Effect of soil organic matter content. The soils contained a high percent (29%) organic matter (closed circles) or low percent (11%) organic matter (open circles). (**A**) Mobilization of [^{14}C]-B*a*P from soils into intestinal fluids versus time. Data shown are for 1 mg of [^{14}C]-B*a*P per 10 mg of soil sample; (**B**) [^{14}C]-B*a*P or [^{14}C]-B*a*P metabolites sorbed to Caco-2 cells 5 h after exposure to intestinal fluids with B*a*P bound to soil. Asterisks indicate that the soils with low organic matter were significantly different from the high organic matter content soil at $p < 0.05$ (*), $p < 0.01$ (**).

pected to associate strongly with soil organic matter. Consequently, the higher the percent of organic matter, the higher the fugacity capacity, *Z*, of the soil (where fugacity = concentration/fugacity capacity [23]). Although the fugacity capacity of Caco-2 cells remained constant, the system was not at equilibrium. Hence, any changes in the extent of B*a*P mobilized into the aqueous phase were reflected in the B*a*P concentration in the Caco-2 monolayers, i.e., the transport of PAH into gastrointestinal cells is fugacity-driven.

Comparison between Caco-2 cells and EVA thin films

Because live Caco-2 cells cannot withstand incubation with gastrointestinal fluid for periods longer than 5 to 6 h, we examined an abiotic substitute to measure freely available B*a*P. Ethylene vinyl acetate thin film was chosen because it exhibits quick absorption kinetics resulting in rapid chemical equilibrium [25]. In addition, Wilcockson and Gobas have suggested that there might be a useful relationship between the lipid-normalized concentration in biological tissue and the EVA film concentration [25]. In the present study, we experimentally

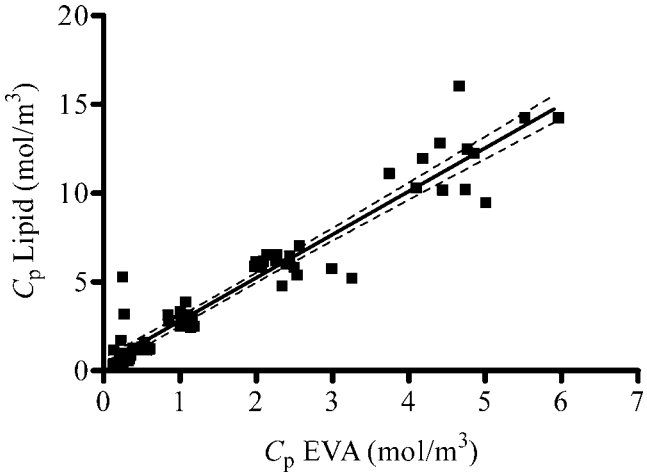

Fig. 2. A comparison of benzo[*a*]pyrene (B*a*P) uptake into Caco-2 cells and ethylene vinyl acetate (EVA) thin film over a range of B*a*P concentrations. Data represent three independent experiments with three replicates for each concentration of B*a*P. For the Caco-2 cells, uptake was normalized to the volume of lipid present in the Caco-2 cells. The solid line represents the best fit using the linear bivariate fit equation, and the dashed lines represent the 95% confidence interval ($r^2 = 0.92$). The C_p represents the concentration in EVA or fixed Caco-2 cells after 14 to 16 h of incubation (at equilibrium) with 0.05, 0.1, 0.2, 0.4, or 0.8 µg of B*a*P per 10 mg of soil.

determined the relationship between Caco-2 lipid and EVA thin film to sorb B*a*P over a range of concentrations.

The uptake of [^{14}C]-B*a*P into EVA and Caco-2 cells from soil spiked with five different concentrations of B*a*P is shown in Figure 2, expressed as the concentration at the plateau (C_p) in each medium for each level of B*a*P. Proteins in Caco-2 cells were cross-linked by pretreatment with paraformaldehyde to permit the system to reach equilibrium and to eliminate any contribution of metabolism. Equilibrium was reached within 12 h under all conditions (data not shown), therefore, the extent of uptake by cells or EVA film was measured at 14 and 16 h. The EVA values were normalized per volume of EVA and Caco-2 values were normalized to the volume of lipid present, as described in Minhas et al. [23]. A linear relationship was found between B*a*P uptake into Caco-2 lipids and EVA thin film over the range of B*a*P concentrations measured (Fig. 2).

From these data, the relative comparison of B*a*P uptake by Caco-2 lipids and EVA thin film could be calculated using a linear bivariate fit equation ($r^2 = 0.92$) with 95% confidence (Fig. 2) according to the following equation:

$$C_{\text{Lipid}} = 2.44 \cdot C_{\text{EVA}} \quad (2)$$

where C_{Lipid} is the concentration of B*a*P in Caco-2 monolayers at equilibrium (mol/m^3) and C_{EVA} is the concentration of B*a*P in EVA films at equilibrium (mol/m^3).

These results confirm our previous finding using another PAH, chrysene [23], in which we calculated that $Z_{\text{Caco-2}}$ was slightly larger than Z_{EVA}. Here, we experimentally confirm that there is a linear relationship between uptake of [^{14}C]-B*a*P by Caco-2 lipids and EVA thin film. The differences in the absolute B*a*P uptake suggest that the fugacity capacity of biological lipids is somewhat greater than EVA for B*a*P. Nevertheless, EVA remains a good surrogate for intestinal absorption because of the linear relationship within the range of B*a*P concentrations tested.

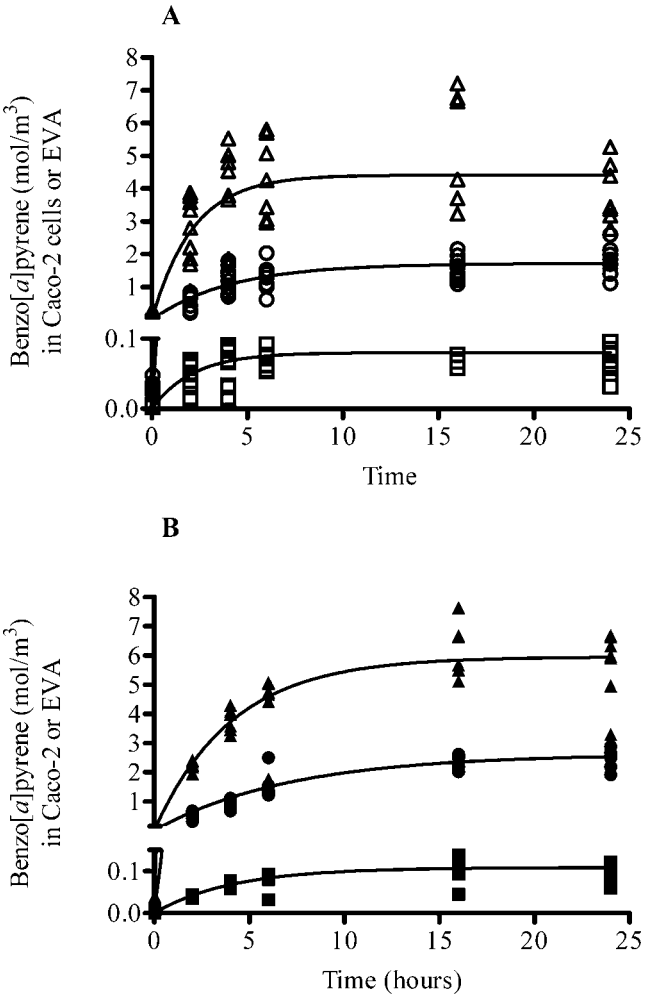

Fig. 3. Uptake of [^{14}C]-benzo[*a*]pyrene from digested soils containing (**A**) low (open symbols) or (**B**) high (solid symbols) percent organic matter into ethylene vinyl acetate (EVA) or fixed Caco-2 monolayers. Data are presented for three independent experiments, each with three replicates. For the Caco-2 cells, uptake was determined for whole cells and was normalized to the volume of lipid present in the Caco-2 cells. The squares represent the whole Caco-2 cells; triangles represent the lipid fractions of the Caco-2 cells; and the circles represent ethylene vinyl acetate coating.

Sorption of [^{14}C]-BaP bound to soil: Comparison of Caco-2 cells with EVA

We compared the mobilization and sorption of soil-bound [^{14}C]-B*a*P from high and low organic matter soils at equilibrium. Soils were incubated in simulated gastric and intestinal fluids, and then the intestinal fluid-soil mixture was applied to cover slips with Caco-2 cells and EVA thin films. To obtain estimates of chemical equilibrium, we fixed cover slips coated with Caco-2 monolayers with paraformaldehyde, as described previously in Minhas et al [23].

The [^{14}C]-B*a*P uptake into EVA and Caco-2 from the two soils is shown in Figure 3. The EVA accumulated greater than 20 times more B*a*P compared to whole Caco-2 cells; however, when the data were normalized to the lipid volume of the cells, the biological lipid bound approximately 2.5-fold more B*a*P per m^3 than EVA, regardless of organic matter content (Fig. 3). This was consistent with the results illustrated in Figure 2, which indicated that the fugacity capacity of Caco-2 lipid for B*a*P was approximately twofold higher than EVA.

At equilibrium, we found that there was no significant difference between low and high organic matter soils in the amount of [^{14}C]-B*a*P sorbed to fixed Caco-2 cells and EVA thin film. This was in contrast to the results obtained using Caco-2 cells after 5 h: In this experiment, cells accumulated significantly more [^{14}C]-B*a*P from low organic matter soil (Fig. 1B). To determine whether the observed differences were due to differences in kinetics, a first-order elimination model was fit to the data shown in Figure 3 (see *Kinetics analysis* section).

The data shown in Figure 3 using fixed Caco-2 cells indicate that, when 1 μg of B*a*P bound to low organic matter soil is added to the gastrointestinal fluids, an average of 4 to 5 mol/m^3 accumulated in the Caco-2 lipid after 5 h (Fig. 3A). Using live Caco-2 cells, we determined that the average uptake into Caco-2 lipid was the same, 4 mol/m^3 (Fig. 1B). The similarity between the uptake values with live and metabolically inert cells suggests that the major mechanism of B*a*P uptake into Caco-2 cells is passive diffusion.

Effect of contaminant-soil contact time on the mobilization and uptake of [^{14}C]-BaP bound to PAH-contaminated sediment

Many researchers have suggested that organic compounds such as PAHs become less available in the soil over time and, therefore, probably exert less toxic effect [1,5]. Sydney Harbor, Nova Scotia has been the depository of industrial and anthropogenic contaminants and, as a result, the sediment contains elevated amounts of oils and tar ([29]; http://www.hc-sc.gc.ca/sr-sr/pubs/funding-finance/tsri-irst/sum-som/summary-contaminants-sommaire_e.html), principally PAHs (35.4 mg/kg), 5 to 10% of which is B*a*P, and other organic conntaminants such as PCBs, as well as metals (Cd, Hg, Pb, and Zn) ([29]; http://www.hc-sc.gc.ca/sr-sr/pubs/funding-finance/tsri-irst/sum-som/summary-contaminants-sommaire_e.html). Therefore, we measured mobilization of [^{14}C]-B*a*P from spiked Sydney Harbor sediments aged for 7 d or four months, as well as uptake into Caco-2 cells and EVA. When sediment was exposed to [^{14}C]-B*a*P for four months, B*a*P uptake into Caco-2 cells (fixed) or EVA decreased significantly compared to nonaged sediments (Fig. 4A and B). The differences observed with EVA film were larger and more consistent than those observed with Caco-2 cells, likely because of greater reproducibility of the EVA technique (compare Fig. 4A and 4B). These data are in agreement with our earlier observation that Caco-2 lipids have a two- to threefold greater fugacity capacity for B*a*P uptake compared to EVA.

Kinetic analysis

Because sorption is so important to the fate of hydrophobic contaminants in soil, it is essential to characterize both the thermodynamics and kinetics of sorption [34]. As stated above, sorption of PAHs to a solid matrix and its physicochemical interactions with soil particles often result in strong binding and slow release rates that significantly influence their bioavailability [6]. Desorption often is considered the rate-limiting step for soil systems [5].

To obtain estimates of equilibria in sorptive epithelia, first-order elimination models were fitted to the data obtained from fixed Caco-2 cells and EVA films using nonlinear regression (Eqn. 1). A one-compartment model was considered to be appropriate because the amount of benzo[*a*]pyrene in the aqueous phase remained constant soon after the introduction of the intestinal components (Table 2).

The rate elimination or desorption rate (k, h^{-1}) was signif-

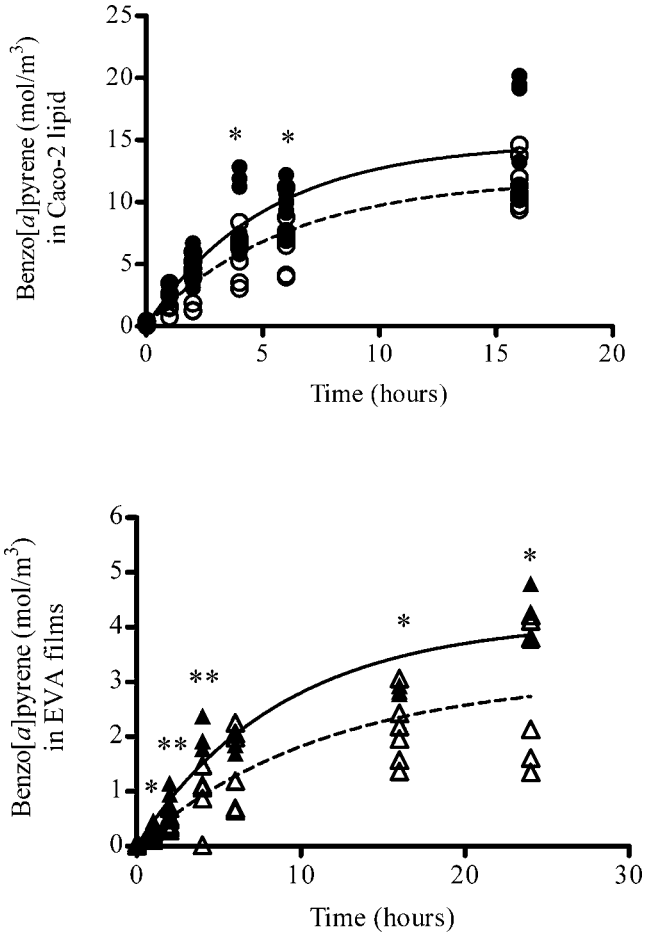

Fig. 4. The effect of aging on partitioning of [^{14}C]-benzo[*a*]pyrene ([^{14}C]-B*a*P) bound to Sidney Harbor (NS, Canada) sediment into Caco-2 cell lipids (**A**), or ethylene vinyl acetate (EVA) film (**B**) during gastrointestinal digestion in vitro. Values shown are from three independent experiments. Uptake of 1 μg of [^{14}C]-B*a*P from digested sediments aged for four months (dashed line and open symbols) or 7 d; nonaged (solid line and closed symbols) into ethylene vinyl acetate (EVA) (triangles); or fixed Caco-2 monolayers (circles). Asterisks indicate that the contaminated sediment with aged B*a*P was significantly different from the nonaged sediment at $p < 0.05$ (*) or $p < 0.01$ (**).

Table 2. First-order rate constant of [^{14}C]-benzo[*a*]pyrene ([^{14}C]-B*a*P) elimination from Caco-2 cells or ethylene vinyl acetate (EVA) thin films from two soil samples and from Sydney Harbor (NS, Canada) sediment. All soils/sediment were spiked with [^{14}C]-B*a*P 7 d prior to the assay except the aged sediment, which was stored for four months at room temperature in the dark prior to analysis.
SE = standard error

Soil type	Sorptive surface	k (h^{-1}) ± SE	r^2
High organic matter	Caco-2	0.23 ± 0.04	0.76
	EVA	0.09 ± 0.02	0.89
Low organic matter	Caco-2	0.46 ± 0.12	0.59
	EVA	0.24 ± 0.04	0.76
Sediment (four months aged)	Caco-2	0.17 ± 0.02	0.85
	EVA	0.09 ± 0.03	0.72
Sediment (nonaged)	Caco-2	0.20 ± 0.03	0.84
	EVA	0.11 ± 0.02	0.91

icantly higher for low organic matter soil when compared to high organic matter soil for both Caco-2 and EVA. This suggests that the presence of organic matter slowed the desorption rate of B*a*P from soil matrices. Sun and Li [35] reported that, as the organic matter content of their soil samples was reduced from 18.7% to 0.6%, the percentage of pyrene accumulated by earthworms increased from 1.9 to 18.5% for nonaged soils and from 0.9 to 3.7% for soils aged 120 d.

In addition, the B*a*P desorption rate from Caco-2 lipids consistently was twofold higher than EVA for all matrices tested. The more rapid kinetics observed with Caco-2 cells probably were due to the greater surface area available for absorption/desorption in Caco-2 cells. Reeves et al. [5] measured desorption rate constants for 13 different PAHs from coal tar–contaminated soil using three aqueous extraction media (phosphate-buffered water, distilled water, and acetic acid) and Tenax beads. The rate constants ranged from 0.03 to 0.9 h^{-1}, similar to those obtained in our study. They found that the majority of the values in the desorption experiments were not significantly different between soils, nor between aged and nonaged samples of the 13 different PAHs tested, with only anthracene and indeno[1,2,3-*cd*]pyrene exhibiting significant differences. No differences in desorption rate constants were observed between aged and nonaged field-contaminated sediments for both EVA and Caco-2 cells (Table 2). The lack of effect of aging on the rate of B*a*P desorption with Sydney Harbor sediment confirmed data obtained by L. Meloche (Simon Fraser University, Burnaby, BC, Canada, Master's thesis) using thin-film solid-phase extraction method [25]. In that study, they observed no significant differences in rates of PCB uptake between aged (102 d) and nonaged marine sediment.

Sydney Harbor sediment had rate constants similar to those found using high organic matter soil, despite the fact that the percent of organic matter of the sediment was comparable to low organic matter soil. Furthermore, the surface area available for desorption was expected to be greater for sediment because 66% of the particles by mass were <63 μm in diameter (Table 1). Although Sydney Harbor sediment has approximately the same percentage of organic matter as the low organic matter soil, the type of organic matter likely differed between the two samples: Nonaqueous phase liquid and carbon particles likely are present in sediment compared to predominantly humic and fulvic acids in the pristine soils. Desorption rate constants of PAH from soot and soot-like materials have been shown to be extremely slow; approximately 10^{-6} h^{-1} [36]. The k (h^{-1}) values obtained in the present study are several orders of magnitude higher than those obtained with soot alone, indicating that the added [^{14}C]-B*a*P may be bound to organic matter other than soot-like materials.

CONCLUSIONS

The in vitro model with digestion and exposure to a membrane or membrane surrogate is sensitive to matrix effects on bioavailability. Differences in soil organic matter concentration were reflected in the extent of B*a*P mobilization from soil and sediment. The lipid-normalized concentration of B*a*P in Caco-2 cells showed a linear relationship with the concentration in EVA thin film over a range of B*a*P concentrations. Furthermore, the similarity between the uptake values with live and metabolically inert cells suggests that the major route of B*a*P uptake into Caco-2 cells is passive diffusion. Together, these data indicate that EVA thin film is a good surrogate for passive membrane uptake of HOCs.

Regardless of the amount or quality of organic matter present, the rate of desorption from Caco-2 cells consistently was twofold greater than with EVA thin film. The more rapid kinetics observed with cells likely are due to the greater surface area of desorption of the Caco-2 cells.

The rough estimates for transit times in healthy humans following ingestion of a standard meal (i.e., solid, mixed foods) in the small intestines showed that 50% of the ingested food would be emptied in 2.5 to 3.4 h [37,38], with maximum prolonged transit up to 6 h due to presence of solids. Hence, for in vitro testing, nonequilibrium conditions (5 h) are more relevant for the human health risk assessment. However, although concentrations in the bioaccessible fraction paralleled the bioavailable fraction under nonequilibrium conditions, the absolute amount of [^{14}C]-B*a*P accumulated by Caco-2 cells was three orders of magnitude greater than the mobilized fraction. Thus, by measuring only the mobilized fraction, one can underestimate the bioavailable fraction.

Acknowledgement—The authors gratefully acknowledge the Natural Sciences and Engineering Research Council of Canada and Vizon SciTec for financial support.

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