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PRELIMINARY PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS FOR BENZO[A]PYRENE AND DIBENZO[DEF, P]CHRYSENE IN RODENTS

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants generated as byproducts of natural and anthropogenic combustion processes. Despite significant public health concern, physiologically based pharmacokinetic (PBPK) modeling efforts for PAHs have so far been limited to naphthalene, plus simpler PK models for pyrene, nitropyrene, and benzo[a]pyrene (B[a]P). The dearth of published models is due in part to the high lipophilicity, low volatility, and myriad metabolic pathways for PAHs, all of which present analytical and experimental challenges. Our research efforts have focused upon experimental approaches and initial development of PBPK models for the prototypic PAH, B[a]P, and the more potent, albeit less studied transplacental carcinogen, dibenzo[def, p]chrysene (DBC). For both compounds, model compartments included arterial and venous blood, flow limited lung, liver, richly perfused and poorly perfused tissues, diffusion limited fat, and a two compartment theoretical gut (for oral exposures). Hepatic and pulmonary metabolism was described for both compounds, as were fractional binding in blood and fecal clearance. Partition coefficients for parent PAH along with their diol and tetraol metabolites were estimated using published algorithms and verified experimentally for the hydroxylated metabolites. The preliminary PBPK models were able to describe many, but not all, of the available data sets, comprising multiple routes of exposure (oral, intravenous) and nominal doses spanning several orders of magnitude. Supported by Award Number P42 ES016465 from the National Institute of Environmental Health Sciences.

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Conflict of Interest Statement

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Keywords

PBPK modeling; benzo[a]pyrene; dibenzo[def, p]chrysene; polycyclic aromatic hydrocarbons

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants with natural and anthropogenic sources. PAHs are generated as by-products of incomplete combustion, and are also present in many fossil fuels (e.g. crude oil, coal) (U.S.EPA, 1991b). PAHs are lipophilic and non-volatile, and are therefore found primarily in soils, sediments, and adsorbed to particulate matter in the air (Baan *et al.*, 2009). Many PAHs are thought to be carcinogenic to animals and humans, including the prototypic PAH, benzo[a]pyrene (B[a]P), and the less well-studied, more potent dibenzo[def, p]chrysene (DBC; formerly referred to as dibenzo[a, l]pyrene).

DBC has been observed to be a highly potent carcinogen in studies in laboratory animals (Cavalieri *et al.*, 1989; Cavalieri *et al.*, 1991; Higginbotham *et al.*, 1993; Lavoie *et al.*, 1993; Prahalad *et al.*, 1997). DBC exposure has been shown to cause skin tumors in SENCAR mice exposed dermally (Cavalieri *et al.*, 1989; Cavalieri *et al.*, 1991; Higginbotham *et al.*, 1993; Lavoie *et al.*, 1993), mammary tumors in Sprague Dawley rats exposed intramammarily (Cavalieri *et al.*, 1991), and lung and liver cancers in CD-1 and A/J mice exposed intraperitoneally (Pralhad *et al.*, 1997; Platt *et al.*, 2004). DBC has been found to be approximately 100-fold more potent in producing lung adenomas than B[a]P (Pralhad *et al.*, 1997). Recently, DBC has been shown to cross the placenta in B6129SF1/J mice, causing T-cell lymphoma, lung adenoma, and liver lesions in offspring of mothers exposed to single doses of 15 mg/kg DBC (Yu *et al.*, 2006; Castro *et al.*, 2008). The International Agency for Research on Cancer (IARC) currently classifies DBC as a 2B, or possibly carcinogenic to humans (IARC, 2010).

Isolated from coal tar and identified in 1933, B[a]P is one of the earliest recognized and best studied chemical carcinogens (reviewed in (Phillips, 1983)). B[a]P is classified as a 2A or probable human carcinogen by the International Agency for Research on Cancer (IARC), based on the strong weight of evidence of animal carcinogenicity and mechanistic data rather than human epidemiological studies (Baan *et al.*, 2009; IARC, 2010). While lung cancer is induced in humans by mixtures of PAHs, there is inadequate human data on exposure to B[a]P alone. Exposures to B[a]P via oral, inhalational, and dermal routes have been demonstrated to be carcinogenic in a variety of laboratory animals, including rodents and primates (IARC, 1973; U.S.EPA, 1991b; U.S.EPA, 1991a).

DBC and B[a]P share many similarities in their physical chemical properties, as well as their presumed mechanisms of toxicity. Both are very lipophilic, non-volatile, high molecular weight PAHs, with six and five aromatic rings, respectively. DBC and B[a]P, like most PAHs, are thought to induce carcinogenesis through the interaction of electrophilic metabolites with cellular macromolecules (e.g., DNA and proteins). Both compounds have complex metabolic pathways centering upon reactive bay and fjord regions of their structures, with diol epoxide and o-quinone metabolites as the primary carcinogenic

metabolites (Figure 1) (Xue and Warshawsky, 2005). While cytochrome P450 (CYP) enzymes play an important role in each of their metabolic pathways, the responsible isoforms appear to vary for each chemical: CYP1A1 and CYP1B1 isoforms both contribute significantly to the oxidation of B[a]P, while CYP1B1 appears to be more relevant in the oxidation of DBC (Gelboin, 1980; Conney, 1982; Shimada *et al.*, 1999). CYP1A1 has little constitutive expression, but is highly inducible in many tissues, including lung and liver (Dey *et al.*, 1999). CYP1B1 is constitutively expressed in lung and a variety of hormonal tissues (e.g., adrenal, thymus, ovary, testes, and mammary glands), and is also inducible in liver tissue (Walker *et al.*, 1995; Buesen *et al.*, 2002; Zhang *et al.*, 2003).

Physiologically based pharmacokinetic (PBPK) models are mathematical descriptions of physiology and biochemistry that facilitate extrapolations between different organisms and exposure scenarios. In recent years, PBPK models have been used increasingly in the risk assessment process to provide a link between laboratory studies and human toxicity, as well as a quantitative basis for developing human exposure limits. Despite the ubiquity and toxicity of many PAHs, PBPK models for the vast majority of these compounds, particularly those of higher molecular weight, have not been developed. Indeed, with the exception of models for lower molecular weight PAHs, such as pyrene (Haddad *et al.*, 1998) and naphthalene (Willems *et al.*, 2001), there are no well explicated PBPK models for any member of this family of compounds, perhaps because of the experimental challenges associated with their high lipophilicity and low volatility, or their complex and extensive metabolism that presents both analytical and model development challenges. No PBPK models exist in the literature for DBC, despite its potency and potential transplacental carcinogenicity. The only published PBPK model for B[a]P in rodents is rudimentary (Roth and Vinegar, 1990), while those described for humans have not been evaluated against any pharmacokinetic data (Chiang and Liao, 2006; Cifroy *et al.*, 2011).

In this paper, we present pharmacokinetic data for DBC administered orally in female B6129SF1/J mice, the same strain of mice used in transplacental carcinogenicity studies, as well as preliminary PBPK models for DBC and B[a]P in rodents. While development of the preliminary DBC model is the primary goal of this work, because of the insufficient data available in the literature, as well as the relative importance of B[a]P as a representative PAH carcinogen and ubiquitous environmental contaminant, a PBPK model of B[a]P has also been developed as a tool to inform and evaluate DBC modeling efforts. This work provides a foundation for the ongoing development of mechanistic models of PAHs, facilitating the identification of important data gaps and design of experiments to aid in the translation of results from animal studies to relevant human exposures.

Methods and Materials

Reagents and Chemicals

DBC, 11,12-DBC diol, 11,12,13,14-DBC tetraols, and 7,8,9,10-B[a]P tetraols were synthesized according to previously reported methods (Krzeminski *et al.*, 1994; Luch *et al.*, 1998; Sharma *et al.*, 2004). B[a]P (for use as an internal standard), sodium sulfate, sulfuric acid, acetone, methanol, tetrahydrofuran, and ethyl acetate were purchased from Sigma (St. Louis, MO, USA). All solvents were of HPLC grade.

Animals

Female B6129SF1/J mice (19.9 ± 0.9 g, $n=36$) from Jackson Laboratory (Bar Harbor, ME, USA) were housed individually in suspended plastic cages with chipped bedding, in rooms maintained at $21 \pm 2^\circ\text{C}$ and $50 \pm 10\%$ relative humidity with a 12-h light/dark cycle. Mice were given a minimum acclimation period of seven days before experiments were begun. Lab diet certified rodent chow and water were provided *ad libitum*. The animal facility is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). All animal protocols were approved by the Institutional Animal Care and Use Committee at Pacific Northwest National Laboratory and studies were performed in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH, 2011).

Pharmacokinetic Studies

To develop and evaluate the initial PAH PBPK models, pharmacokinetic data were culled from the literature for B[a]P, and since no comparable data are available, a pharmacokinetic study of DBC was undertaken in female B6129SF1/J mice, the same strain of mice used by Yu et al. (2006) in their transplacental carcinogenicity studies.

Mice ($n=36$, 19.9 ± 0.9 g) were dosed by oral gavage to 15 mg/kg DBC dissolved in corn oil (0.2 ml/kg body weight), based on the dose level used in the prior DBC transplacental carcinogenesis study (Yu *et al.*, 2006). At 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 12, 24, and 48 hours post-exposure, subgroups of three mice were sacrificed by CO_2 asphyxiation followed by exsanguination through the vena cava. Animals designated for 48 hr sacrifice were individually housed in all-glass metabolism cages for separate collection of urine and feces over dry ice. Whole blood was collected in heparinized vials, tissues were dissected and all samples were flash frozen and stored at -80°C until analyses.

Sample Preparation and Analysis

Samples were prepared and analyzed as follows: 250 mg whole blood were spiked with 5 μL of an 8.6 μM B[a]P solution as an internal standard. 250 μL 0.9 M sulfuric acid and 250 mg sodium sulfate were added to each sample, and vortex mixed for 30 seconds. The samples were then thrice extracted with 0.5 mL ethyl acetate and centrifuged for 10 minutes at $1400 \times g$, and the combined supernatant of each sample evaporated to dryness under a gentle stream of nitrogen. Samples were reconstituted in 100 μL methanol.

DBC and B[a]P were quantitated by reverse phase high pressure liquid chromatography (HPLC) using an Agilent 1100 HPLC system (Santa Clara, CA, USA) equipped with a fluorescence detector. 20 μL of reconstituted sample were injected onto an Ascentis 25 cm $\times$ 4.6 mm, 5 μm C18 column (Sigma Aldrich, St. Louis, MO, USA). A water:acetonitrile gradient from 45:55 to 0:100 was employed from 0 to 10 minutes, and then held at 100% acetonitrile until 22 minutes, at a constant flow rate of 0.95 mL/min. Excitation and emission wavelengths were 245 and 430 for 11,12,13,14-DBC tetraols, and 360 and 430 for 11,12-DBC diol, and 235 and 430 for B[a]P and DBC. Elution times were 5.5, 6.1, and 6.6 min for 11,12,13,14-DBC tetraols, 10.4 min for 11,12-DBC diol, 16.6 min for B[a]P, and

19.0 min for DBC. The limits of reliable quantitation (LOQ) for DBC were 0.0026 – 3.8 μM .

PBPK Model Development

Modeling Strategy—Model development was heavily focused on B[a]P, for which a greater body of literature was available, and then used as a template for the development of the DBC model. Model structures were kept as simple as possible while still describing observed pharmacokinetics. Greater emphasis was placed on independent measures of biochemical constants, rather than optimized parameters. Because of the relative simplicity of IV pharmacokinetics as compared with other routes of administration, a model of IV exposure for B[a]P was developed first, and used to refine distribution and clearance terms. The B[a]P model was then extended to include oral exposure, and absorption parameters were optimized. This model was then extrapolated to DBC and assessed alongside preliminary pharmacokinetic results.

Model Description—Models for both B[a]P and DBC ultimately had identical structures, consisting of compartments representing blood, liver, fat, lung, and lumped richly and poorly perfused tissues (Figure 2). All compartments except fat were well-mixed and flow-limited; fat was described by diffusion-limited distribution. The inclusion of each compartment in the model relates to PAH disposition and toxicity, as well as the intended purposes of the model. Liver and lung are both metabolically active tissues, and are therefore vulnerable to toxicity, and ultimately carcinogenicity, induced by reactive PAH metabolites. Additionally, both serve as sites of entry to systemic circulation: liver for oral exposures, and lung for eventual simulation of inhalation exposures in subsequent models. Fat was included in the model for its role as a sink for the highly lipophilic PAHs (DBC Log $K_{OW} \sim 7.2$; B[a]P Log $K_{OW} \sim 6.1$), and to adjust body composition in subsequent models of pregnancy. The remaining compartments represent tissue groups combined on the basis of blood perfusion, to maintain mass balance. Absorption of orally administered PAH occurred via a two compartment theoretical gut (Staats *et al.*, 1991) in which first order rate equations described absorption into the liver, transfer between gut compartments, and elimination into feces. Bioavailability was addressed through the rates of gut motility and absorption, rather than the inclusion of a fractional absorption coefficient, so that the quantity of parent PAH recovered in the feces varied dynamically based on exposure considerations (e.g. vehicle). Based on reports that B[a]P is readily bound and sequestered in rat plasma proteins and lipoproteins (Aarstad *et al.*, 1987), fractional binding of B[a]P and DBC in blood was described. Saturable metabolism (oxidation) described by the kinetic parameters V_{MAX} and K_M occurred in liver and lung compartments.

Model Parameterization—Model parameters are summarized in Tables 1 and 2. Physiological parameters are taken from Brown *et al.* (Brown *et al.*, 1997) unless otherwise noted. Tissue volumes were scaled linearly with body weight, cardiac output was scaled as $(\text{body weight})^{0.75}$, and tissue perfusion rates were set as a fractions of cardiac output.

The rate of stomach emptying (KSI, 1/min) for rats was obtained directly from the literature (Roth *et al.*, 1993), while that for mice was calculated through exponential regression of

stomach contents data for CD-1 mice (Osinski *et al.*, 2002). For both rats and mice, the rates for transfer between the intestines and the feces (KIF, 1/min) were based on the rates of transfer between the jejunum, ileum, and colon of rats (Roth *et al.*, 1993).

Metabolic parameters for the oxidation of B[a]P were those measured by Wiersma & Roth (Wiersma and Roth, 1983a) using hepatic and pulmonary microsomes from Sprague Dawley rats. Originally reported values of V_{MAX} were scaled from units of nmol B[a]P metabolized per minute per mg microsomal protein to those used in the model (nmol B[a]P metabolized per minute per g liver) using study- and tissue-specific microsomal protein yields. During model development, it became apparent that the measured V_{MAX} for B[a]P oxidation in lung was insufficient to describe local dosimetry. For this reason, the measured value for hepatic microsomes was scaled for lung using microsomal protein content and tissue volume. As appropriate data are not yet available for DBC metabolism kinetics, values for B[a]P were used directly in the DBC model.

Attempts to experimentally measure partition coefficients for DBC and B[a]P according to the methods of Jepson *et al.* (Jepson *et al.*, 1994) were thwarted by substantial binding to filters and plastic housing; therefore, partition coefficients were estimated using algorithms based on octanol:water partitioning coefficients and tissue composition (Poulin and Krishnan, 1995; Poulin and Theil, 2000). Attempts to experimentally measure partition coefficients for the tetraol metabolites of B[a]P were successful due to significantly less binding to the filters, and the results confirmed the adequacy of the algorithm approach. Estimated partition coefficients for DBC, B[a]P, and 7,8,9,10-B[a]P tetraols appear alongside measured partition coefficients for 7,8,9,10-B[a]P tetraols in Table 2.

Parameters describing fractional binding in blood, permeability of fat, and absorption from stomach and intestines were estimated using AcsIX parameter estimation and/or visual fitting to pharmacokinetic data as follows. Data from Schlede *et al.* (Schlede *et al.*, 1970) describing blood and fat concentrations of B[a]P in female Sprague Dawley rats intravenously exposed to 0.0556 mg/kg B[a]P in rat plasma were used to estimate fractional binding and fat permeability.

Estimation of oral absorption parameters from a single data source was hindered by the limited availability of oral pharmacokinetic data and the variation in vehicle used between data sets. Oral absorption and gut motility are influenced by fat content of vehicle (e.g. aqueous vs. corn oil) (Trout *et al.*, 1978; Palin *et al.*, 1982; Kim *et al.*, 1990). Thus, oral absorption parameters are study specific. For oral absorption of B[a]P in the rat, intestinal absorption (KAI) was first fitted to blood data from male Sprague Dawley rats collected after duodenal infusion of 6.0 – 10.0 ng/kg/min B[a]P in an aqueous vehicle (Krebs ringer bicarbonate with 4% BSA) (Foth *et al.*, 1988). KAI was fixed at its estimated value, and gastric absorption (KAS) was then fitted to blood data from male Sprague Dawley rats orally gavaged with 0.0008 – 0.001 mg/kg B[a]P, in the same vehicle (Foth *et al.*, 1988). Employing these estimated absorption rates for oral exposures in a corn oil vehicle yielded inadequate simulation of data, in which absorption occurred too rapidly and too completely (not shown); therefore, KAS and KAI were simultaneously optimized to blood data from male C57BL/6J mice orally gavaged with 15 mg/kg B[a]P in corn oil (Uno *et al.*, 2004). The

resulting values were used directly in the DBC model as initial estimates of bioavailability, for simulation of mice orally gavaged with 15 mg/kg DBC in corn oil.

Model Evaluation—The performance of the initial B[a]P model was compared to observed data not used in model parameterization, from Moir *et al.* (Moir *et al.*, 1998), Wiersma & Roth (Wiersma and Roth, 1983b), Roth & Vinegar (Roth and Vinegar, 1990), and Foth *et al.* (Foth *et al.*, 1988), while the DBC model was evaluated against pharmacokinetic data developed as part of this work. For each comparison, only the appropriate study- and species-specific physiological parameters (e.g. body weight) were changed in the model to perform simulations. If model simulations fell within a factor of two of observed data, the model structure and biochemical parameters were considered reasonable first estimates. However, the main purpose of developing preliminary PBPK models for these PAHs is to identify critical data gaps or needs and to more effectively design further studies that will be needed to adequately translate results from *in vivo* and *in vitro* animal studies to relevant human exposures (Table 3). Thus, when simulations deviated substantially from observed data, we have attempted to explain the disparity and used the opportunity to propose possible improvements to model structure and parameterization. Because of the dearth of pharmacokinetic data, the DBC model remains preliminary and could not be thoroughly evaluated at this time. However, the B[a]P model, which has a more extensive supporting database, should provide valuable insights for future studies with DBC. Sensitivity analyses were also performed for the preliminary B[a]P and DBC models, and are further discussed in supplementary material to this manuscript.

Software, Algorithms, and Model Code—PBPK models for B[a]P and DBC were developed using AcsIX (Aegis Corporation, Huntsville, AL, USA) as a system of algebraic and differential equations. The Gear algorithm was used for integration of double precision variables. Optimization of specific model parameters, discussed in greater detail below, was achieved using the AcsIX parameter estimation feature, with heteroskedasticity = 2 and the Nelder-Mead algorithm, and the fitting criterion was maximization of the log-likelihood function. Starting values for parameter estimation were determined from visual fitting of simulations to data. Model codes are available upon request.

Results

B[a]P Model Simulations – IV Exposures

Female Sprague Dawley rat data of Schlede et al. (Schlede et al., 1970)—

Female Sprague Dawley rats were injected via tail vein with 0.0556 mg/kg tritiated B[a]P in rat plasma. Concentrations of B[a]P were determined via thin layer chromatography in whole blood, fat, and liver at several intervals after dosing (Schlede *et al.*, 1970), and observed data and model simulations appear in Figure 3. Blood and fat data were used to estimate fractional binding ($F_b = 0.90$) and fat permeability ($PA_{FatPC} = 0.25$), respectively, as described above; these values are comparable to those used with other lipophilic compounds such as chlorpyrifos ($\log K_{OW} \sim 4.8$) (Timchalk *et al.*, 2002) and dioxin ($\log K_{OW} \sim 6.8$) (Emond *et al.*, 2006). Model prediction of blood concentrations (Figure 3A) were reasonable, though observed blood concentrations dropped more rapidly than model

predicted values initially ($t < 2$ hours), then slowed while the model simulation continued to decline. Model prediction of B[a]P concentrations in fat were also reasonable using the estimated permeability coefficient, as shown in Figure 3B. However, liver concentrations of B[a]P were significantly under-predicted by the model for the duration of the data set (Figure 3C). While limits of reliable quantitation are not given by the authors, it is unlikely, given the diminishing concentrations reported and the use of radioactivity measurements, that data were approaching the limits of reliable quantitation. It is possible that metabolic parameters measured in male Sprague Dawley rats are inappropriate for use in modeling females; however, the slower hepatic metabolism required to better describe the observed liver concentrations would negatively impact prediction of blood concentrations from the same study. Redistribution of metabolism from hepatic and pulmonary compartments alone to other tissues could improve model predictions, but additional data on metabolic parameters as well as pharmacokinetic behavior would be necessary to support such alterations of the model. Finally, binding of B[a]P to macromolecules in liver tissue, which is currently described only in the blood compartment, is another plausible explanation for the observed behavior.

Male Sprague Dawley rat data of Wiersma & Roth (Wiersma and Roth, 1983b)

—Male Sprague Dawley rats were given IV bolus doses of 0.03 mg/kg tritiated B[a]P in rat plasma, and whole blood samples taken serially via left femoral artery. Concentrations of B[a]P in whole blood were determined by liquid-liquid extraction of B[a]P from aqueous metabolites, and measurement of radioactivity (Wiersma and Roth, 1983b; Roth and Vinegar, 1990). Model prediction of blood concentrations were accurate through 200 minutes, at which point observed data plateau and the model predicts continued loss from blood (Figure 4). Despite the two fold lower dose, these data are nearly indistinguishable in magnitude from those observed by Schleder *et al.* (Schleder *et al.*, 1970) (Figure 3A). While both data sets indicate a slowing of redistribution/elimination from blood around 200 minutes, it is unclear whether the magnitude of either data set is correct. The methods employed by Wiersma & Roth (Wiersma and Roth, 1983b) may have allowed unidentified lipophilic metabolites to be quantified as B[a]P, thereby inflating observed concentrations. Regardless, model prediction of early phase blood concentrations are accurate, while terminal phase kinetics could perhaps benefit from the inclusion of dynamic, rather than simple fractional binding processes, or interaction with mobile lipid pools. Redistribution from other tissue compartments, or enterohepatic recirculation could also contribute to the observed kinetics.

Male Wistar rat data of Moir et al. (Moir et al., 1998)

—Male Wistar rats received bolus doses of 2, 6, or 15 mg/kg ¹⁴C-B[a]P in 20% emulphor via penile vein. Pharmacokinetics of B[a]P in whole blood, fat, liver, and lung were assessed in serially sacrificed rats by liquid-liquid extraction of B[a]P and metabolites, followed by analysis of B[a]P concentrations by HPLC (Moir *et al.*, 1998). For perspective, these doses were two to three orders of magnitude higher than those so far discussed. Model prediction of B[a]P in blood was highly accurate for 2 and 15 mg/kg doses, while observed data for 6 mg/kg dose were somewhat over-predicted (Figure 5A). However, there is little separation between data for 2 and 6 mg/kg despite the three-fold higher dose level. Similarly, model prediction of

B[a]P in fat had high fidelity to observed data, though 2 and 6 mg/kg data were again essentially indistinguishable in magnitude (Figure 5B). While peak liver concentrations were accurately predicted, simulation of the redistribution and elimination of B[a]P from liver tissue deviated from observed data, which plateaued 10 – 20 fold above simulations (Figure 5C). Though not as marked a deviation from observed liver concentrations as with data from Schlede *et al.* (Schlede *et al.*, 1970), this underestimation supports the need for investigation into hepatic binding and the relative rates of hepatic vs. extra-hepatic metabolism. In Figure 5D, observed concentrations of B[a]P in lung tissue are shown alongside model simulations. Significant variability in the data hinder analyses of model simulations to some extent, but lung concentrations, particularly in the terminal phase, appear to be under-predicted, especially at higher doses. B[a]P appears to linger in lung tissue, perhaps due to macromolecular binding.

Male Sprague Dawley rat data of Foth et al. (Foth et al., 1988)—Individual male Sprague Dawley rats received either an IV bolus dose via penile vein of 0.002 mg/kg B[a]P (Figure 6A) or an IV infusion via left femoral vein of 3.45 or 6.6 ng/kg/min (Figure 6B) in Krebs ringer bicarbonate with 4% BSA, and serial samples of whole blood were taken from the jugular vein or right femoral vein, respectively. B[a]P concentrations were determined via extraction and thin layer chromatography (Foth *et al.*, 1988). In Figure 6A, model prediction of initial B[a]P concentrations in blood were accurate over two orders of magnitude, but observed data plateau markedly at around 2 hours (~0.25 nM) while model predicted concentrations continue to drop until around 6 hours (~0.001 nM). In Figure 6B, model simulations of B[a]P in whole blood during and after IV infusion also under-predict observed data, albeit by a much smaller (0.5 – 2.5 fold) margin. Limits of reliable quantitation are not discussed by the authors, so we cannot rule out the possibility that blood concentrations fell within background noise levels in the case of the IV bolus data (Figure 6A). Alternatively, B[a]P adsorbed to cannulae material at early high concentration time points could be contaminating and artificially elevating terminal phase samples. In both experiments, single animals were used, rather than employing statistical replicates; thus there is no indication of inter-individual variability. It is also plausible that dynamic (saturable), rather than fractional binding is necessary to describe the wide range of pharmacokinetic data covering several orders of magnitude in dose.

B[a]P Model Simulations – Oral Exposure

Male Sprague Dawley rat data of Foth et al. (Foth et al., 1988)—Individual male Sprague Dawley rats were exposed to B[a]P via duodenal infusion at rates of 6 – 10 ng/kg/min or via oral bolus at 0.0008 – 0.001 mg/kg in Krebs ringer bicarbonate with 4% BSA, and serially blood samples taken via the jugular vein. B[a]P concentrations were determined via extraction and thin layer chromatography (Foth *et al.*, 1988). Intestinal absorption (KAI) was initially optimized from duodenal infusion data, resulting in a value of 0.04 min^{-1} . Model simulation of B[a]P in blood during and after duodenal infusion appears alongside data in Figure 6C. There is a high degree of variability in the data, including poor separation between different doses; however, model predictions are generally highly consistent with the data as a whole. There may be some indication that terminal phase blood concentrations are under-predicted, but variability in the data hinders assessment. It is

unclear why observed blood concentrations for 8.8 and 10.0 ng/kg/min exposures increase well after cessation of exposure, while the 6.0 ng/kg/min exposure data behave in an expected manner (i.e. decline appropriately).

Gastric absorption (KAS) was optimized from oral bolus data, with a final value of 0.0005 min^{-1} . Model prediction of blood concentrations of B[a]P subsequent to oral bolus exposure appear in Figure 6D. Model predictions for both doses are within a factor of two of observed data during the absorption and initial redistribution/elimination phases. Terminal phase concentrations are under-predicted, as observed data plateaued around 0.02 nM and simulations indicate continued clearance. Once again, possible culprits for deviation include data being below limits of reliable quantitation, adsorbed B[a]P contaminating low concentration samples, or the potential necessity of including dynamic binding processes in the model.

For both duodenal infusion and oral bolus exposures, as well as previously reported IV bolus and infusion exposures, individual animals were used, rather than biological replicates; thus inter-individual variability may underlie some of the unexpected features of the observed data, *e.g.* poor separation of observed blood concentrations between different dose levels.

Male C57BL/6J mouse data of Uno et al. (Uno et al., 2004)—Male C57BL/6J mice received bolus doses of 15 mg/kg B[a]P in corn oil, and whole blood samples were taken after serial sacrifices, extracted, and concentrations assessed by HPLC (Uno *et al.*, 2004). Probably because of marked differences in vehicle (oil vs. aqueous buffer), but perhaps also due to species differences, the absorption parameters optimized to rat data of Foth *et al.* (Foth *et al.*, 1988) lead to significant over-prediction of these data. Thus KAI and KAS were simultaneously optimized to these data, resulting in values of 0.013 and 0.001 min^{-1} , respectively. Model simulation appears alongside observed data in Figure 7. Simulation fit was reasonable for peak blood concentrations, while somewhat higher (approximately five fold) than observed data during the redistribution and elimination phases. Notably, this is the only data set for which terminal phase concentrations in blood, or in fact any tissue, were over-estimated.

DBC Pharmacokinetic Study and Model Simulations

Pharmacokinetic data for DBC in whole blood from female B6129SF1/J mice exposed to 15 mg/kg DBC in corn oil via oral gavage appear alongside model simulation in Figure 8. Peak blood concentrations occurred two to four hours after exposure and declined thereafter, indicating that oral absorption of DBC may occur more slowly than for B[a]P administered under comparable exposure conditions; additionally, peak concentrations of DBC were significantly higher than B[a]P (Figure 7). DBC was still detectable in blood 48 hours after administration, at a concentration of 0.007 μM .

DBC concentrations in mouse blood subsequent to oral administration were under-predicted by the preliminary DBC model by approximately two fold (Figure 8, solid line). Based on its higher lipophilicity, we increased the fractional binding coefficient for DBC to 0.975 from the value optimized for B[a]P (0.90); this markedly improved the fidelity of model predictions (Figure 8, dotted line). Measured blood concentrations of DBC during the

terminal phase ($t > 360$ min) were under-predicted by a factor of 2 – 10, indicating that DBC may be sequestered in blood to a greater degree, or that metabolism may proceed more slowly, than for B[a]P.

Discussion

A PBPK model was developed and evaluated for B[a]P in rats, and a preliminary PBPK model developed for DBC in mice based on pharmacokinetic data generated as part of this research. These models create a foundation for contextualizing the broad base of literature on this class of persistent environmental contaminants. B[a]P is classified as a human carcinogen, and a large body of literature supports its carcinogenicity in rodents and humans. DBC, while much less studied, is considered to be more potent than B[a]P, and has recently been observed to cause transplacental carcinogenesis in mice (Yu *et al.*, 2006; Castro *et al.*, 2008). The models we present provide a valuable tool for guiding experimental research on poorly understood aspects of the disposition of DBC and B[a]P, and eventually for elucidating the relationship between animal toxicity experiments and realistic human exposures. By integrating all of the available pharmacokinetic data for these two PAHs into a single framework, we have been able to identify consistent trends as well as discrepancies between data sets and chemicals that may not be obvious when evaluating individual studies. In Table 3, the primary data gaps and impediments for continued model development are presented, and these issues are further discussed below.

These models currently describe only the parent compounds, a distinct limitation of model utility, as in both cases carcinogenesis is associated with reactive metabolites of B[a]P and DBC. For B[a]P, there are no pharmacokinetic data on individual metabolites, nor are there adequate *in vitro* or *in vivo* data describing individual reactions from its metabolic pathways. For DBC, there is no published information regarding metabolism, and thus the preliminary DBC model relies on kinetic values derived from B[a]P. We are currently working to extend both models to include metabolite sub-models, a process which requires additional pharmacokinetic analyses as well as *in vitro* metabolic studies describing individual steps of their metabolic pathways. A key to successfully completing these studies will be continued custom synthesis of metabolite standards, as most are not available commercially, as well as continued analytical methods development for these metabolites.

Binding of B[a]P and DBC in blood was described using a simple fractional binding coefficient, rather than a more physiologically realistic description of association and dissociation rate constants and binding capacity. A simplified approach was considered more appropriate based on the limited data available to support description of binding. While binding of B[a]P to various constituents of serum has been explored, only information about fractional binding is currently available (Aarstad *et al.*, 1987). Standard approaches to measurement of binding kinetics, such as equilibrium dialysis or ultrafiltration, are complicated by the high lipophilicity of B[a]P (log KOW ~ 6.1), which causes profound adsorption to or absorption by plastic materials commonly used in experimentation. We found that increasing fractional binding in the DBC model relative to the B[a]P value greatly improved model predictions. This is consistent with the physical chemical properties of DBC, which is significantly more lipophilic than B[a]P, and thus likely to associate more

profoundly with proteins and mobile lipids in the largely aqueous blood compartment. Sensitivity analyses (discussed at length in Supplementary Materials) indicated that the fractional binding coefficient was the most sensitive parameter in the models by a wide margin, underscoring the importance of experimental work to develop and support this feature.

The simplified description of oral absorption as occurring through a two compartment theoretical GI tract has been commonly employed for lipophilic chemicals (Fisher *et al.*, 2000). In order to adequately describe the available data on oral absorption of B[a]P, absorption rates had to be fitted to each data set. However, given the variation in experimental dosing regimens, this is not surprising. The data of Foth *et al.* (Foth *et al.*, 1988) used an aqueous vehicle for exposure of Sprague Dawley rats, while that of Uno *et al.* (Uno *et al.*, 2004) used corn oil for exposure of C57BL6/J mice. While inter-species differences could be contributing, the differences in vehicle certainly contribute to the variation in absorption.

Deviation of B[a]P model predictions from observed data occurred in simulation of several data sets. As seen in Figures 3 and 5, observed terminal phase concentrations of B[a]P in liver and lung were significantly higher than model predictions, possibly indicating macromolecular interactions in these tissues (*e.g.* binding). Terminal phase concentrations of B[a]P in blood were also under-predicted for some, but not all, of the available data (*e.g.*, simulations of the lowest available data on IV bolus exposures, the 0.03 mg/kg and 0.002 mg/kg IV exposures of Wiersma & Roth (Wiersma and Roth, 1983b) and Foth *et al.* (Foth *et al.*, 1988), respectively, were significantly below observed concentrations). While it is possible that our fractional description of binding in blood is insufficient at such low exposures, it is notable that in neither paper were the reliable limits of quantitation reported. This, as well as the distinct plateau visible in each data set, may indicate that measured concentrations were too near background to accurately quantify. The data of Foth *et al.* (Foth *et al.*, 1988), in particular, merits further discussion: our model predictions of each of the reported data sets deviated in some manner and to some degree (Figure 6). Specific aspects of experimental design (*i.e.*, no biological replicates, the use of plastic vascular catheters, and unreported limits of reliable quantitation) would generally have precluded the use of these data in model development and evaluation; however, because of the uniqueness of the data, we chose to include them. Foth *et al.* are the only researchers to report and compare B[a]P pharmacokinetics after multiple routes of administration. In particular, their investigation of duodenal infusion and oral bolus exposures were invaluable for model development, and were thus used, albeit with caution.

Despite deviations in fit, the B[a]P model is able to reasonably predict concentrations of B[a]P in blood and several tissues of rodents following exposures covering three orders of magnitude and both IV and oral routes of exposure. Additionally, because of the similarities between B[a]P and DBC, we were able to use the B[a]P model as a template for DBC model development.

Until now, pharmacokinetic data for B[a]P have almost exclusively been evaluated using non-compartmental modeling approaches. Non-compartmental analyses facilitate estimation

of descriptive parameters such as volumes of distribution, clearance rates, and biological half-lives through empirical descriptions of observed data. However, because they do not consider physiological constraints or biochemical intricacies, such as blood flow, partitioning, or binding, non-compartmental analyses have very limited utility for extrapolation and predictions under varying exposure conditions or in different organisms. PBPK models, such as the ones described here, rely on the incorporation of physiological and biochemical information to develop models that are capable of extrapolation and prediction. This will be especially important as work proceeds to incorporate life-stage information (pregnancy, growth and development) to extrapolate results from transplacental carcinogenicity studies in the future.

A single PBPK model for intravenous dosing of B[a]P in rats has been reported by Roth & Vinegar (Roth and Vinegar, 1990), but model structure and parameterization were not well described. The model structure was comparable to that reported here, and also used the *in vitro* metabolic parameters of Wiersma & Roth (Wiersma and Roth, 1983a) to describe hepatic and pulmonary metabolism. Partition coefficient values, not specifically reported, were achieved through some method of fitting or estimation to observed data. While binding in blood, lung, and liver was included in the model, the authors did not describe whether it was fractional or dynamic, nor did they report the degree of binding (i.e. parameter values) or the basis for its inclusion. Additional PBPK models for B[a]P in humans have been reported more recently (Chiang and Liao, 2006; Cifroy *et al.*, 2011), and while their structures and parameterization are better described, neither has been evaluated against pharmacokinetic data.

The models we describe here for B[a]P and DBC are thus the first well-explicated PBPK models for high molecular weight PAHs that include extensive evaluation against available pharmacokinetic data in rats and mice, covering a wide range of doses and routes of exposure. During model development, we added only what complexity was needed to describe the available pharmacokinetic data, thereby creating a model for B[a]P with few optimized parameters. The B[a]P model reasonably predicts B[a]P in blood and several tissues following exposures covering three orders of magnitude, as well as both IV and oral routes of exposure. While model predictions were generally adequate, under-prediction of liver and lung concentrations indicate that further research is warranted for these tissues. Disparities between data sets also underscore the necessity of undertaking clarifying pharmacokinetic studies.

Because of their similar physical chemical properties and mechanisms of toxicity, as well as the comparative dearth of available data, the preliminary DBC model is heavily reliant on the B[a]P model for its parameterization. Evaluation against our initial pharmacokinetic data support this approach, as model predictions were generally within a factor of two. Increasing fractional binding of DBC in blood to 97.5% (compared to 90% for B[a]P) brought model predictions in line with observed data, and is plausible based on the higher lipophilicity of DBC. The preliminary DBC model will certainly benefit from additional pharmacokinetic studies and chemical specific parameterization, but in its current iteration is a useful guide for continuing experimental studies, as well as a promising foundation for further model

development into additional routes of exposure (specifically inhalation), other organisms including humans, and important life stages (e.g. pregnancy).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research Highlights

- We developed PBPK models for benzo[a]pyrene (B[a]P) and dibenzo[def, p]chrysene (DBC) in rodents
- The B[a]P model accurately predicted data from multiple sources over a wide range of doses
- The DBC model was based on the B[a]P model as less chemical specific information is available
- The DBC model accurately predicted preliminary pharmacokinetic data
- The DBC model underscored existing data gaps on metabolism, binding and pharmacokinetics

Figure 1.
Major metabolic pathways for benzo[a]pyrene and dibenzo[def, p]chrysene.

Figure 2.

Schematic of PBPK model structures for B[a]P and DBC in rodents. Parent PAHs are administered intra-venously, intra-arterially, orally, or duodenally as data require (see text). For oral and duodenal exposures, PAH moves through and is absorbed from a theoretical two-compartment GI tract. Subsequent to exposure and uptake, PAH moves between physiological compartments (blood, lung, fat, liver, richly and poorly perfused tissues) based on blood flow rates (Q) and concentrations (C), and in the case of fat, tissue permeability. Saturable metabolism occurs in liver and lung compartments. Tables 1 and 2 provide a list of model parameter definitions and values.

Figure 3.

Data (symbols) and model simulations (lines) of the concentrations of B[a]P in (A) whole blood, (B) fat, and (C) liver of female Sprague Dawley rats (180 ± 5 g) administered 0.056 mg/kg B[a]P in rat plasma via tail vein injection (data from Schlede *et al.* (1970)). Blood and fat data were used for optimization of fractional binding and fat permeation coefficients, respectively.

Figure 4.

Data (symbols) and model simulation (line) of the concentration of B[a]P in whole blood of male Sprague Dawley rats (250 ± 50 g) administered 0.03 mg/kg B[a]P in rat plasma via injection into the left carotid artery (data from Wiersma and Roth (1983b); Roth and Vinegar (1990)).

Figure 5. Data and model simulations of the concentrations of B[a]P in (A) whole blood, (B) fat, (C) liver, and (D) lung of male Wistar rats (324 ± 39 g) administered 2 (····, ○), 6 (---, ●), or 15 (—, ●) mg/kg B[a]P in 20% emulphor via the penile vein (data from Moir *et al.* (1998)).

Figure 6.

Data and simulations of the concentration of B[a]P in serially sampled whole blood of individual male Sprague Dawley rats (380 ± 80 g) exposed to (A) a bolus dose of 0.002 mg/kg B[a]P via the penile vein, (B) an infusion of 3.45 (····,○) or 6.64 (—,●) ng/kg/min B[a]P for 90 minutes via the left femoral vein, (C) a duodenal infusion of 6.0 (····,○), 8.8 (---, ●), or 10.0 (—,●) ng/kg/min B[a]P for 90 minutes, and (D) a bolus doses of 0.00081 (····,○), or 0.00101 (—,●) mg/kg B[a]P via oral gavage (data from Foth *et al.* (1988)). Data from duodenal infusion and oral bolus exposures were used for optimization of rates of intestinal absorption and gastric absorption, respectively.

Figure 7.

Data (symbols) and simulation (line) of the concentration of B[a]P in whole blood of male C57BL/6J mice (20 g) administered bolus doses of 15 mg/kg B[a]P in corn oil via oral gavage (data from Uno *et al.* (2004)). Data were used for optimization of intestinal and gastric absorption rates.

Figure 8.

Data (symbols) and simulations (lines) of the concentration of DBC in whole blood of female B6129SF1/J mice (19.9 ± 0.9 g) administered bolus doses of 15 mg/kg DBC in corn oil via oral gavage. Increased fractional binding (....., FB = 0.96) improved model predictions compared to the fractional, FB = 0.86).

Table 1

Physiological and biochemical parameters for B[a]P and DBC PBPBK Models

Parameter	Symbol	Units	Rat	Mouse	Source/Comments
Body Weight	BW	kg	0.25	0.025	Default; study specific values used where available
Cardiac Output	QCC	mL/min	1.756	1.253	(Brown et al., 1997)
<i>Fractional Tissue Volumes (% Body Weight)</i>					
Arterial Blood	VARTC	--	0.025	0.017	(Brown et al., 1997)
Venous Blood	VVENC	--	0.05	0.033	(Brown et al., 1997)
Fat	VFATC	--	0.065	0.07	(Brown et al., 1997)
Blood in Fat (% of Fat Volume)	VFATBLDC	--	0.02	0.02	(Brown et al., 1997)
Liver	VLIVC	--	0.037	0.055	(Brown et al., 1997)
Lung	VLNGC	--	0.005	0.005	(Brown et al., 1997)
Richly Perfused Tissues	VRICHC	--	0.098	0.1	1 - (VARTC + VVENC + VFATC + VLIVC + VLNGC + VPOORC)
Poorly Perfused Tissues	VPOORC	--	0.6	0.6	(Brown et al., 1997)
<i>Fractional Blood Flows (% Cardiac Output)</i>					
Fat	QFATC	--	0.07	0.059	(Brown et al., 1997)
Liver	QLIVC	--	0.183	0.161	(Brown et al., 1997)
Richly perfused tissues	QRICHC	--	0.4	0.48	(Brown et al., 1997)
Poorly Perfused Tissues	QPOORC	--	0.347	0.3	1 - (QFATC + QLIVC + QRICHC)
Fractional binding in blood	FB	--	0.9	rat value	Optimized to blood data from Schlede <i>et al.</i> (1970)
Fat permeation coefficient	PAFATPC	--	0.25	rat value	Optimized to fat data from Schlede <i>et al.</i> (1970)
Absorption rate from theoretical stomach	KAS	1/min	0.0005	0.001	Rat: optimized to oral bolus data from Foth <i>et al.</i> (1988). Mouse: optimized to oral bolus data from Uno <i>et al.</i> (2004).
Absorption rate from theoretical intestines	KAI	1/min	0.04	0.013	Rat: optimized to duodenal infusion data from Foth <i>et al.</i> (1988). Mouse: optimized to oral bolus data from Uno <i>et al.</i> (2004).
Gastric emptying rate	KSI	1/min	0.006	0.02	Rat: Roth <i>et al.</i> (1993). Mouse: Exponential regression of stomach contents data of CD-1 mice from Osinski <i>et al.</i> (2002).
Fecal elimination rate	KIF	1/min	0.0045	rat value	Roth <i>et al.</i> (1993)
Maximal rate of metabolism in liver	VMAXLIVPA	nmol/min/mL	44.7	rat value	(Weirisma & Roth, 1983a)
Maximal rate of metabolism in lung	VMAXLNGPA	nmol/min/mL	18.13	rat value	Liver V _{MAX} scaled to lung microsomal protein content
Michaelis constant for metabolism in liver	KMLIVPA	nmol/mL	5.5	rat value	(Weirisma & Roth, 1983a)
Michaelis constant for metabolism in lung	KMLNGPA	nmol/mL	0.22	rat value	(Weirisma & Roth, 1983a)

Table 2

Partition coefficients for B[a]P, the tetrol metabolites of B[a]P, and DBC.

Parameter	Symbol	Rat			Mouse		
		B[a]P ^a	B[a]P-tetrols ^a	B[a]P-tetrols ^b	B[a]P ^a	DBC ^a	DBC ^a
Fat:Blood	PFATP	496.38	153.59	--	496.38	496.56	
Liver:Blood	PLIVP	13.31	4.71	3.84	13.31	13.32	
Lung:Blood	PLNGP	14.54	5.15	6.87	7.49	7.49	
Richly perfused tissues:Blood ^c	PRICHP	13.31	4.71	3.84	13.31	13.32	
Poorly perfused tissues:Blood ^d	PPOORP	6.99	2.77	1.88	6.99	6.99	

^a Calculated using an algorithm that incorporates tissue composition and octanol:water partition coefficients (Poulin and Theil, 2000).

^b Measured according to the method of Jepson et al. (1994).

^c Liver value used for richly perfused tissues

^d Muscle value used for poorly perfused tissues

Table 3
Primary data gaps and required experimental work for continuing PAH PBPK model development.

Model Feature or Data Gap	Required Data	Comments
<i>Model extensions</i>		
Metabolite sub-models	Metabolite PK data; metabolism kinetic parameters; partition coefficients	Requires continued access to or custom synthesis of potential metabolites, and improved analytical methods.
Pregnancy	Maternal and fetal PK data; fetal metabolic parameters; partition coefficients	Requires improved sensitivity of methods to analyze individual fetal tissues, as well as genotyping of individual fetuses for Ah responsiveness
Human extrapolated model	Human metabolic parameters; partition coefficients; any available PK data	Sources for human PK data may be limited to biomonitoring data from poorly defined exposures, or controlled studies at ultra-low doses due to potential toxicity
<i>Model refinements</i>		
Clarification of B[a]P PK	Additional PK studies of B[a]P, particularly after oral administration	
Blood binding	Refined fractional values; binding kinetic parameters for dynamic binding if possible	Traditional measurement techniques for association and dissociation constants are complicated by adsorption to apparatus
Tissue binding	Fractional values; binding kinetic parameters for dynamic binding if possible	
Extrahepatic metabolism	<i>In vitro</i> metabolic parameters for other tissues	Isoforms may have different tissue specificity, activity
<i>Additional areas of interest</i>		
Ontogeny of metabolism	Metabolic data at different developmental stages	Access to late-term human fetal tissues is problematic
Induction of enzymes	Repeated exposure PK data; induced metabolic parameters	
Receptor binding	Interactions with Ah receptor	

PK, pharmacokinetic; Ah, aryl hydrocarbon.