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Toxicokinetics of Benzo[a]pyrene in Humans: Extensive Metabolism as Determined by UPLC-Accelerator Mass Spectrometry Following Oral Micro-Dosing

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Abstract

Benzo[a]pyrene (BaP), is a known human carcinogen (International Agency for Research on Cancer (IARC) class 1). The remarkable sensitivity (zepto-attomole ¹⁴C in biological samples) of accelerator mass spectrometry (AMS) makes possible, with *de minimus* risk, pharmacokinetic (PK) analysis following [¹⁴C]-BaP micro-dosing of humans. A 46 ng (5 nCi) dose was given thrice to 5 volunteers with minimum 2 weeks between dosing and plasma collected over 72 hours. [¹⁴C]-BaP_{eq} PK analysis gave plasma T_{max} and C_{max} values of 1.25 hours and 29–82 fg/mL, respectively. PK parameters were assessed by non- compartment and compartment models. Intervals between dosing ranged from 20–420 days and had little impact on intra-individual

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The authors have no conflicts of interest to disclose.

Compliance with Ethical Standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in this study.

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variation. DNA, extracted from peripheral blood mononuclear cells (PBMCs) of 4 volunteers, showed measurable levels (LOD ~ 0.5 adducts/10¹¹ nucleotides) in two individuals 2–3 hours post-dose, approximately three orders of magnitude lower than smokers or occupationally-exposed individuals. Little or no DNA binding was detectable at 48–72 hours. In volunteers the allelic variants *CYP1B1**1/*1, *1/*3 or *3/*3 and *GSTM1**0/0 or *1 had no impact on [¹⁴C]-BaP_{eq} PK or DNA adduction with this very limited sample. Plasma metabolites over 72 hours from two individuals (one *CYP1B1**1/*1 and one *CYP1B1**3/*3) were analyzed by UPLC-AMS. In both individuals, parent [¹⁴C]-BaP was a minor constituent even at the earliest time points and metabolite profiles markedly distinct. AMS, coupled with UPLC, could be used in humans to enhance the accuracy of pharmacokinetics, toxicokinetics and risk assessment of environmental carcinogens.

Keywords

Benzo[*a*]pyrene; Pharmacokinetics; Polycyclic Aromatic Hydrocarbons; Accelerator Mass Spectrometry; Micro-Dosing; Metabolites

Introduction

Benzo[*a*]pyrene (BaP), the most studied polycyclic aromatic hydrocarbon (PAH), is a high priority (8th/785) environmental chemical of concern for human exposure according to the Agency for Toxic Substances and Disease Registry (ATSDR, 2013). In preclinical models, BaP exposure results in multiple toxicities including cardiovascular, developmental, immunological and reproductive as well as multiple cancers (International Agency for Research on Cancer (IARC), 2010; World Health Organization (WHO), 1998; U.S. Environmental Protection Agency (EPA), 2017). The EPA (2017) recently modified the oral BaP cancer risk slope factor (an upper bound estimate of risk) from 7.3 to 1 (mg/kg-day)⁻¹ which, based on an estimated daily exposure (non-smoking adult in the U.S.) of 270–750 ng, would correspond to a 3.9×10⁻⁶–1.1×10⁻⁵ (1 in 93,000 to 1 in 260,000) lifetime excess risk of developing cancer. BaP is used by the EPA as the reference compound for determination of cancer risk for environmental PAH mixtures (Relative Potency Factor (RPF) with BaP=1) (EPA, 2010).

Formation of covalent DNA adducts, following exposure to BaP, has long been used as a biomarker in preclinical models (Godschalk et al., 1997; Ross et al., 1995; Zuo et al., 2014), or *in vitro* and *ex vivo* studies with human cells, postmortem or biopsy tissue (Alexandrov et al., 2002; Lodovici et al., 1998; Monien et al., 2015; Schults et al., 2013; Shiilzaki et al., 2017) and human epidemiology studies, primarily with occupationally exposed populations or smokers (Borska et al., 2014; Boysen and Hecht, 2003; Divi et al., 2002; Kriek et al., 1993; Tuominen et al., 2002). The major adduct associated with mutation and carcinogenesis (both rodents and humans) is formed from reaction of (+)-*anti*-BaP-7,8-dihydrodiol-9,10-epoxide ((+)-*anti*-BaPDHDE), the most mutagenic of four possible enantiomers) and the N² position of guanine is the preferred site for adduction (Jernström and Gräslund 1994). In A/J mice, often used in lung cancer studies, BaP-induction of adenomas correlates with time-integrated DNA adducts (Ross et al., 1995). In the Muta Mouse model oral administration of

BaP leads to an order of DNA adduction (spleen > liver > lung > glandular stomach > colon > forestomach) distinct from that of target organ mutation sensitivity (colon > forestomach > spleen > glandular stomach > liver = lung) or tumor incidence (forestomach > spleen > lung) (Hakura et al., 1998a; b; 1999; 2000). Zuo et al., (2014) enhanced the prediction of target organs by also incorporating BaP-induced alteration in gene expression. Assessment of DNA adducts in peripheral blood mononuclear cells (PBMCs), a relatively non-invasive approach, of smokers or as a result of occupational exposures yields BaP-DNA adducts in the 1–50 per 10⁸ nucleotide range (Alexandrov et al., 2002; Kriek et al., 1993; Pavanello et al., 2004).

Accelerator mass spectrometry (AMS), due to its remarkable sensitivity to ¹⁴C, is finding increasing use in study of environmental carcinogens (Enright et al., 2016; Felton and Turteltaub, 1994; Ognibene et al., 2004). Human subjects have been dosed with [¹⁴C]-labeled 2-amino-3-methylimidazo[4,5-*f*]quinolone (IQ), 2-amino-3,8-dimethylimidazo[4,5-*b*]quinoxaline (MeIQx), 2-amino-3-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), tamoxifen, aflatoxin B₁, dibenzo[*def*,*p*]chrysene (DBC) and BaP (Brown et al., 2006; Enright et al., 2016; Hummel et al., 2018; Jubert et al., 2009; Lightfoot et al., 2000; Madeen et al., 2015; 2016). The majority of these studies with carcinogens have assayed for target organ DNA binding (Lightfoot et al., 2000; Felton and Turteltaub, 1994) (dosing prior to surgical resection) but some have examined pharmacokinetics (Hummel et al., 2018; Madeen et al., 2015; 2016). The graphite combustion technique allows for measurement of total [¹⁴C] but not chemical identification.

In the present study a dose of 46 ng of [¹⁴C]-BaP was given orally to five volunteers on three separate cycles with a minimum two week “washout” interval. Blood was collected over 72 hours to determine pharmacokinetics. Covalent binding of [¹⁴C]-BaP to DNA from PBMCs was assayed in 4 of the 5 individuals over time and preliminary analysis of the [¹⁴C]-BaP metabolite profile in two individuals conducted by a recently developed UPLC-AMS interface developed for biological samples (Ognibene et al., 2015; Thomas et al., 2011).

Materials and Methods

This study was conducted under FDA-IND #117175 and IRB approval #5644 from Oregon State University and LLNL-approved IRB Protocol 2017–008.

Volunteers:

Subjects of both sexes, 21–65 years of age, were recruited. All subjects were white not of Hispanic origin. Exclusion criteria included women that were pregnant or could become pregnant, smoking (tobacco or other substances) in the past 3 months or living with one or more smokers, regular use of medications that affect gut motility or nutrient absorption, a history of gastrointestinal surgery or gastrointestinal disorder, current or history of kidney or liver disease, prior high-dose ¹⁴C exposure from medical tests or occupational PAH exposure (e.g. roofers, asphalt pavers, fire-fighters, etc.). We did not recruit individuals considered to be members of vulnerable populations, including those 20 years of age or younger, pregnant women, prisoners, non-English speakers, non-literate subjects or adults lacking capacity to consent. Following receipt of a signed informed consent, volunteers were given a physical exam by the study physician and blood chemistry analysis performed

(including a pregnancy test for women). The demographics of the five individuals participating in the present study are given in Table 1. Three male and two females subjects 30–63 years of age with BMIs of 18.5–36.5 were enrolled and dosed with [^{14}C]-BaP on three separate dosings to follow a 0–72 hour time course. In order to minimize the contribution of daily BaP exposure due to this study, for two weeks prior to micro-dosing of [^{14}C]-BaP and during the 72 hour study, subjects were requested to refrain from consuming smoked meats or cheeses and charcoal-broiled meat. A dietary questionnaire was completed by each subject for the 3 days prior to dosing. Levels of dietary consumption of BaP were estimated using the guidelines of Dieziel et al., (2011).

Dosing and Blood Collections:

Subjects fasted overnight before Day 1 of each cycle. Each cycle was performed thrice with a minimum two week “washout” between cycles. The actual interval between cycles varied considerably (20–420 days) based on subject availability. A catheter was placed in an appropriate vein and a time zero blood sample taken prior to swallowing a capsule containing 46 ng (5 nCi) of [7- ^{14}C]-BaP (27 nCi/nmol), with water. The [7- ^{14}C]-BaP was from American Radiolabeled Chemicals, Inc. (St. Louis, MO, custom synthesis) and purified by reverse phase HPLC, capsules prepared and blood (8 mL at each time point) drawn at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 2.5, 3, 4, 8, 24, 48 and 72 hours as described in Hummel et al., (2018).

Plasma Extraction and Determination of [^{14}C]-BaP_{eq} by AMS:

Aliquots of plasma (0.75 mL or 1.5 mL for metabolic profiles) from each time point were extracted within two hours after collection according to the method of Crowell et al., (2011). The protocol is described in detail in Hummel et al., (2018). Samples were evaporated and flame-sealed in a quartz tube containing Cu(II) and combusted to 900°C, producing CO₂ which was then transferred to a septa sealed glass tube containing Zn and Co and heated to 525°C, producing graphite on the Co catalyst. The graphite was loaded into an aluminum sample holder for AMS analysis, performed on the 250 kV single stage AMS, constructed and maintained by the Center for Accelerator Mass Spectrometry at LLNL (ratio of ^{14}C :C with a precision of 3% and sensitivity of 0.7 attomol ^{14}C per mg total carbon) (Ognibene et al., 2015). As in previous studies (Madeen et al., 2015; 2016; Hummel et al., 2018) biochemical samples are measured 4–10 times with at least 10,000 ^{14}C counts or for 30 seconds (each replicate) (Ognibene et al., 2015).

Pharmacokinetic Analysis:

Pharmacokinetics of total BaP equivalents (BaP and BaP metabolites, [^{14}C]-BaP_{eq}) were evaluated using non-compartmental and compartmental analyses. Area under the curve (AUC) values to the last measured time point and extrapolated to infinity (using last 3 time points) were calculated using the trapezoidal rule (Gibaldi and Perrier 1982). Mean resident times were calculated as a ratio of AUC under the 1st moment curve extrapolated to infinity to AUC extrapolated to infinity. Non-compartmental half-lives were calculated as the product of mean resident times with the natural log of two. A two-compartment pharmacokinetic model was used to evaluate the time course of [^{14}C]-BaP_{eq} (Equations 1–3), where “A0”, “A1”, and “A2” represent the amount (fg) of [^{14}C]-BaP_{eq} in the absorption,

central, and peripheral compartments, respectively; “ k_a ”, “ k_e ”, “ k_{12} ”, and “ k_{21} ” are first-order rate constants. The concentration of the central compartment (C_1) was calculated by normalizing the amount with the volume of distribution (V_1 , L, Equation 4).

$$\frac{dA_0}{dt} = -(k_a \times A_0) \quad (1)$$

$$\frac{dA_1}{dt} = (k_a \times A_0) - (k_e \times A_1) - (k_{12} \times A_1) + (k_{21} \times A_2) \quad (2)$$

$$\frac{dA_2}{dt} = (k_{12} \times A_1) - (k_{21} \times A_2) \quad (3)$$

$$C_1 = \frac{A_1}{V_1} \quad (4)$$

Model parameters were optimized using a maximum log likelihood objective and the Nelder-Mead algorithm. Initial values were set by adjusting parameters visually. Software used to statistically analyze data was “R: A language and environment for statistical computing” Version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria).

DNA Extraction from PBMCs and Determination of BaP_{eq} Covalent Binding to DNA:

Peripheral blood mononuclear cells (PBMCs) were isolated from blood of each volunteer at each time point and DNA isolated for determination of total [^{14}C]-BaP_{eq} bound using the graphite AMS assay as previously described (Felton and Turteltaub, 1994). DNA (average yield $24 \pm 8 \mu\text{g/mL}$ whole blood) was extracted with a DNA Isolation Kit for Mammalian Blood (Roche Diagnostics, Indianapolis, IN). The DNA pellet was washed twice with 100% and once with 70% ethanol prior to solubilizing in nuclease-free water for determination of the concentration and purity via a Nanodrop ND-1000 (Thermo Scientific, Wilmington, DE). Samples were prepared as 200 ng/ μL solutions. Twenty μg of DNA were analyzed by graphite AMS. The limit of adduct detection (LOD) for the 20 μg DNA sample was 0.2 fg (10 fg [^{14}C]-BaP adducts/mg DNA) or approximately 0.5 adducts/ 10^{11} nucleotides.

Genotyping for *CYP1B1**1 vs. *3 and *GSTM1**1 vs. *GSTM1**0 Alleles:

*CYP1B1**1 and *3 alleles were distinguished by Sanger sequencing. PCR products were amplified from PMBC DNA (extraction described above) in a 20 μL reaction containing 200 ng DNA, 250 nM forward and reverse primers, and 10 μL SsoFast™ Supermix (Bio-Rad). The product was cleaned with Exo-SAP-IT™ (Applied Biosystems) per manufacturer’s instructions and sequenced at the Oregon State University Center for Genome Research and

Biocomputing on an ABI 3730 capillary sequencer using ABI's Big Dye Terminator chemistry. Sequences were visualized using FinchTV v. 1.5 (Supplemental Fig. 1). *GSTM1* nulls were determined using a conventional PCR amplification. PCR components were the same as described above using *GSTM1* specific primers to detect homozygotes or heterozygotes for a *GSTM1* allele. Primers for human beta globin (*HBB*) were used as a control in the event an individual was a *GSTM1* null. See Supplemental Table 1 for a list of primer sequences. Products were visualized on 10% TBE gels (Supplemental Fig. 2).

UPLC-AMS Analysis of [¹⁴C]-BaP and Metabolites Extracted from Human Plasma:

The following BaP metabolite standards were obtained from the PAH repository of the Oregon State University Superfund Research Program (<http://limsweb.science.oregonstate.edu/>, accessed 8–22-2018); BaP-9,10-diol, BaP-7,8-diol, BaP-1,6-quinone, BaP-3,6-quinone, BaP-6,12-quinone, BaP-7,8-dione, 3-hydroxy-BaP, 7-hydroxy-BaP, 9-hydroxy-BaP, BaP and DBC (internal standard). Resolution of BaP and metabolites was performed with a Waters Acquity H series UPLC. The column utilized was a Waters Acquity UPLC BEH C18 1.7 µm, 2.1 × 50 mm (Part # 186002350) with a guard column (part #186003981). Solvent A was HPLC-grade H₂O with 0.3% formic acid and solvent B was HPLC grade (PEG free) acetonitrile. Initial conditions were 40% B for 2 minutes followed by a linear gradient to 60% B over 5 minutes with a hold at 60% for an addition 5 minutes following which solvent B was increased by a linear gradient to 80% over 10 minutes, and then to 100% over 5 minutes followed by a 3 minute gradient return to 40% B. The samples were maintained at 20°C in an autosampler. The column temperature was maintained at 28°C. Five µL was the injection volume and the column was eluted at 0.25 mL/minute. BaP and metabolite standards were prepared in acetonitrile and stored at –80°C until analysis. The plasma extracts of [¹⁴C]-BaP and metabolites (100 µL) in ethyl acetate were evaporated (Speed Vap, vacuum only with no centrifugation) to dryness and the residue redissolved in acetonitrile and transferred to the autosample vials. BaP and metabolites (Supplemental Table 2) were analyzed by a Waters Acquity UPLC PDA detector. Analysis of plasma extract containing [¹⁴C]-BaP and metabolites was by collection of the UPLC eluate onto a nickel moving wire (periodically indented to hold solvent) that passed through a cleaner prior to deposition of the eluate. The wire with eluate is passed through a drying oven followed by a combustion oven to convert the sample to ¹⁴CO₂ before entering the 250 kV AMS. The 250 kV [¹⁴C]-AMS detector employed has previously been validated as an analytical instrument for pharmaceutical development (Keck et al. 2010). With respect to the moving wire interface with the UPLC and AMS we have previously established the LLOQ at 200 zmol [¹⁴C] (Thomas et al. 2011). Thus, with a specific activity of [¹⁴C]-BaP (27 nCi/nmol) and a molecular weight of 252 ng/nmol, the LLOQ is 1.2 fg. The accuracy was 1–3% with a precision (coefficient of variation) between 1–6% (Keck et al. 2010). A more complete description of the system is provided by Thomas et al., (2011) and <https://www.youtube.com/watch?v=oFi17Vho44k> (accessed 8–22-2018).

Results

Pharmacokinetics of [^{14}C]-BaP_{eq}

Pharmacokinetic parameters in plasma of volunteers were compared as a function of the interval between dosing. The relatively small difference between cycles (with the exception of 1 dose with subject BaP002) indicates little intra-individual variation in BaP pharmacokinetics, even with up to 60 weeks between runs, and variation did not correlate to days between doses (Fig. 1). The small number of individuals analyzed does not allow for any conclusions with respect to impact of gender, age, race/ethnicity, or BMI (Table 1). Although not statistically significant (due to the small cohort), compared to the 6-membered ring PAH, [^{14}C]-DBC, (29 ng), 46 ng of [^{14}C]-BaP was taken up faster ($T_{\max}$ of 1.25 and 2.25 hours for [^{14}C]-BaP_{eq} and [^{14}C]-DBC_{eq}, respectively, $p=0.09$), but reached similar $C_{\max}$ levels (70 ± 20 and 69 ± 40 fg/mL, respectively) (Table 2). The $T_{1/2}$ (non-compartmental) was 70 ± 27 and 37 ± 30 hours, respectively for [^{14}C]-BaP_{eq} and [^{14}C]-DBC_{eq} (not statistically significant, $p=0.11$) (Table 2). The average $\text{AUC}_{0-72\text{hr}}$ and $\text{AUC}_{0-\infty}$ for [^{14}C]-BaP_{eq} were 1400 and 2615 (fg/mL $\times$ hr), respectively (Table 2 and Fig. 1), both similar to [^{14}C]-DBC_{eq}. A two-compartmental model yielded a $T_{1/2\alpha}$ and $T_{1/2\beta}$ for plasma [^{14}C]-BaP_{eq} of 1.1 ± 0.3 and 89 ± 42 hours, respectively and the volume of distribution ranged from 235–1146 L. These kinetic constants were not significantly different from [^{14}C]-DBC_{eq}. Overall, these comparisons demonstrate similar kinetic profiles in plasma of total (parent [^{14}C]-PAH and metabolites) following exposures to [^{14}C]-BaP or [^{14}C]-DBC but the extent of metabolism of [^{14}C]-BaP is markedly greater.

Time Course of Covalent Binding of [^{14}C]-BaP_{eq} to PBMC DNA

In four of the five volunteers, PBMCs were isolated from plasma at various time points up to 72 hours post-dose to assess covalent binding of [^{14}C]-BaP_{eq} to DNA. In two volunteers, (BaP002 and BaP003), no DNA adduction was detected at any time point (Fig. 2, top panel). With volunteers BaP004 and BaP009 a small amount of adduction is seen at 2 and 2.5 hours, respectively (Fig. 2). With the possible exception of the 24 hour time point for volunteer BaP004, no DNA adduction is observed 24–72 hours post-dosing consistent with previous studies with both [^{14}C]-DBC_{eq} (Madeen et al., 2015; 2016) and [^{14}C]-BaP_{eq} (Hummel et al., 2018). A recently developed ultrasensitive high-resolution mass spectrometric assay (Villata et al., 2017) achieved a similar LOD (1 adduct/ 10^{11} nucleotides) with BaP in human lung.

Time Course of [^{14}C]-BaP Metabolites in Plasma of Two Volunteers

The [^{14}C]-BaP metabolite profile for volunteers BaP002 and BaP009 was assessed over the 72 hour time course utilizing the moving wire UPLC-AMS system developed by the Biomedical Research group at LLNL (Thomas et al., 2011; Ognibene et al., 2015; Madeen et al., 2016). Unlike the case with [^{14}C]-DBC (Madeen et al., 2016), [^{14}C]-BaP (21.86 min) was a minor component even at the earliest time points post-dosing (Fig. 3). Two major metabolites dominated the profile of each volunteer. With volunteer BaP002, the earliest (60–90 minutes) metabolite (M2, 1.41 min) to appear post-dosing in plasma eluted in the region of the gradient where BaP-dihydrodiols (could not identify based on retention time) and a later ($C_{\max}$ of 3 hours) eluting metabolite (M4, 7.66 min) in the region where quinones (could not distinguish between the 1,6-, 3–6- or 6,12-quinone) are seen. In the plasma

extract from volunteer BaP009, M2 is also a major metabolite and is seen in plasma with a time course similar to volunteer BaP002. A second major metabolite (M5, 12.86 min) which has a $C_{\max}$ 45 minutes post-dosing, with a smaller peak at 90 minutes, elutes with a retention time matching 3-hydroxy-BaP (Fig. 3).

***CYP1B1* (*1/*1, *1/*3 and *3/*3) and *GSTM1* (*1 and *0/*0) Allelic Variants**

This study is not sufficiently powered to correlate genotype to pharmacokinetic constants, metabolite profiles or formation of covalent [^{14}C]-BaP_{eq}-DNA adducts in PBMCs. We did observe that, in the two-compartment model, k_{12} ($r^2=0.86$, $p=0.023$) and k_{ela} ($r^2=0.75$, $p=0.056$) rates increased with the number of *CYP1B1**3 alleles whereas the $t_{1/2\alpha}$ decreased ($r^2=0.671$, $p=0.090$) (data not shown). Both volunteers BaP002 and BaP009 were genotyped as *GSTM1* nulls and *CYP1B1**3/*3 and *CYP1B1**1/*1, respectively (Table 1 and Supplemental Figs. 1 and 2).

Discussion

In the present report five volunteers, fasted overnight, were given capsules on three separate occasions with the same dose of [^{14}C]-BaP. It should be noted that (with the exception of BaP002) there was markedly low intra-individual variation in the PK profile of [^{14}C]-BaP_{eq} independent of the interval between doses. As in rodent models (Crowell et al., 2011), BaP is fairly rapidly eliminated from plasma. We found that at doses 5–15-times lower than the estimated daily exposure to BaP in the U.S., very little parent compound was present in plasma, even at the earliest time points, perhaps indicative of extensive hepatic and/or intestinal first-pass metabolism.

Comparison of PK parameters of [^{14}C]-BaP_{eq} to [^{14}C]-DBC_{eq} by graphite AMS displayed few marked differences. It should be recognized that our PK parameters, determined by graphite AMS, incorporate all [^{14}C]-BaP (parent and metabolites), not just the parent PAH; thus PK parameters are impacted by protein binding, partitioning, transport, etc. Incorporation of the recently developed UPLC “moving wire” AMS interface, that allows for speciation of [^{14}C]-PAHs and metabolites, demonstrated a marked difference in extent of metabolism. An interesting observation, yet unexplained, is that, when comparing [^{14}C] recovered (volunteers BaP002 and BaP009) as [^{14}C]-BaP_{eq} using graphite (Figure 1) to UPLC-AMS (Figure 3), the latter is 26–33% lower than the former (based on AUC_{0–72hr}). Compared to [^{14}C]-DBC, [^{14}C]-BaP was extensively metabolized such that parent [^{14}C]-BaP was a minor component even at the earliest time points. The more rapid metabolism of BaP, compared to DBC, is consistent with *in vitro* studies at much higher concentrations (Crowell et al., 2014; Smith et al., 2017) whereas the rates of phase 1 and phase 2 metabolism of BaP-7,8-DHD and DBC-11,12-DHD are similar (Smith et al., 2017).

The metabolic profile of [^{14}C]-BaP exhibited some differences and similarities in the two individuals examined. In one individual (BaP002), a BaP quinone, not seen in the profile from BaP009, was observed although the second of the two major metabolites was the same and markedly more polar, probably a dihydrodiol (possibly a tetrol for which no standard was examined). Another striking difference was the lack of formation of any phenols with BaP002 whereas 3-hydroxy-BaP was a major metabolite found in plasma from BaP009. The

later eluting smaller 3-hydroxy-BaP peak in plasma may be indicative of enterohepatic recirculation.

PAHs, such as BaP and DBC, have multiple mechanisms of carcinogenesis. Metabolic activation by CYPs in the 1 family (especially CYP1B1) to bay or fjord region epoxides are rapidly hydrolyzed by epoxide hydrolase to two stereo isomers ((+/-)-BaP-7,8-DHD or (+/-)-DBC-11,12-DHD) followed by a second CYP1-epoxygenation to the potent electrophilic *anti*- or *cis*-dihydrodiol-epoxides (4 enantiomers each of BaP-7,8-DHD-9,10-E or DBC-11,12-DHD-13,14-E). BaP readily forms covalent DNA adducts, predominantly the N²-(7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-C10-yl)-2'-deoxyguanosine (N²-BaP-DHDE-dG) whereas DBC-11,12-DHD-13,14-E has a preference for adduction at N⁶-adenosine. Additional mechanisms of carcinogenesis involve reduction of the DHD by aldo-keto reductase (AKR) to electrophilic and redox active catechols (Penning, 2014; Quinn and Penning, 2008). In our UPLC system the BaP-7,8-dione had a retention time of 9.34 minutes and was well resolved from the three quinones and phenols (we did not have a standard of the BaP-7,8-catechol). With PAHs, one-electron oxygenations by peroxidases also produce electrophiles (Zamzow et al., 1989). Carcinogenicity can also result from PAH-dependent alteration of expression of genes important in tumor production through binding to the aryl hydrocarbon receptor (AhR) (Shimizu et al., 2000).

DBC, a six-membered PAH with both a bay- and fjord-region, is a potent carcinogen in preclinical rodent models (probable compared to BaP. In the study with [¹⁴C]-DBC, the major plasma metabolite observed was tentatively identified as DBC-11,12-DHD yet we found no evidence of covalent DNA adduction in PBMCs (Madeen et al., 2016). In this study with [¹⁴C]-BaP, although extensive metabolism was evident, little or no covalent DNA adduction was observed 48–72 hours after dosing. Two out of four volunteers examined over the 72 hour time-course showed [¹⁴C]-BaP DNA binding at an early time point (2 and 2.5 hr for volunteers BaP004 and BaP009, respectively), which we originally believed was random noise, however, DNA from PBMCs from BaP009 gave similar results over 2 separate dosing time-courses. We are continued to examine this issue with a planned dose-response study.

Can studies such as this be useful in prediction of cancer risk from truly environmentally relevant levels of exposure to carcinogenic PAHs or other carcinogens? There are limitations to this study including sample size and a single dosage not reflective of steady-state concentrations with daily exposures. The EPA has set an oral cancer slope factor (upper bound for risk) for BaP of 1 (mg/kg-day)⁻¹. The lifetime risk-specific dose equating to 1 cancer in 10⁶ is 1.0 × 10⁻⁶ mg/kg-day which is approximately twice the risk if the dose used here (5.2 × 10⁻⁷ mg/kg) were administered every day of the volunteer's lives. If we utilized covalent DNA adduction to predict cancer susceptibility (Bartsch et al., 1998; Kriek et al., 1993; Lee et al., 2002), the limited DNA adduct data we have to date would indicate a risk markedly less than 1 in 10⁶. Due to extensive metabolism of [¹⁴C]-BaP, observed by UPLC-AMS, the plasma C_{max} following our single dose of 46 ng ranged from 9–15 fM (two volunteers). Utilizing our PBPK model (Crowell et al., 2011), the estimated C_{max} in liver would be 0.12–0.2 pM, over 10⁶-fold lower than the calculated K_m (0.725 μM) in human liver microsomes (Crowell et al., 2014) and the IC₅₀ in a BaP competitive binding assay with human AhR (Flaveny et al., 2009). Thus it would seem unlikely that BaP would impact

the expression of AhR-dependent gene expression following this exposure although no alterations in the transcriptome were examined in this study.

It is also useful to relate our data to studies with human cells *in vitro* and BaP plasma concentrations and degree of PBMC DNA adduction found *in vivo* with human populations. Peak plasma levels of [¹⁴C]-BaP in this study are more than 3 orders of magnitude lower than the BaP median level of 4–19 pg/mL from a study by Pleil et al., (2010), examining a healthy student population and about 30,000 fold lower than concentrations found to have no adverse impact on Hepa1c1c7 hepatocytes (Madureira et al., 2014). The levels of BaP-DNA adducts in PBMCs from occupationally exposed workers have been reported to be 1–10 adducts per 10⁸ nucleotides (Boysen and Hecht, 2003; Carstensen et al., 1999; Divi et al., 2002; Pavanello et al., 2005). The levels of covalent DNA adduction in PBMCs from volunteers in our study were at least 3 orders of magnitude lower, again indicating little to no measurable risk from this exposure.

Somewhat surprisingly, history of exposure (smoking, occupational) has been reported as not significantly elevating BaP-DHDE-DNA adducts in PBMCs or other tissues (Boysen and Hecht, 2003; Carstensen et al., 1999). Other studies report modestly higher levels in smoker's compared to non- or ex-smokers (Lodovici et al., 1998; Villalta et al., 2017). Examination of 10 human lung biopsy samples by Monien et al., (2015) found no detectable (LOD 1–3 adducts per 10⁸ nucleotides) BaP-DHDE-dG adducts. Recent advances in methodology, leading to increased specificity and sensitivity for assaying BaP-DNA adducts, have improved (LOD of ~ 1 adduct in 10¹¹ nucleotides). Villalta et al., (2017) reported levels of (predominantly 7*R*,8*S*,9*S*,10*R*) BaP-DHDE-N²-dG adducts in lung biopsies from smokers and non-smokers as 3.1 and 1.3 adducts per 10¹¹ nucleotides, respectively.

Another potential use of UPLC-AMS technology, if expanded, is identification of resistant and susceptible individuals. Measures of BaP-DNA adducts in humans is likely driven by gene-environment interactions. A number of studies have documented enhanced cancer susceptibility for individuals expressing the *GSTM1**0 (null) allele (approximately 50% in Caucasians) (Alexandrov et al., 2002; Boysen and Hecht, 2003; Pavanello et al., 2002, 2005; Rojas et al., 1998; Wenzlaff et al., 2005a). As all volunteers examined for [¹⁴C]-BaP DNA adduction in PBMCs were *GSTM1* nulls, this study could not examine the impact of *GSTM1* expression.

Studies suggest individuals expressing the *CYP1B1**3 allelic variant (about 39% in Caucasians) have an enhanced risk of some cancers including lung (Shah et al., 2008; Wenzlaff et al., 2005b; Xu et al., 2012), ovarian (Goodman et al., 2001), and head and neck cancer in smokers (Thier et al., 2002), but not for colorectal cancer (Xie et al., 2012). An inverse relationship is seen for prostate cancer (Beuten et al., 2008). Individuals with both *CYP1B1**3 (or *CYP1A1**2, not examined in this study) and *GSTM1**0 alleles have an enhanced lung cancer risk compared to either alone (Alexandrov et al., 2002; Shah et al., 2008).

The CYP1B1.3 (L432V) enzyme is expressed at about 50% the level of CYP1B1.1, is less responsive to induction by BaP (human lymphocytes) and exhibits a 25% lower K_{cat} for BaP

epoxygenation to the 7,8-DHD (Aklillu et al., 2005; Helmig et al., 2014). These observations would seem to be inconsistent with an enhanced lung cancer risk if CYP1B1-dependent formation of BaP-7,8-DHD-9,10-E were critical. The two individuals examined for [¹⁴C]-BaP metabolic profiles in plasma were homozygous for either *CYP1B1**1 or *CYP1B1**3. The extent of metabolism and profile of metabolites was distinct suggesting further genotype-dependent studies are warranted. The same could be said for the *GSTM1* allelic variant.

A limitation of this study was failure to positively identify BaP metabolites. Work is in progress at LLNL to send the UPLC sample (spiked with unlabeled metabolites standards) elute through a splitter, a UV/VIS scanning detector and conventional MS/MS for positive identification of peaks with the same retention time as the AMS ¹⁴C peaks.

These studies complement and expand PK determinants of PAHs such as BaP and DBC (Crowell et al., 2014) and their metabolites (Smith et al., 2017) *in vitro* with human liver microsomes of BaP at concentrations at least 6 orders of magnitude greater than the C_{max} in liver (based on the PBPK model from Crowell et al., 2011)). Such an approach is consistent with the philosophy of “the best model for humans is humans”.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Aklillu E, Øvrebø S, Botnen IV, Otter C, Ingelman-Sundberg M (2005) Characterization of common *CYP1B1* variants with different capacity for benzo[a]pyrene-7,8-dihydrodiol epoxide formation from benzo[a]pyrene. *Cancer Res* 65:5105–11, doi:10.1158/0008-5472.CAN-05-0113. [PubMed: 15958554]
- Alexandrov K, Cascorbi I, Rojas M, Bouvier G, Kriek E, Bartsch H (2002) *CYP1A1* and *GSTM1* genotypes affect benzo[a]pyrene DNA adducts in smokers' lung: comparison with aromatic/hydrophobic adduct formation. *Carcinogenesis* 23:1969–77, PMID:. [PubMed: 12507920]
- ATSDR (2013), http://www.atsdr.cdc.gov/spl/resources/ATSDR_2013_SPL_Detailed_Data_Table.pdf., accessed 08–22–2018.
- Bartsch H, Rojas M, Alexandrov K, Risch A (1998) Impact of adduct determination on the assessment of cancer susceptibility. *Recent Res Cancer Res* 154:86–96, PMID:. [PubMed: 10026994]
- Beuten J, Gelfond JAL, Byrne JJ, Balic I, Crandall A, Johnson-Pais TL, Thompson IM, Price DK, Leach RJ (2008) *CYP1B1* variants are associated with prostate cancer in non-Hispanic and Hispanic Caucasians. *Carcinogenesis* 29:1751–7, doi:10.1093/carcin/bgm300. [PubMed: 18544568]
- Borska L, Andrys C, Krejsek J, Palicka V, Chmelarova M, Hamakova K, Kremlacek J, Fiala Z (2014) Oxidative damage to nucleic acids and benzo(a)pyrene-7,8-diol-9,10-epoxide-DNA adducts and chromosomal aberration in children with psoriasis repeatedly exposed to crude coal tar ointment

and UV radiation. *Oxid Med Cell Longev* 2014:302528, doi: 10.1155/2014/302528. [PubMed: 25197429]

Boysen G, Hecht SS (2003) Analysis of DNA and protein adducts of benzo[a]pyrene in human tissues using structure-specific methods. *Mutat Res* 543:17–30, 10.1016/S1383-5742(02)00068-6. [PubMed: 12510015]

Brown K, Tompkins EM, White INH (2006) Applications of accelerator mass spectrometry for pharmacology and toxicological research. *Mass Spectrom Rev* 25:127–4, doi:10.1002/mas.20059. [PubMed: 16059873]

Carstensen U, Hou S-M, Alexandrie A-K, Högstedt B, Tagesson C, Warholm M, Rannug A, Lambert B, Axmon A, Hagmar L (1999) Influence of genetic polymorphisms of biotransformation enzymes on gene mutations, strand breaks of deoxyribonucleic acid, and micronuclei in mononuclear blood cells and urinary 8-hydroxydeoxyguanosine in potroom workers exposed to polyaromatic hydrocarbons. *Scand J Work Environ Health* 25:351–60, doi:10.5271/sjweh.445. [PubMed: 10505661]

Crowell SR, Amin SG, Anderson KA, Krishnegowda G, Sharma AK, Soelberg JJ, Williams DE, Corley RA (2011) Preliminary physiologically based pharmacokinetic models for benzo[a]pyrene and dibenzo[def,p]chrysene in rodents. *Toxicol Appl Pharmacol* 257:365–76, doi:10.1016/j.taap.2011.09.020. [PubMed: 22001385]

Crowell SR, Hanson-Drury S, Williams DE, Corley RA (2014) *In vitro* metabolism of benzo[a]pyrene and dibenzo[def,p]chrysene in rodent and human hepatic microsomes. *Toxicol Lett* 228:48–55, doi:10.1016/j.toxlet.2014.04.004. [PubMed: 24769260]

Diezel NC, Strickland PT, Platz EA, Abubaker S, Buckley TJ (2011) Comparison of standard methods for assessing dietary intake of benzo[a]pyrene. *Cancer Epidemiol Biomarkers Prev* 20:962–70, doi:10.1158/1055-9965.EPI-10-1344. [PubMed: 21430297]

Divi RL, Beland FA, Fu PP, Von Tungeln LS, Schoket B, Camara JE, Ghei M, Rothman N, Sinha R, Poirier MC (2002) Highly sensitive chemiluminescence immunoassay for benzo[a]pyrene-DNA adducts: validation by comparison with other methods, and use in human biomonitoring. *Carcinogenesis* 23:2043–9, PMID: 12507927 [PubMed: 12507927]

Enright HA, Malfatti MA, Zimmermann M, Ognibene T, Henderson P, Turteltaub KW (2016) Use of accelerator mass spectrometry in human health and molecular toxicology. *Chem Res Toxicol* 29:1976–86, doi:10.1021/acs.chemrestox.6b00234. [PubMed: 27726383]

EPA (2010) Development of a relative potency factor (RPF) approach for polycyclic aromatic hydrocarbon (PAH) mixtures (External Review Draft), U.S. EPA, Washington, D.C., EPA/635/R-08/012A, 622 p.

EPA (2017) Toxicological Review of Benzo[a]pyrene, Integrated Risk Information System, National Center for Environmental Assessment, Office of Research and Development, EPA, Washington, D.C., [CASRN 50–32-8]. EPA/635/R-17-003, https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=136 (accessed 08–22–2018).

Felton JS, Turteltaub KW (1994) Accelerator mass spectrometry for measuring low-dose carcinogen binding to DNA. *Environm Hlth Perspect* 102:450–2, doi:10.1289/ehp.94102450.

Flaveny CA, Murray IA, Chiaro CR, Perdew GH (2009) Ligand selectivity and gene regulation by the human aryl hydrocarbon receptor in transgenic mice. *Molec Pharmacol* 75:1412–20, doi:10.1124/mol.109.054825. [PubMed: 19299563]

Gibaldi M, Perrier D (1982) *Pharmacokinetics*, M. Dekker, New York.

Godschalk RW, Vermeer IT, Kriek E, Floot B, Schilderman PA, Moonen EJ, Kleinjans JC, van Schooten FJ (1997) Comparison of 32P-postlabeling and HPLC-FD analysis of DNA adducts in rats acutely exposed to benzo(a)pyrene. *Chem-Biol Interact* 104:41–54, 10.1016/S0009-2797(97)03765-4. [PubMed: 9158694]

Goodman MT, McDuffie K, Kolonel LN, Terada K, Donlon TA, Wilkens LR, Guo C, Le Marchand L (2001) Case-control study of ovarian cancer and polymorphisms in genes involved in catechol estrogen formation and metabolism. *Cancer Epidemiol Biomarkers Prev* 10:209–16, PMID: 11303589 [PubMed: 11303589]

Hakura A, Sonoda J, Tsutsui Y, Mikami T, Imade T, Shimada M, Yaguchi S, Yamanaka M, Tomimatsu M, Tsukidate K (1998a) Toxicity profiles of benzo[a]pyrene in the male LacZ transgenic mouse

(MutaMouse) following oral administration for 5 consecutive days. Regul Toxicol Pharmacol 27:273–9, doi:10.1006/rtp.1998.1218. [PubMed: 9693078]

- Hakura A, Tsutsui Y, Sonoda J, Kai J, Imade T, Shimada M, Sugihara Y, Mikami T (1998b) Comparison between in vivo mutagenicity and carcinogenicity in multiple organs by benzo[a]pyrene in the LacZ transgenic mouse (Muta Mouse). Mutat Res 398:123–30, 10.1016/S0027-5107(97)00248-0. [PubMed: 9626972]
- Hakura A, Tsutsui Y, Sonoda J, Mikami T, Tsukidate K, Sagami F, Kerns WD (1999) Multiple organ mutation in the lacZ transgenic mouse (Muta mouse) 6 months after oral treatment (5 days) with benzo[a]pyrene. Mutat Res 426:71–7, 10.1016/S0027-5107(99)00046-9. [PubMed: 10320752]
- Hakura A, Tsutsui Y, Sonoda J, Tsukidate K, Mikami T, Samami F (2000) Comparison of the mutational spectra of the LacZ transgene in four organs of the MutaMouse treated with benzo[a]pyrene: target organ specificity. Mutat Res 447:239–47, 10.1016/S0027-5107(99)00213-4. [PubMed: 10751607]
- Helmig S, Wenzel S, Maxeiner H, Schneider J (2014) CYP1B1 mRNA inducibility due to benzo(a)pyrene is modified by the *CYP1B1* L432V gene polymorphism. Mutagenesis 29:237–40, doi:10.1093/mutage/geu010. [PubMed: 24695682]
- Hummel JM, Madeen EP, Siddens LK, Uesugi SL, McQuistan T, Anderson KA, Turteltaub KW, Ognibene TJ, Bench G, Krueger SK, Harris S, Smith J, Tilton SC, Baird WM, Williams DE (2018) Pharmacokinetics of [¹⁴C]-benzo[a]pyrene (BaP) in humans: impact of co-administration of smoked salmon and BaP dietary restriction. Fd Chem Toxicol 115:136–47, doi:10.1016/j.fct.2018.03.003.
- IARC (2010), Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. Monographs on the evaluation of carcinogenic risks to humans Lyon, France.
- Jernström B, Gräslund A (1994) Covalent binding of benzo[a]pyrene 7,8-dihydrodiol 9,10-epoxides to DNA: molecular structures, induced mutations and biological consequences. Biophys Chem 49:185–99, 10.1016/0301-4622(93)E0087-L. [PubMed: 8018817]
- Jubert C, Mata J, Bench G, Dashwood R, Pereira C, Tracewell W, Turteltaub K, Williams D, Bailey G (2009) Effects of chlorophyll and chlorophyllin on low-dose aflatoxin B₁ pharmacokinetics in human volunteers. Cancer Prev Res 2:1015–22, doi:10.1158/1940-6207.CAPR-09-0099.
- Keck BD, Ognibene T, Vogel JS (2010) Analytical validation of accelerator mass spectrometry for pharmaceutical development. Bioanalysis 2:469–85, doi: 10.4155/bio.10.14. [PubMed: 21083256]
- Kriek E, Van Schooten FJ, Hillebrand MJX, Van Leeuwen FE, Den Engelse L, De Looft AJA, Dijkmans APG (1993) DNA adducts as a measure of lung cancer risk in humans exposed to polycyclic aromatic hydrocarbons. Environm Hlth Perspect 99:71–5, doi:10.1289/ehp.939971.
- Lee BM, Yoo SD, Kim S (2002) A proposed methodology of cancer risk assessment modeling using biomarkers. J Toxicol Environ Health A 65:341–54, doi:10.1080/15287390252808019. [PubMed: 11936215]
- Lightfoot TJ, Coxhead JB, Cupid BC, Nicholson S, Garner RC (2000) Analysis of DNA adducts by accelerator mass spectrometry in human breast tissue after administration of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine and benzo[a]pyrene. Mutat Res 472:119–27, 10.1016/S1383-5718(00)00134-0. [PubMed: 11113704]
- Lodovici M, Akpan V, Giovannini L, Migliani F, Dolara P (1998) Benzo[a]pyrene diol-epoxide DNA adducts and levels of polycyclic aromatic hydrocarbons in autoptic samples from human lungs. Chem-Biol Interact 116:199–212, 10.1016/S0009-2797(98)00091-X. [PubMed: 9920462]
- Madeen E, Corley RA, Crowell S, Turteltaub K, Ognibene T, Malafatti M, McQuistan TJ, Garrad M, Sudakin D, Williams DE (2015) Human *in vivo* pharmacokinetics of [¹⁴C]dibenzo[*def,p*]chrysene by accelerator mass spectrometry following oral microdosing. Chem Res Toxicol 28:126–34, doi: 10.1021/tx5003996. [PubMed: 25418912]
- Madeen EP, Ognibene TJ, Corley RA, McQuistan TJ, Henderson MC, Baird WM, Bench G, Turteltaub KW, Williams DE (2016) Human microdosing with carcinogenetic polycyclic aromatic hydrocarbons: in vivo pharmacokinetics of dibenzo[*def,p*]chrysene and metabolites by UPLC accelerator mass spectrometry. Chem Res Toxicol 29:1641–50, doi:10.1021/acs.chemrestox.6b00169. [PubMed: 27494294]

- Madureira DJ, Weiss FT, Midwood PV, Helbling DE, Sturla SJ, Schirmer K (2014) Systems toxicology approach to understand the kinetics of benzo[a]pyrene uptake, biotransformation, and DNA adduct formation in a liver cell model. *Chem Res Toxicol* 27:443–53, doi:10.1021/tx400446q. [PubMed: 24446834]
- Monein BH, Schumacher F, Herrmann K, Glatt H, Turesky RJ, Chesné C (2015) Simultaneous detection of multiple DNA adducts in human lung samples by isotope-dilution UPLC-MS/MS. *Analy Chem* 87:641–8, doi:10.1021/ac503803m.
- Ognibene TJ, Bench G, Brown TA, Vogel JS (2004) The LLNL accelerator mass spectrometry system for biochemical ¹⁴C-measurements. *Nucl Instr Meth Phys Res* 23:12–5, doi:10.1021/ac026334j.
- Ognibene TJ, Haack KW, Bench G, Brown TA, Turteltaub KW (2015) Operation of the “small” BioAMS spectrometers at CAMS: past and future prospect. *Nucl Instr Meth Phys Res Section B* 361:54–7, doi:10.1016/j.nimb.2015.05.019.
- Pavanello S, Siwinska E, Mielzynska D, Clonfero E (2004) *GSTM1* null genotype as a risk factor for *anti*-BPDE-DNA adduct formation in mononuclear white blood cells of coke-oven workers. *Mutat Res* 558:53–62, doi:10.1016/j.mrgentox.2003.10.019. [PubMed: 15036119]
- Pavanello S, Pulliero A, Siwinska E, Mielzynska D, Clonfero E (2005) Reduced nucleotide excision repair and *GSTM1*-null genotypes influence *anti*-B[a]PDE-DNA adduct levels in mononuclear white blood cells of highly PAH-exposed coke oven workers. *Carcinogenesis* 26:169–75, doi:10.1093/carcin/bgh303. [PubMed: 15471894]
- Penning TM (2014) Human aldo-keto reductases and the metabolic activation of polycyclic aromatic hydrocarbons. *Chem Res Toxicol* 27:1901–17, doi:10.1021/tx500298n. [PubMed: 25279998]
- Pleil JD, Stiegel MA, Sobus JR, Tabucchi S, Ghio AJ, Madden MC (2010) Cumulative exposure assessment for trace-level polycyclic aromatic hydrocarbons (PAHs) using blood and plasma analysis. *J Chromatogr B Analyt Technol Biomed Life Sci* 878:1753–60, doi:10.1016/j.jchromb.2010.04.035.
- Quinn AM, Penning TM (2008) Comparisons of (±)-benzo[a]pyrene-trans-7,8-dihydrodiol activation by human cytochrome P450 and aldo-keto reductase enzymes: effect of redox state and expression levels. *Chem Res Toxicol* 21:1086–94, doi:10.1021/tx700345v. [PubMed: 18402469]
- Rojas M, Alexandrov K, Cascorbi I, Brockmöller J, Likhachev A, Pozharisski K, Bouvier G, Auburtin G, Mayer L, Koop-Schneider A, Roots I, Bartsch H. (1998) High benzo[a]pyrene diol-epoxide DNA adduct levels in lung and blood cells from individuals with combined *CYP1A1 MspI/Msp-GSTM1*0/*0* genotypes. *Pharmacogenetics* 8:109–18, PMID:. [PubMed: 10022748]
- Ross JA, Nelson GB, Wilson KH, Rabinowitz JR, Galati A, Stoner GD, Nesnow S, Mass MJ (1995) Adenomas induced by polycyclic aromatic hydrocarbons in strain A/J mouse lung correlate with time-integrated DNA adduct levels. *Cancer Res* 55:1039–44, PMID:. [PubMed: 7866986]
- Schults MA, Chiu RK, Nagle PW, Wilms LC, Kleinjans JC, van Schooten FJ, Godschalk RW (2013) Genetic polymorphisms in catalase and CYP1B1 determine DNA adduct formation by benzo[a]pyrene *ex vivo*. *Mutagenesis* 28:181–5, doi:10.1093/mutage/ges070. [PubMed: 23325794]
- Shah PP, Singh AP, Singh M, Mathur N, Mishra BN, Pant MC, Parmar D (2008) Association of functionally important polymorphisms in cytochrome P4501B1 with lung cancer. *Mutat Res* 643:4–10, doi:10.1016/j.mrfmmm.2008.05.001. [PubMed: 18573508]
- Shiizaki K, Kawanishi M, Yagi T (2017) Modulation of benzo[a]pyrene-DNA adduct formation by CYP1 inducer and inhibitor. *Genes Environ* 39:14, doi:10.1186/s41021-017-0076-x. [PubMed: 28405246]
- Shimizu Y, Nakatsuru Y, Ichinose M, Takahashi Y, Kume H, Mimura J, Fujii-Kuriyama Y, Ishikawa T (2000) Benzo[a]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. *Proc Natl Acad Sci (USA)* 97:779–82, 10.1073/pnas.97.2.779. [PubMed: 10639156]
- Smith JN, Mehinagic D, Nag S, Crowell SR, Corley RA (2017) *In vitro* metabolism of benzo[a]pyrene-7,8-dihydrodiol and dibenzo[def,p]chrysene-11,12 diol in rodent and human hepatic microsomes. *Toxicol Lett* 269:23–32, doi:10.1016/j.toxlet.2017.01.008. [PubMed: 28119020]

- Thier R, Brüning T, Roos PH, Bolt HM (2002) Cytochrome P450 1B1, a new keystone in gene-environment interactions related to human head and neck cancer? Arch Toxicol 76:249–56, doi: 10.1007/s00204-002-0349-3. [PubMed: 12107641]
- Thomas AT, Ognibene T, Daley P, Turteltaub K, Radousky H, Bench G (2011) Ultrahigh efficiency moving wire combustion interface for online coupling of high-performance liquid chromatography (HPLC). Anal Chem 83:9413–7, doi: 10.1021/ac202013s.
- Tuominen R, Baranczewski P, Warholm M, Hagmar L, Möller L, Rannug A (2002) Susceptibility factors and DNA adducts in peripheral blood mononuclear cells of aluminum smelter workers exposed to polycyclic aromatic hydrocarbons. Arch Toxicol 76:178–86, doi:10.1007/s00204-002-0331-0. [PubMed: 11967624]
- Villalta PW, Hochalter JB, Hecht SS (2017) Ultrasensitive high-resolution mass spectrometric analysis of a DNA adduct of the carcinogen benzo[a]pyrene in human lung. Anal Chem 89:12,735–42, doi:10.1021/acs.analchem.7b02856.
- Wenzlaff AS, Cote ML, Bock CH, Land SJ, Schwartz AG (2005a) GSTM1, GSTT1, and GSTP1 polymorphisms, environmental tobacco smoke exposure and risk of cancer among never smokers: a population based study. Carcinogenesis 26:395–401, doi:10.1093/carcin/bgh326. [PubMed: 15528218]
- Wenzlaff AS, Cote ML, Bock CH, Land SJ, Santer SK, Schwartz DR, Schwartz AG (2005b) CYP1A1 and CYP1B1 polymorphisms and risk of lung cancer among never smokers: a population-based study. Carcinogenesis 26:2207–12, doi:10.1093/carcin/bgi191. [PubMed: 16051642]
- WHO, Benzo[a]pyrene (1998) WHO FoodAdditives Series 28 in <http://www.inchem.org/documents/ehc/ehc202.htm#SubSectionNumber:7.1.6> (accessed 08–22–2018), United Nations environment programme, international labour organization, World Health Organization, international programme on chemical safety, environmental health criteria 202, selected non-heterocyclic, polycyclic aromatic hydrocarbons, ISBN 92 4 157202 7, (NLM Classification: QD 341.H9), ISSN 0250–863X.
- Xie Y, Liu GQ, Miao XY, Liu Y, Zhou W, Zhong DW (2012) CYP1B1 leu431val polymorphism and colorectal cancer risk among Caucasians: a meta-analysis. Tumour Biol 33:809–16, doi:10.1007/s13277-011-0298-7. [PubMed: 22190224]
- Xu W, Zhou Y, Hang X, Shen D (2012) Current evidence on the relationship between CYP1B1 polymorphisms and lung cancer risk: a meta-analysis. Mol Biol Rep 39:2821–9, doi:10.1007/s11033-011-1041-6. [PubMed: 21674184]
- Zamzow D, Jankowiak R, Cooper RS, Small GJ, Tibbels SR, Cremonesi P, Devanesan P, Rogan EG, Cavalieri EL (1989) Fluorescence line narrowing spectrometric analysis of benzo[a]pyrene-DNA adducts formed by one-electron oxidation. Chem Res Toxicol 2:29–34, PMID: [PubMed: 2519228]
- Zuo J, Brewer DS, Arlt VM, Cooper CS, Phillips DH (2014) Benzo[a]pyrene-induced DNA adducts and gene expression profiles in target and non-target organs for carcinogenesis in mice. BMC Genomics 15:880, doi:10.1186/1471-2164-15-880. [PubMed: 25297811]

HIGHLIGHTS

- There is little intra-individual variation in $T_{1/2}$, $C_{\max}$ or $T_{\max}$ of [^{14}C]-benzo[a]pyrene
- [^{14}C]-Benzo[a]pyrene is rapidly absorbed from GI and eliminated from plasma
- UPLC-accelerator mass spectrometry shows benzo[a]pyrene is rapidly metabolized
- Little or no covalent adduction to DNA in PBMCs occurs (< 1 in 10^{11} base pairs)
- Two individuals showed marked differences in the profile of metabolites in plasma

Fig. 1. Time Intervals Between Dose and Plasma Levels of $[^{14}\text{C}]\text{-BaP}_{\text{eq}}$ Determined by the Graphite Method over 72 Hours

Volunteers were fasted overnight and dosed (9:00–10:00 AM) with 46 ng $[7\text{-}^{14}\text{C}]\text{-BaP}$ (5 nCi) in a food-grade cellulose capsule with 100 mL of water. Blood was drawn at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 8, 12, 24, 48 and 72 hours. Plasma was extracted with ethyl acetate and $^{14}\text{C}/\text{C}$ determined by the graphite method at the Center for Accelerator Mass Spectrometry (AMS), Lawrence Livermore National Laboratory. The 1 kv AMS has a precision of 3% and sensitivity of 0.7 attomol ^{14}C per mg carbon. Additional experimental details are provided in Materials and Methods. The levels of $[^{14}\text{C}]\text{-BaP}_{\text{eq}}$ in fg/mL plasma

are depicted over 72 hours for each of the three dosing or cycles for the five volunteers. Results are shown for the first (C1-●), second (C2-■) and third cycles (C3-▲). The legend inset gives days elapsed, at the beginning of C2 and C3, since the first dose (C1). The raw data can be found as an Excel file in the Supplementary Materials.

Fig. 2. Time Intervals Between Dose and PBMC [^{14}C]-BaP_{eq} DNA Adducts Determined by the Graphite Method over 72 Hours

PBMCs were isolated and nuclear DNA extracted, at each of the time points for plasma collection, as described in Materials and Methods. The time course data for volunteers BaP002 (●), BaP003 (■) and BaP004 (▲) was following a single dosing of 46 ng, 5 nCi [^{14}C]-BaP (top panel) and from two separate dosing to volunteer BaP009 (36 day interval between dosing BaP009a-● and BaP009b-■) (bottom panel). Twenty μg DNA was analyzed with an LOD of 10 fg [^{14}C]-BaP/mg DNA, equivalent to 0.5 adducts/ 10^{11} nucleotides). The data are expressed as the number of [^{14}C]-BaP_{eq} covalent adducts per 10^{11} nucleotides.

Fig. 3. [^{14}C]-BaP and Metabolite Profile by UPLC-AMS as a Function of Time Post-Dosing for Volunteers BaP002 and BaP009

Blood was collected from volunteers BaP002 and BaP009 at intervals from 0–72 hours and plasma isolated and extracted with ethyl acetate. The extract was taken up in acetonitrile and [^{14}C]-BaP and metabolites resolved utilizing UPLC interfaced via a “moving wire” into a 250 kV AMS. The UPLC conditions and operation of the “moving wire” interface were as described in Materials and Methods. Metabolites 1–3 (M1●, M2■ and M3▲) eluted early in the gradient (Supplemental Table 2) and are possibly dihydrodiols or tetrols. The metabolite designated “quinone” (M4▼) eluted at 7.66 minutes. At this time we cannot, with confidence, designate this peak as either the 1,6-, 3,6- or 6,12-quinone of [^{14}C]-BaP. This peak is unlikely [^{14}C]-BaP-7,8-dione as that standard elutes somewhat later in the gradient as a single peak (Supplemental Fig. 3). The single phenol (M5◆) observed co-elutes with 3-hydroxy-[^{14}C]-BaP. The metabolite identities are tentative and based on similar retention times to the BaP standards available (see Materials and Methods for list). Parent [^{14}C]-BaP (◆) was well resolved from the metabolites and was a minor component in the profiles from both volunteers even at early time points.

Table 1.Volunteer^a Demographics and *CYP1B1* and *GSTM1* Genotypes

	Age	Gender	Height (cm)	Weight (kg)	BMI	CYP1B1 *1 and *3	GSTM1*1 and *0
BaP002^b	63	M	175	99.8	32.5	*3/*3	*0/*0
BaP003	38	M	190	131.8	36.5	*1/*1	*0/*0
BaP004	46	F	173.5	55.6	18.5	*1/*3	*0/*0
BaP009	55	F	157	77.8	31.6	*1/*1	*0/*0
BaP014	30	M	183	68.5	20.5	*1/*3	*1/*0 or *1/*1 ^c

^aRace/ethnicity- All volunteers were Caucasian, not Hispanic^bThe numbers are non-continuous as 10 other enrolled subjects did not meet eligibility criteria (screen fail) or voluntarily withdrew prior to beginning the study or completing 3 cycles.^cDoes not distinguish between *1/*1 or *1/*0

Table 2.

Plasma Pharmacokinetics of [¹⁴C]-BaP_{eq} and [¹⁴C]-DBC_{eq} Following Micro-Dosing^a

[¹⁴ C]-PAH	T _{max} (hr)	C _{max} (fg/mL)	AUC _{0-72hr} (fg × hr × mL ⁻¹)	AUC _{0,∞} (fg × hr × mL ⁻¹)	T _{1/2} (hr) ^b	T _{1/2α} (hr) ^c	T _{1/2β} (hr) ^c	V (L) ^c
[¹⁴ C]-BaP	1.25±0.44 (0.75–2.0)	70±20 (29–82)	1400±483 (649–2060)	2615±962 (1769–4306)	70±27 (31–114)	1.1±0.3 (0.7–1.6)	89±42 (38–156)	452±386 (235–1146)
[¹⁴ C]-DBC ^d	2.25±0.95 (1.5–4.0)	69±40 (23–124)	1345±920 (315–2851)	2193±1947 (315–4892)	37±30 (9–93)	1.4±0.3 (1.0–1.9)	42±16 ^e (20–70)	327±183 (116–646)

^aMean ± SE with (range), of 3 separate dosing of 5 volunteers for [¹⁴C]-BaP_{eq} (Hummel et al., 2018) and 6 volunteers for [¹⁴C]-DBC_{eq} (Madeen et al., 2015)

^bNon-compartmental model

^cTwo-compartmental model

^dData taken from Madeen et al., (2015)

^eOne outlier of 2683 hours was not used in the calculation