Human in Vivo Pharmacokinetics of $^{14}$CDibenzo[def,p]chrysene by Accelerator Mass Spectrometry Following Oral Microdosing

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ABSTRACT: Dibenzo(def,p)chrysene (DBC), (also known as dibenzo[a,l]pyrene), is a high molecular weight polycyclic aromatic hydrocarbon (PAH) found in the environment, including food, produced by the incomplete combustion of hydrocarbons. DBC, classified by IARC as a 2A probable human carcinogen, has a relative potency factor (RPF) in animal cancer models 30-fold higher than benzo[a]pyrene. No data are available describing the disposition of high molecular weight (>4 rings) PAHs in humans to compare to animal studies. Pharmacokinetics of DBC was determined in 3 female and 6 male human volunteers following oral microdosing (29 ng, 5 nCi) of $^{14}$CD-DBC. This study was made possible with highly sensitive accelerator mass spectrometry (AMS), capable of detecting $^{14}$CD-DBC equivalents in plasma and urine following a dose considered of de minimus risk to human health. Plasma and urine were collected over 72 h. The plasma Cmax was 68 ± 44.3 fg·mL$^{-1}$ with a Tmax of 2.25 ± 1.04 h. Elimination occurred in two distinct phases: a rapid ($\alpha$)-phase, with a T1/2 of 5.8 ± 3.4 h and an apparent elimination rate constant ($K_{\alpha}$) of 0.17 ± 0.12 fg·h$^{-1}$, followed by a slower ($\beta$)-phase, with a T1/2 of 41.3 ± 29.8 h and an apparent $K_{\beta}$ of 0.03 ± 0.02 fg·h$^{-1}$. In spite of the high degree of hydrophobicity (log $K_{ow}$ of 7.4), DBC was eliminated rapidly in humans, as are most PAHs in animals, compared to other hydrophobic persistent organic pollutants such as, DDT, PCBs and TCDD. Preliminary examination utilizing a new UHPLC-AMS interface, suggests the presence of polar metabolites in plasma as early as 45 min following dosing. This is the first in vivo data set describing pharmacokinetics in humans of a high molecular weight PAH and should be a valuable addition to risk assessment paradigms.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are produced by the incomplete combustion of carbon and are of concern as environmental toxicants/carcinogens. The most recent list of the priority chemicals at remediation sites by the Agency for Toxic Substances Disease Registry (ATSDR) includes individual PAHs or PAH mixtures as three of the top 10 chemicals of concern. Major sources of environmental exposure include wood smoke, creosote, and burning of fossil fuels and tobacco. PAHs consisting of four rings or less constitute the low molecular weight, volatile class. These PAHs tend to be less toxic and are typically classified as level 3 or insufficient weight of evidence as carcinogens in humans. The major route of exposure to low molecular weight PAHs, such as naphthalene, pyrene, and phenanthrene, is inhalation. The high molecular weight PAHs include five or more aromatic rings. The majority of exposure (>95%), to high molecular weight PAHs in nonsmokers, is through the diet in a variety of foods including breads and cereal grains, vegetables, and smoke-cured or barbequed meats. The high molecular weight group contains the majority of the carcinogenic PAHs, including benzo[a]-pyrene (BaP, class 1, known human carcinogen) and dibenzo[def,p]chrysene (DBC, CAS 191-30-0, class 2A, probable human carcinogen).

Dietary intake of total PAHs in the U.S. has been estimated to be between 160 and 1600 ng/day. Some studies have reported intake levels of BaP alone at 40–2800 ng/day. A 2005 report from the FAO/WHO Joint Expert Committee on Food Additives and Contaminants listed a mean BaP daily intake of 280 ng per 70 kg individual with 700 ng considered as a high-level intake. Finally, a report by Menzie et al. estimated total carcinogenic PAH intake at 3120 ng/day for a nonsmoking male, 19–50 years of age, of which 96.2% was from the diet. The European Union established a maximum limit for BaP in smoked meats at 5,000 ng/kg fresh weight. Data on DBC contamination of food is scarce. Veyrand et al. addressed DBC (also known as dibenzo[a,l]pyrene) as a...
component of food from a French market, estimating a daily exposure of 0.129 ng/kg BW in French adults and 0.208 ng/kg BW in French children.11

Crowell et al. developed a physiologically based pharmacokinetic (PBPK) model for DBC and BaP, relying upon previous studies of in vitro (human and rodent liver) BaP metabolism and the metabolic profile in vivo following oral gavage in rodents.12 Administering an oral dose of 15 mg/kg DBC to mice resulted in a T_{max} between 2 and 4 h, with detectable DBC in blood 48 h post gavage. The T_{1/2} was found to be between 3.4 and 4.8 h.12,13 The higher log K_{ow} of DBC (7.4), compared to that of BaP (6.1), is believed to contribute to a prolonged sequestration in blood and other tissues. The DBC model development relied heavily upon BaP due, in part, to the lack of available data on DBC metabolism.

Accelerator mass spectrometry (AMS) measures the ratio of 14C/C with 14C detection limits in the attomole range per mg total carbon,14,15 allowing the use of a “microdose”, defined as a dose at least 2 orders of magnitude below that which would be expected to yield a pharmacological effect. AMS is increasingly being utilized by pharmaceutical companies to assess pharmacokinetic parameters, metabolism, and excretion in humans during drug development (Phase 0 trials).16–18 These studies almost exclusively utilized 14C as the radiolabel, and doses of 100 μCi have been standard.17 AMS is an attractive method to determine human pharmacokinetics, DNA binding, and other potential biomarkers of importance in risk assessment of compounds with potential toxicity. For example, AMS has been utilized to determine DNA binding of [14C]-labeled compounds with potential toxicity. For example, AMS has been utilized to determine DNA binding of [14C]-labeled amino acid pyrolys products or cooked meat mutagens, MeIQ, PhIP, MeIQx,19–22 as well as BaP23 and the pharmacokinetics of the potent human dietary hepatocarcinogen, aflatoxin B1.24 The sensitivity provided by AMS allows for safe microdosing of human volunteers with chemical carcinogens, such as DBC, at environmentally relevant doses, providing pharmacokinetic parameters for risk assessment that do not rely solely on high-dose animal studies.

In this study, we determined the human in vivo pharmacokinetics of DBC, following microdosing, utilizing AMS. Total elimination of DBCeq (parent and metabolites), detected as [14C], are used to determine the pharmacokinetics in plasma and urine. Solid sample AMS is not able to distinguish between labeled parent compound and metabolites. Total plasma distribution and urinary elimination are compared to total plasma distribution and urinary elimination scaled from rodent models.

## EXPERIMENTAL PROCEDURES

### Human Volunteers

All protocols and procedures, including plans for recruitment and volunteer informed consent documents, were approved by the OSU Institutional Review Board. The use of radioisotopes was reviewed and approved by an OSU Radioactive Use Agreement under the oversight of the Oregon Health Authority. Healthy adult males and infertile (postmenopausal or tubally ligated) females, between the ages of 20–65, were recruited for this study. To protect confidentiality, all specimens were deidentified at the time of collection.

Additional inclusion criteria were as follows: good general health; nonsmoking; not using medications that can affect gut motility; and no history of gastrointestinal surgeries, kidney or liver disease, gastrointestinal diseases such as Crohn’s, ulcerative colitis, or gastritis. A medical examination was conducted by a licensed physician. The screening assessment included a careful menopausal history and a urine pregnancy test for all women. Women who were pregnant or capable of becoming pregnant were excluded from the study in an abundance of caution, due to the proven transplacental toxicity of DBC in high dose rodent models.25–27 A total of 9 volunteers were enrolled. Individual body weight, height, BMI, age, gender, ethnicity, and race were recorded (Table 1).

### Justification of the Dose Used in This Study

The 29 ng DBC dose utilized in this study is equivalent to the BaP content of a 5.2 oz. serving of smoked meat at the European Union maximum legal limit3 or 28% of the average daily dietary PAH intake.28 This chemical dose was chosen to be relevant to typical dietary exposure in humans. The specific activity of [l-ring-U-14C]-DBC was 5.14 nCi/nmol, resulting in 5 nCi of [14C] in a 29 ng dose. This β particle radiation dose of 5 nCi can be compared to the internal radiation of consuming 5 bananas, each containing 1 nCi.29 It would require a dose of 200 study capsules to equal the radioactive dose of a single [14C]-urea diagnostic test for Helicobacter pylori.30 The radioactive dose was chosen to be detectable by AMS without appreciable risk to volunteers consistent with the policy of utilizing doses as low as Reasonably Achievable (ALARA) or Practical (ALARP).31

### Chemicals

Dibenzo[def]chrysene (formerly known as dibenzo-[a,l]pyrene) [l-ring-U-14C], MRL Part No. U479C, 5.14 mCi/mmol, was obtained from the NCI Chemical Carcinogen Standards Reference Repository, Midwest Research Institute (Kansas City, MO). The reagents used included Ultima Flo M scintillation cocktail, sulfuric acid, HPLC grade acetonitrile, Milli-Q water, ethyl acetate, potassium sulfate, 95% ethanol (PCCA 50–3161), lactose monohydrate (PCCA 30–3329) and size 0 cellulose capsules (Spectrum, Irvine CA). Chromatography utilized a Luna 3 μm particle C18 100 Å 150 × 3 mm Phenomenex HPLC column with a C18 2 mm guard column on an Agilent 1100 HPLC with UV detector.

### [14C]-DBC Dosing Solution

A portion of the stock solution of [14C]-DBC (1.11 mCi/mL, 6.53 mg/mL benzene) was chosen to be dryness under aragon and then purified, prior to the preparation of dosing solutions, to ≥99% radiochemical purity via reverse-phase HPLC. HPLC elution conditions were isocratic 90% ACN, 10% H2O with a flow rate of 1 mL/min at a column temperature of 35 °C. The A_{280} peak containing purified [14C]-DBC (6.0 to 6.5 min retention time) was collected over several HPLC runs. The pooled eluent was evaporated to dryness under a stream of argon and resuspended in ethanol thrice to produce the dosing solution. An HPLC run of the dosing solution was collected in 15-s fractions for scintillation counting. 29 ng DBC/30 μL dose incorporated into each cellulose capsule. The stock solution was stored at −80 °C under argon and protected from light. All neat DBC powder was handled by a trained carcinogen
specialist in an enclosed glovebox, per the OSU Extreme Carcinogen handling protocol. DBC solutions were stored in a locked laboratory dedicated to carcinogens, and all waste was discarded in accordance with OSU radiation safety and hazardous material protocols.

**Capsule Manufacturing and Quality Control.** Capsules were prepared by filling empty cellulose capsules with pharmaceutical grade lactose monohydrate. Thirty microliters of 0.16 nCi/μL dosing solution was applied to the capsule and sealed by allowing the ethanol to evaporate, inverting the capsule to create a lactose layer above the dosing solution to prevent any possible integrity loss upon moistening, and the capsule cap was pinched closed over the capsule body. Every capsule batch manufactured included a dosing capsule per volunteer plus at least 3 extra for quality control. Capsules were stored at −20 °C until time of use and utilized within 5 days of preparation. Quality control was performed by scintillation counting 3 randomly chosen capsules per batch, individually dissolved by vortex in 5 mL of water prior to the addition of 15 mL of scintillation cocktail. The variability between capsules per batch was ≤5%. The exact dosage a volunteer received, measured by scintillation, is included in Table 2.

**Dosing and Sample Collection Protocols.** At 8 a.m., volunteers who had fasted overnight were orally administered a cellulose capsule containing [14C]-DBC (target dose of 29 ng DBC, 5 nCi [14C)], which was swallowed with 100 mL of water. Food and water were made available at 10 a.m. Blood was drawn by a registered nurse at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3, 4, 8, 12, 24, 48, and 72 h post-consumption and collected into glass vacutainer tubes containing anticoagulant, EDTA. An indwelling i.v. catheter was used for the consumption and collected into glass vacutainer tubes containing the 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3, 4, 8, 12, 24, 48, and 72 h post-food and water were made available at 10 a.m.

**Extraction of Plasma and Collection of Urine.** Plasma samples were collected at a separate test of extraction efficiency of the [14C]-DBC microdosing ethanol stock solution. Following ethyl acetate extraction and reconstitution with 50:50 methanol/water (v/v) used for AMS analysis (described below), the recovery was 51 ± 10% (n = 3) as determined by AMS. In a separate test of extraction efficiency, fresh or thawed frozen plasma, 0.75 mL, was spiked with 1.25 nCi [14C]-DBC solution prior to extraction resulting in 55 ± 7% recovery of [14C]-DBC as detected by scintillation counting. This process was repeated with several [14C]-DBC concentrations and with detection by AMS and scintillation counting. Overall, across a number of concentrations, 55 ± 7% (at the low end of previously reported recoveries of 61–101% in human studies with pharmaceuticals, perhaps due to the much lower mass of the dose utilized in this study) was the average DBC recovery when reconstituted in methanol/water, and this value was used for efficiency corrections in the PK parameters reported below. Recent work by Crowell et al. had determined this to be the optimal extraction method for DBC, as well as the diol, and tetrox metabolites.

**AMS of Plasma and Urine Samples.** Upon arrival at LLNL, samples were stored at −80 °C until processing. Plasma extract samples were reconstituted with 100 μL of 50:50 methanol/water and converted to graphite by the method of Ognibene et al. Urine samples (100 μL) were converted, without prior processing, by the same graphitization method. The urine data was not normalized for creatinine. All voided urine was collected, pooled by time point, and volumetrically recorded prior to aliquoting and storing pooled samples. Briefly, the samples were evaporated and flame-sealed in a quartz tube containing Cu(II) and combusted to 900 °C, producing CO2. The CO2 is then transferred to a sealed glass tube containing Zn and Co and heated to 525 °C, producing graphite on the Co catalyst. The graphite is then loaded into an aluminum sample holder for AMS analysis.

**Determination of Pharmacokinetic Parameters.** Pharmacokinetic analysis of data utilized an Excel based add-on developed at Allergan, Inc. (Irvine, CA). Briefly, the formulas rely on non-compartmental analysis of six functions: peak concentrations in plasma

| 0–6 h | 6–12 h | 12–24 h | 24–48 h | 48–72 h | elim. total | oral dose DBC (ng) | % excreted |
|-------|--------|---------|---------|---------|------------|------------------|------------|
| V1    | 0.085  | 0.049   | 0.044   | 0.046   | 0.006      | 0.230            | 23.9       | 0.96       |
| V3    | 0.044  | 0.109   | 0.038   | 0.029   | 0.000      | 0.220            | 24.5       | 0.90       |
| V5    | 0.033  | 0.184   | 0.123   | 0.119   | 0.073      | 0.532            | 25.2       | 2.11       |
| V6    | 0.179  | 0.075   | 0.107   | 0.056   | 0.024      | 0.441            | 29.8       | 1.48       |
| V8    | 0.071  | 0.046   | 0.111   | 0.058   | 0.000      | 0.285            | 23.9       | 1.20       |
| V9    | 0.052  | 0.028   | 0.063   | 0.043   | 0.014      | 0.200            | 28.5       | 0.70       |
| V10   | 0.237  | 0.104   | 0.097   | 0.027   | 0.028      | 0.494            | 25.6       | 1.93       |
| V11   | 0.113  | 0.059   | 0.052   | 0.056   | 0.017      | 0.297            | 29.7       | 1.00       |
| V13   | 0.124  | 0.056   | 0.045   | 0.033   | 0.005      | 0.263            | 28.5       | 0.92       |
| average | 0.124  | 0.056   | 0.045   | 0.033   | 0.005      | 0.263            | 28.5       | 0.92       |

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**Table 2. Total Nanograms of [14C]-DBC eq Eliminated in Urine during Each Collection Interval**

As PAHs can adsorb to plastic, care was taken to use glass containers (amber when possible) for protection from light and argon or nitrogen capping to prevent oxidation of samples. Urine was aliquoted in 1 mL volumes for shipping, requiring no additional processing prior to AMS preparation.

**Determination of Pharmacokinetic Parameters.** Pharmacokinetic analysis of data utilized an Excel based add-on developed at Allergan, Inc. (Irvine, CA). Briefly, the formulas rely on non-compartmental analysis of six functions: peak concentrations in plasma
We were able to empirically determine the environmentally relevant in vivo pharmacokinetics of \[^{14}C\]-DBC in human volunteers utilizing AMS. The pool of nine volunteers included both sexes and was characterized by an age range from 20 to 65 years of age and BMIs from 23 to 35 (Table 1). No recruitment selection was made by volunteer physical characteristics. This study was intended to assess the range of pharmacokinetics in a cross section of human volunteers; as such, it is not statistically powered to assess parameters such as gender, age, or BMI. Differences in age could lead to differences in gut motility, possibly resulting in changes in absorption. The range of BMI likely results in a range of blood volumes and distribution. Because of a necessary change in plasma collection and processing method, three volunteers were excluded from the plasma pharmacokinetic analysis (Table 3 and Figure 2); however, urine data from all nine volunteers are included (Table 2 and Figure 1).

There were some similarities in the temporal pattern (but not magnitude) of absorption/excretion between individuals (Figures 1 and 2). Levels of \[^{14}C\] in plasma rose rapidly following oral administration of \[^{14}C\]-DBC to volunteers that had been fasted overnight (Tables 3 and 4, and Figure 2). An $T_{max}$ at 2.25 h was observed, followed by a rapid $\alpha$-phase elimination prior to a slower $\beta$-phase elimination. This study was limited to the appearance and disappearance of \[^{14}C\]-labeled DBC$_{eq}$ from plasma and the levels appearing with time in urine (a true mass balance study would have required fecal analysis as well as determination of tissue levels (not possible in a human study without subsequent diagnostic surgery)). Considering differences in absorption based on motility, distribution based on BMI, and metabolism based on polymorphisms, the pharmacokinetics were notably consistent.

Recovery of urinary \[^{14}C\] over 72 h yielded 1.24 ± 0.49% of the dose administered (Table 2). Because of the high hydrophobicity ($\log K_{ow}$ 7.4), the majority of DBC is likely unabsorbed and eliminated in the feces unlike 3- or 4-ring PAHs, which were reported to be rapidly absorbed from the intestine. However, if we had collected urine over a longer period and observed a pattern similar to plasma, it could be concluded that sequestered DBC is released slowly from the system. Oral bioavailability could be expected to be strongly linked to the fat content of vehicle administered at dosing.

The fasted volunteers administered a dosing capsule with water likely limited bioavailability. Previous high dosing studies of BaP in rodents have resulted in minimal elimination in urine, 0.22–0.35% and 0.10–0.08% of oral dose administered, detected as known metabolites. Oral gavage of mice with 15 mg/kg of DBC with corn oil vehicle yielded 4.9 to 7% urinary excretion of the total oral dose. Administering an oral dose of 15 mg/kg DBC to mice resulted in a $T_{max}$ between 2 and 4 h, with detectable DBC in blood 48 h post-gavage.

### Table 3. Plasma Pharmacokinetics of DBC$_{eq}$ in Human Volunteers

| Vol | C$_{max}$ (fg·mL$^{-1}$) | $T_{max}$ (h) | AUC$_{0-t}$ (fg·h·mL$^{-1}$) | $K_{el}$ (fg·h$^{-1}$) | AUC$_{0-\infty}$ (fg·h·mL$^{-1}$) | $T_{1/2}$ (h) | $\alpha$-phase $K_{el}$ (fg·h$^{-1}$) | $\alpha$-phase AUC$_{0-t}$ (fg·h·mL$^{-1}$) | $\alpha$-phase $T_{1/2}$ (h) | $\beta$-elim $K_{el}$ (fg·h$^{-1}$) | $\beta$-elim AUC$_{0-t}$ (fg·h·mL$^{-1}$) | $\beta$-elim $T_{1/2}$ (h) | $\beta$-elim AUC$_{0-\infty}$ (fg·h·mL$^{-1}$) | Urinary DBC$_{eq}$ clearance (CL, mL/min) | SD |
|-----|-----------------|-------------|-----------------|-----------------|-----------------|-------------|-----------------|-----------------|-------------|-----------------|-----------------|-------------|-----------------|-----------------|-----------------|-----------------|
| V1  | 124.1           | 1.50        | 1616            | 0.03            | 1616            | 23.5        | 6.8             | 0.10            | 462.1       | 23.6            | 0.03            | 594.0       | 15.5           | 485.0           | 46.2            | 22.7            |
| V6  | 118.2           | 1.50        | 2850            | 0.03            | 3690            | 27.5        | 11.7            | 0.06            | 179.7       | 86.6            | 0.01            | 151.0       | 10.2           | 46.2            | 485.0           | 22.7            |
| V8  | 30.4            | 1.50        | 589             | 0.03            | 699             | 25.9        | 4.8             | 0.14            | 30.7        | 28.3            | 0.02            | 30.7        | 10.2           | 46.2            | 485.0           | 22.7            |
| V9  | 41.6            | 1.50        | 899             | 0.03            | 604             | 13.1        | 1.7             | 0.14            | 30.7        | 28.3            | 0.02            | 30.7        | 10.2           | 46.2            | 485.0           | 22.7            |
| V10 | 75.0            | 1.50        | 1921            | 0.03            | 678             | 14.2        | 5.8             | 0.12            | 30.7        | 28.3            | 0.02            | 30.7        | 10.2           | 46.2            | 485.0           | 22.7            |
| V13 | 25.3            | 1.50        | 309             | 0.03            | 338             | 21.1        | 3.8             | 0.12            | 30.7        | 28.3            | 0.02            | 30.7        | 10.2           | 46.2            | 485.0           | 22.7            |
| Avg | 68.8            | 2.25        | 1274            | 0.02            | 2254            | 42.1        | 3.4             | 0.12            | 30.7        | 28.3            | 0.02            | 30.7        | 10.2           | 46.2            | 485.0           | 22.7            |
DISCUSSION

As environmental PAHs occur in complex mixtures, ingestion/elimination kinetics in humans from PAH exposures are difficult to discern. The translation of high dose animal experiment data for human risk assessment is often criticized as being of questionable relevance for human exposures. Because of the sensitivity of AMS, we are able to assess the in vivo human metabolic parameters of one of the most carcinogenic PAHs in animal models, DBC, while maintaining risk to volunteers at a de minimus level. Our pharmacokinetic data set following human DBC exposure at environmentally relevant levels offer an excellent biomonitoring tool for agencies charged with risk assessment and modeling to humans of high molecular weight carcinogenic PAHs.

PAHs are pro-carcinogens, requiring enzymatic activation to electrophilic metabolites such as epoxides, dihydrodiol-epoxides, and quinones.45 These electrophiles have numerous nucleophilic targets in the cell including DNA. In the case of DBC, cytochrome P450 (P450)-dependent 11,12-epoxyge- nation followed by hydrolysis (epoxide hydrolase) and a second epoxygenation produces the 11,12-dihydrodiol-13,14-epoxide (DBCDE). Of the four possible enantiomers, the (\(-\)\)anti-11R,12S-dihydrodiol-13S,14R-epoxide or (\(-\)\)anti-DBCDE is thought to be the most efficient at forming DNA adducts at a number of sites on purine bases47 especially at N6-dA and N2-dG. Understanding the extent of metabolism, both bioactivation and detoxication (conjugation by SULTs, UGTs, and GSTs), following exposure to environmentally relevant doses would greatly add to the impact of Bio-AMS studies such as this one. The same is true for the determination of the extent and identity of DNA adducts in peripheral blood mononucleocytes following microdosing.

A current strength and drawback to the solid sample Bio-AMS technology employed in this study is that only total [\(^{14}\text{C}\)] label is detected. While providing sensitivity in the attomole range, this method is not capable of speciation, i.e., chemical identification and quantification of the parent compound and metabolites. Previously, to identify metabolites, plasma extracts would have to be separated by HPLC and collected as discrete fractions, based on the retention time of metabolite standards, prior to graphitization.14 This approach is burdened with the same limitations of HPLC fraction collection for uncoupled traditional MS. HPLC fraction selection is based on the retention time of commercially available standards for down- stream MS analysis. Only select fractions can be analyzed due to study limitations, including manual sample preparation.

New instrumentation at LLNL provides liquid sample AMS through the interface of UHPLC and AMS and determination of the metabolite profile.48 Moving wire coupled UHPLC-Bio-AMS (liquid sample Bio-AMS) technology combusts column eluent to [\(^{14}\text{C}\)] CO\(_2\) prior to AMS carbon isotope detection across an entire chromatographic run. Parent, metabolite, and all conjugated species present from a biological matrix such as plasma, urine, or isolated DNA adducts can be detected and quantitated. Standards are necessary for identification based on retention time, but all species resulting from metabolism would be represented in the [\(^{14}\text{C}\)] tracing. Future work will focus on utilizing liquid sample Bio-AMS to identify the species and concentration of PAH metabolites over time and examination of interindividual differences such as genetics or previously environmental exposures, elucidating gene-environmental interactions at microdoses. One such study is currently underway.
in our laboratory. A targeted total of 75 enrollees will be dosed with 5 nCi (46 ng) of [14C]-BaP. Plasma and urine levels of parent [14C]-BaP and [3H]-BaP metabolites will be assessed over a 72 h period employing UHPLC-AMS48 (FDA IND #117175; OSU IRB #5644). Preliminary results to date (Figure 3) show that the majority of the 14C counts in plasma coelute with DBC with minor amounts of [14C]-DBC-11,12-dihydrodiol tentatively identified by coelution with unlabeled standard (synthesized and provided by Dr. Shantu Amin, Penn State University). The apparent peak eluting early (2–2.5 min) is unknown. It should be noted that this is the first sample run by UHPLC and is from plasma (not urine, where metabolites should predominant). Extraction at later time points (or pooling of some time points) and from higher volumes of plasma should allow us to better quantify these putative metabolites.

We used a previously published PBPK model developed for the rat12 to evaluate how well the human [14C]-DBCeq pharmacokinetic profile compared with that of other species. For this comparison (Figure 4), we scaled the initial step in the human metabolism of DBC from rate constants determined from rats. Otherwise, the human PBPK model was based upon average anatomy and physiology of a 70 kg adult male under the dosing conditions used in this study. When human metabolism was scaled from the rat, the PBPK simulation of parent DBC pharmacokinetics in plasma was remarkably close to the measured data during the first 12 h postdosing. At later time points, the model simulations of DBC kinetics began to

**Table 4. Plasma DBCeq As Determined by AMS (fg DBCeq/mL Plasma)**

| time (h) | 0.00 | 0.25 | 0.50 | 0.75 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 4.0 | 8.0 | 12.0 | 24.0 | 48.0 | 72.0 |
|----------|------|------|------|------|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|
| V1       | 0.0  | 0.0  | 13.8 | 44.1 | 88.4| 124 | 91.7| 61.5| 50.1| 45.7| 33.8| 33.6 | 36.5 | 18.0 | 0.0 |
| V6       | 0.0  | 0.0  | 27.9 | 53.7 | 87.3| 110 | 107 | 107 | 107 | 93.4| 69.3| 65.1 | 34.9 | 26.5 | 21.2|
| V8       | 0.0  | 0.0  | 0.4  | 15.4 | N.D.| 34.7| 30.8| 26.5| 27.9| 41.6| 8.1 | 20.4 | 9.0  | 0.0  | 0.0 |
| V9       | 0.0  | 0.0  | 8.9  | 23.7 | 39.5| 61.1| 54.8| 75.0| N.D.| 37.8| 22.6| 17.6 | 17.3 | 33.0 | 26.1|
| V10      | 0.0  | 0.0  | 0.0  | 1.4  | 7.1 | 13.9| 16.1| 18.6| 16.5| 23.3| 13.4| 5.2  | 4.3  | 5.5  | 3.2 |
| V13      | 0.0  | 1.5  | 12.3 | 29.5 | 55.3| 63.1| 58.1| 48.2| 45.3| 39.8| 24.8| 24.5 | 21.5 | 13.3 | 7.8 |
| mean     | 0.0  | 3.6  | 11.6 | 18.7 | 34.4| 46.7| 37.8| 38.4| 31.9| 29.0| 24.0| 22.6 | 14.7 | 11.7 | 11.0|
| SD       | 0.0  | N.D. | 27.9 | 53.7 | 87.3| 118 | 107 | 110 | 107 | 93.4| 69.3| 65.1 | 34.9 | 26.5 | 21.2|

**Figure 3.** UHPLC-AMS of [14C]-DBC and putative metabolites from plasma of volunteer 5 3 h following dosing. Plasma samples were extracted twice with 50 μL of ethyl acetate and the extract transferred to an HPLC vial with a 300 μL insert. The ethyl acetate was evaporated in a vacuum chamber and the residue reconstituted with 50 μL of acetonitrile prior to injection. Five microliters of plasma extract (volunteer 5, 3 h after dosing) was injected onto a Phenomenex Kinetex 2.6 μm C18 100 Å (Part no: 00f-4462-AN) column (150 × 2.1 mm) fitted with a C18 guard column and eluted with 45% acetonitrile for 0–3 min followed by a linear gradient (3–10 min) to 100% acetonitrile, which was maintained from 10 to 13 min before a return to initial conditions (flow rate of 0.12 mL·min⁻¹ (25 °C)). Following passage through a dual channel UV/vis detector (280 and 315 nm), the eluent was deposited onto a moving nickel wire and the [14C]-DBCeq converted to 14CO₂ prior to AMS analysis.48 Multiple channels allow for the simultaneous determination of A280 and A315 as well as counts of 12C and 14C. Only the 14C profile is shown here. The use of unlabeled parent DBC and standards (DBC-(±)-11,12-diol and DBC-(±)-11,12,13,14-tetraol) allow for tentative identification of 14C peaks (top panel). The polar putative metabolite eluting between 2 and 2.5 min is currently unknown but could be a conjugate formed by sulfotransferases or UDP-glucuronosyl transferase (sample not treated with sulfatase or β-glucuronidase prior to analysis). The plasma sample from volunteer 5 (lower panel) shows a detectable peak at 13.5 min, which is the retention time (top panel) of DBC-(±)-diol. The major 14C-containing peak in plasma at 3 h postdosing coelutes with parent DBC.
significantly under predict the total $^{14}$C data, which likely represented an increasing proportion of DBC metabolites. As described above, studies are ongoing to quantitate DBC and its major metabolites in plasma and urine samples from this study to more directly compare with PBPK predictions. These data, along with recently published data on species differences in metabolic rate constants associated with DBC metabolism, will be incorporated into the next generation PBPK model to improve cross-species comparisons. Nevertheless, these initial comparisons based upon simple metabolic scaling assumptions are encouraging.

The pharmacokinetics of phenanthrene (a 3-ring PAH) and its diol and tetrox metabolites have been conducted in humans following oral or inhalation exposure to 10 μg of [D$_{10}$]-phenanthrene and analysis of plasma and urine over time by GC-electron impact-MS/MS. Phenanthrene can act as a surrogate for larger molecular weight carcinogenic PAHs as it has a bay region and is metabolized in a manner similar to that of BaP yet is designated a class C PAH by IARC and has been given an RPF of 0 by EPA. In our subsequent studies, the opportunity to identify macromolecular adducts from peripheral blood mononuclear cells (PBMCs) as potential biomarkers of exposure and perhaps risk following microdosing with 46 ng, we hope to examine the impact of genetic polymorphisms in BaP-metabolizing enzymes as was done by Wang et al. Likewise, we plan to examine [14C]-PAH-derived DNA and protein adducts from peripheral blood mononuclear cells (PBMCs) as potential biomarkers of exposure and perhaps risk following microdosing. The use of moving wire technology may provide potential biomarkers of exposure and perhaps risk following microdosing. The use of moving wire technology may provide potential biomarkers of exposure and perhaps risk following microdosing.

The ability to determine the pharmacokinetic parameters, safely in humans, of carcinogenic chemicals found in the environment and their metabolites is an advancement in risk assessment. Further development and application of this technology could have a major impact in the arena of human environmental health.

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