Published in final edited form as:
Chem Res Toxicol. 2011 February 18; 24(2): 246–252. doi:10.1021/tx100345x.

Immediate Consequences of Cigarette Smoking: Rapid Formation of Polycyclic Aromatic Hydrocarbon Diol Epoxides

Yan Zhong, **Steven G. Carmella**, **Pramod Upadhyaya**, **J. Bradley Hochalter**, **Diane Rauch**, **Andrew Oliver**, **Joni Jensen**, **Dorothy Hatsukami**, **Jing Wang**, **Cheryl Zimmerman**, and **Stephen S. Hecht***
Masonic Cancer Center and Department of Pharmacology, University of Minnesota, 420 Delaware St SE - MMC 806, Minneapolis, MN 55455

Abstract

Polycyclic aromatic hydrocarbons (PAH) are among the likely major causative agents for lung cancer in smokers. PAH require metabolic activation to exert their carcinogenic effects, and one important pathway proceeds through a three-step sequence resulting in the formation of diol epoxides which react with DNA producing adducts that can cause mutations and initiate the carcinogenic process. However, no previous published studies have examined this critical pathway in humans specifically exposed to PAH by inhalation of cigarette smoke. We used a unique approach employing a stable isotope derivative of phenanthrene, the simplest PAH with a bay region, a feature closely associated with PAH carcinogenicity. Twelve subjects each smoked a cigarette to which [D_{10}]phenanthrene had been added. Plasma was analyzed for [D_{10}]*r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene ([D_{10}]PheT), the major end product of the diol epoxide metabolism pathway of phenanthrene. The analysis was performed by gas chromatography-negative ion chemical ionization-tandem mass spectrometry, using [$^{13}C_6$]PheT as internal standard. The results demonstrated that the three-step pathway resulting in formation of diol epoxides, as monitored by [D_{10}]PheT, occurred with remarkable rapidity. Levels of [D_{10}]PheT in plasma of all subjects were maximal at the earliest time points examined, 15–30 min after smoking the cigarette containing [D_{10}]phenanthrene, and decreased thereafter. These results demonstrate that the formation of a PAH diol epoxide occurs rapidly in smokers. Since PAH diol epoxides are mutagenic and carcinogenic, the results clearly demonstrate immediate negative health consequences of smoking which should serve as a major warning to anyone contemplating initiating tobacco use.

Keywords

cigarette smoking; polycyclic aromatic compounds; diol epoxides

Introduction

Lung cancer kills an average of 3,000 people in the world each day (1). An estimated 90% of this horrific toll is due to cigarette smoking, which also causes at least 18 other types of cancer (2). Among the multiple carcinogens and toxicants in cigarette smoke, polycyclic aromatic hydrocarbons (PAH) are widely viewed as important causative agents for lung cancer (3–5). PAH are formed in the incomplete combustion of organic matter, including tobacco, and always occur as mixtures (6). Some PAH are potent carcinogens, readily

*To whom correspondence should be addressed: Masonic Cancer Center, University of Minnesota, MMC 806, 420 Delaware St SE, Minneapolis, MN 55455, USA., phone: (612) 624-7604, fax: (612) 626-5135, hecht002@umn.edu.

inducing tumors of the lung, skin, and other organs upon treatment of laboratory animals (7). One member of this class of compounds, benzo[*a*]pyrene (BaP), is classified as "carcinogenic to humans" by the International Agency for Research on Cancer (6).

PAH require metabolism to exert their mutagenic and carcinogenic effects (7–9). The formation of metabolites that bind covalently to DNA producing potentially mutagenic DNA adducts that can cause miscoding and permanent mutations is an accepted mechanism by which PAH initiate the carcinogenic process (6). Multiple studies clearly demonstrate that certain diol epoxide metabolites of PAH bind readily to DNA and are highly tumorigenic in animal models (6,7,9,10). Further evidence shows that diol epoxide - DNA adducts are present in human lung tissue, including that obtained from smokers, and that diol epoxides of BaP and other PAH can cause mutations in the *p53* tumor suppressor gene similar to those found in lung tumors from smokers (5,11–14).

Formation of diol epoxides from phenanthrene (Phe), the simplest PAH with a bay region, is illustrated in Scheme 1 (15–19). Phe is generally considered non-carcinogenic but it has structural features similar to those of other PAH such as BaP (Figure 1). The first step is cytochrome P450-catalyzed formation of an epoxide, followed by epoxide hydrolase-catalyzed hydration to yield diols such as Phe-(1*R*,2*R*)-diol (1) and Phe-(3*R*,4*R*)-diol (4) (Scheme 1). The diols can then undergo a second epoxidation to produce diol epoxides 2 and 5. Previous metabolic studies of Phe have shown that both Phe bay region diol epoxide (2) and Phe reverse diol epoxide (5) are formed in humans, with the latter predominating (15). Phe bay region diol epoxide (2) is structurally analogous to the highly carcinogenic bay region diol epoxide of BaP (BPDE, Figure 1), considered to be a major ultimate carcinogen of BaP (6). Many PAH diol epoxides react readily with DNA, but their major fate is rapid and facile reaction with H_2O, producing tetraols. In the case of Phe, tetraol enantiomers 3 and 6, collectively termed PheT and having absolute stereochemistry as indicated in Sheme 1, are formed and are usually quantified together (20–22). Tetraols such as PheT are excellent biomarkers for the PAH diol epoxide metabolism pathway because they cannot be formed any other way. In previous studies, we have developed methods for quantitation of PheT in human urine and plasma (20,21,23). PheT, which is found in all human urine samples due to environmental and dietary exposure to Phe, is present in much greater quantities than the corresponding tetraol derived from BPDE, and is therefore a more practical biomarker of PAH exposure plus metabolic activation. Levels of PheT are higher in smokers than in non-smokers (21).

Many studies have investigated PAH metabolism in human tissues in vitro, and some have also quantified PAH metabolites in human urine and blood as well as PAH-DNA adducts in human tissues including lung tissue from smokers (6,8,12,24,25). However, there are to our knowledge no reports in the literature on human PAH pharmacokinetics after exposure by inhalation, from a cigarette or from any other source. Indeed, a recent comprehensive review concludes that "most of the available data on toxicokinetic parameters for PAHs derive from studies… in animals" (6). In the study reported here, we used a double stable isotope labeling strategy to assess the fate of [D_{10}]Phe inhaled by a cigarette smoker. Smokers smoked cigarettes to which [D_{10}]Phe had been added, and [D_{10}]PheT as well as unlabelled PheT were quantified in plasma, using [$^{13}C_6$]PheT as internal standard. The results demonstrate the remarkable rapidity of Phe diol epoxide formation in smokers.

Materials and Methods

Chemicals

[D_{10}]Phe (98%, containing 2% non-deuterated Phe) was purchased from Cambridge Isotope Laboratories. [$^{13}C_6$]PheT was prepared by hydrolysis in 50/50 THF/H_2O of of

[$^{13}C_6$]Phe(1*R*,2*S*)diol-(3*S*,4*R*)epoxide (26). PheT was kindly provided by Drs. Donald M. Jerina and Haruhiko Yagi.

Purification of [D_{10}]Phe

[D_{10}]Phe was re-purified in the University of Minnesota Molecular and Cellular Therapeutics GMP facility by normal phase HPLC followed by recrystallization from ethanol, m.p. 102 °C. Its chemical purity was greater than 99% as established by normal and reverse phase HPLC, UV, MS, and ^{1}H- and ^{13}C-NMR. Its isotopic purity was 98% [D_{10}]Phe, 2% Phe, as determined by ^{1}H-NMR.

Cigarettes containing [D_{10}]Phe

Using a specially designed cigarette spiking instrument which distributes an additive uniformly along the tobacco rod, [D_{10}]Phe, dissolved in 20 μL ethanol, was added to each of 20 Marlboro cigarettes at each of the following levels (μg): 0, 25, 50, 100, and 200. The cigarettes were sent to Dr. David Ashley at the Centers for Disease Control and Prevention, Atlanta, GA, where they were smoked on a machine. Ten cigarettes at each spike level were smoked under ISO conditions (puff volume 35 mL; puff interval 60 sec; puff duration 2 sec; no vent blocking) and 10 were smoked under Health Canada “intense” conditions (puff volume 55 mL; puff interval 30 sec; puff duration 2 sec; ventilation holes blocked). Mainstream smoke total particulate matter from 3 cigarettes was collected on one Cambridge filter pad, and the pads were sent back to the University of Minnesota, where they were analyzed by GC-MS, essentially as described (27), for [D_{10}]Phe, using [$^{13}C_6$]Phe as internal standard. Levels of added [D_{10}]Phe were plotted against levels of [D_{10}]Phe detected in mainstream smoke. The Health Canada intense smoking regimen was chosen for this study because it more closely approximated the subjects’ natural smoking pattern.

Study Design

This study was approved by the University of Minnesota Institutional Review Board and the U.S. Food and Drug Administration. Cigarette smokers were recruited using advertisements on the radio, television or in metropolitan and campus newspapers. The specific inclusion criteria included: smoking at least 10 cigarettes daily for the past year; being in good physical health with no unstable medical condition; having stable mental health e.g. not currently experiencing unstable or untreated psychiatric diagnosis including substance abuse as determined by the DSM-IV criteria; in the case of female subjects, not pregnant or nursing.

Cigarette smokers who were interested in the study called the clinic, were informed about the study, and were screened to determine whether they met specific inclusion criteria. Subjects meeting these criteria were asked to come into the clinic for an orientation visit, to provide informed consent, and to undergo a more thorough screening. They filled out a questionnaire including information on age, gender, medical history, medication use, and smoking history including number of years of smoking, age at onset of smoking, number of cigarettes per day, and brand of cigarettes used. Pregnancy tests were done. Subjects were informed that the study would examine ways in which their bodies metabolized toxic constituents of cigarette smoke. Subject recruitment incentives were used, with an average of $500 per subject for completing the study.

Subjects were asked to smoke the cigarette containing [D_{10}]Phe through a smoking topography device which recorded the number of puffs, puff duration, and puff volume. Subjects first underwent an adaptation trial prior to smoking the cigarette containing [D_{10}]Phe. They were given specific instructions on puff number, inter-puff interval, puff duration and puff volume to achieve the correct conditions. They then engaged in a practice

session with a Marlboro cigarette. Puff volume was assessed by the topography device, and when a specific puff volume and duration were attained, the subject was given a signal to exhale.

Subjects reported to the clinic at 7:00 a.m. and were fitted with an intravenous catheter or heparin lock to allow serial blood sampling without the need for multiple venipunctures. Blood samples of 10 mL each were taken 30 min before smoking the cigarette and 15, 30, 45, 60, 90, 120, 150, 240, 360, 540 or 720, and 1440 min after the completion of smoking the cigarette. Blood samples were collected into anticoagulant-containing vacuum collection tubes and centrifuged to obtain plasma which was frozen at −20 °C until analysis.

Analysis of Plasma

The method was modified from that described previously (23). A mixture of 0.8 mL plasma, 3 mL saline, 12,000 units of β-glucuronidase (recombinant from *E. coli* BL21, Sigma-Aldrich catalog # G8295), and 25 pg (100 fmol) of [$^{13}C_6$]PheT (internal standard) was incubated overnight at 37 °C, and then applied to a preconditioned Oasis MCX mixed mode cation exchange solid-phase extraction cartridge (60 mg, Waters, Milford, MA). The PheT-containing fraction was eluted with 4 mL 40% CH_3OH in H_2O, concentrated to dryness, and passed through a 0.45-μm nylon filter into an HPLC vial (Whatman, Clifton, NJ) with 16.7% CH_3OH. To the vial was added 6 μg of *trans*-1,2-dihydroxy-1,2-dihydronaphthalene as an HPLC UV marker (retention time: 12.5 min). The apparatus for HPLC was the same as reported before (23). The column was eluted with an acetonitrile/1% aq formic acid gradient at 1.0 mL/min. The gradient program was as follows (percentage 1% aq formic acid, time): 95% to 93%, 0 to 8.2 min; 93%, 8.2 to 9.5 min; 93% to 0%, 9.5 to 10 min; 0%, 10 to 13 min. The UV/Vis detector was set at 254 nm. HPLC eluant was collected for 5 min before the peak of the UV marker appeared (collection time: 7.5 – 12 min). The retention time for PheT, [D_{10}]PheT and [$^{13}C_6$]PheT was about 10 min. The collected HPLC fraction was dried, transferred to a glass insert vial with CH_3OH, dried again, and dissolved in 10 μL of *bis*-trimethylsilyltrifluoroacetamide plus 1% trimethylchlorosilane (BSTFA, Regis Technologies, Morton Grove, IL). The samples were heated to 60 °C for 1 h. Two μL were injected on GC-NICI-MS/MS using the splitless mode.

GC-NICI-MS/MS analysis was carried out with a TSQ Quantum instrument (Thermo Scientific, San Jose, CA) with a 0.25 mm (inside diameter) × 30 m, 0.15-μm film thickness, DB-17MS column (Agilent Technologies, Palo Alto, CA), and a 0.53 mm (inside diameter) × 2 m deactivated fused silica pre-column. The analytes were detected as their trimethylsilyl derivatives. The oven temperature program was as follows: 80 °C for 1 min, then 80 to 200 °C at 35 °C/min, then 200 to 215 °C at 3 °C/min, then 215 to 320 °C at 35 °C/min, then hold for 4 min. The injector was operated in the splitless mode. The injection port temperature was 250 °C and the MS transfer line temperature was 320 °C. The flow rate was 1 mL/min He. The NICI-MS/MS conditions were as follows: CI gas, methane at 1.5 ml/min; source temperature, 200 °C; emission current, 350 μA. Selected reaction monitoring with a collision energy of 12 eV, electron energy of −150 eV, and Ar collision gas pressure of 1.0 mTorr was used to detect PheT, [$^{13}C_6$]PheT, and [D_{10}]PheT at *m/z* 372 → *m/z* 210, *m/z* 378 → *m/z* 216, and *m/z* 382 → *m/z* 220 respectively. Quadrupoles 1 and 2 were operated at a resolution of 0.7 amu.

Pharmacokinetic Analysis

Based on the concentration-time data, a noncompartmental analysis was carried out using Phoenix WinNonlin v6.1 (PharSight, Cary, NC). The area under the concentration-time curve ($AUC_{0\text{-}t}$) was calculated with the linear trapezoidal rule up to the last measured time point. The remainder of the AUC ($AUC_{t\text{-}\infty}$) was calculated by dividing the last measured

time point by the rate constant associated with the terminal mono-exponential phase, γ. Summation of the two portions of the AUC results in $AUC_{0\text{-}\infty}$, which is a measure of the exposure of the body to $[D_{10}]$Phe diol epoxides, assessed by $[D_{10}]$PheT. The half-life associated with the terminal phase was determined by dividing 0.693 by γ. The apparent clearance (CL/F) was calculated by dividing the inhaled dose (10 μg $[D_{10}]$Phe) by the $AUC_{0\text{-}\infty}$.

Results

In preliminary experiments, we determined the approximate delivery of $[D_{10}]$Phe in mainstream smoke of cigarettes to which various amounts of $[D_{10}]$Phe had been added to the tobacco. These cigarettes were smoked on a machine, using either the International Organization of Standards (ISO) conditions or the "intense" conditions favored by Health Canada to evaluate smoke emissions. Plots of added $[D_{10}]$Phe versus mainstream smoke levels of $[D_{10}]$Phe were linear. Using these plots, we determined that a cigarette to which 80 μg of $[D_{10}]$Phe had been added delivered approximately 10 μg $[D_{10}]$Phe in its mainstream smoke under Canadian intense conditions. These smoking conditions were duplicated to the extent possible by each subject, by monitoring their smoking with a topography apparatus, such that the inhaled dose of $[D_{10}]$Phe was approximately 10 μg. Blood samples were drawn at baseline and at intervals beginning 15 min after each subject finished smoking the single cigarette, which took approximately 5 min for about 8 puffs.

For the analysis of plasma samples, $[^{13}C_6]$PheT was added as the internal standard. After enrichment steps, quantitation of PheT, $[D_{10}]$PheT, and $[^{13}C_6]$PheT were accomplished by gas chromatography-negative ion chemical ionization-tandem mass spectrometry (GC-NICI-MS/MS) with selected reaction monitoring. The transition monitored for PheT was *m/z* 372 → *m/z* 210 which corresponds to loss of $[OSi(CH_3)_3 + Si(CH_3)_3]$ from its base peak. The corresponding transitions for $[D_{10}]$PheT and $[^{13}C_6]$PheT were *m/z* 382 → *m/z* 220 and *m/z* 378 → *m/z* 216, respectively. Typical chromatograms from this analysis are illustrated in Figure 2A,B. The analysis at baseline, 30 min before a subject smoked the cigarette containing $[D_{10}]$Phe, shows peaks for PheT and the internal standard $[^{13}C_6]$PheT, but no detectable peak for $[D_{10}]$PheT (Figure 2A). The origin of PheT, which is detected in all human urine samples, is Phe in cigarette smoke, the diet, and the general environment. The analysis of the sample collected 15 min. after the subject smoked the cigarette containing $[D_{10}]$Phe shows a clear peak for $[D_{10}]$PheT (Figure 2B). Clean, readily quantifiable peaks such as these were observed in all samples. Positive and negative control samples were included with each set of analyses, and produced the expected values.

The results summarized in Table 1 show that levels of $[D_{10}]$PheT reached their maximum in each subject's plasma within 15–30 min after smoking the cigarette, and decreased thereafter. This is illustrated for one subject in Figure 3, which also shows that levels of PheT remained relatively constant throughout the time period studied, as would be expected due to steady state exposure to Phe. Plots such as that in Figure 3 for the other 11 subjects were similar, and resemble those that would be expected from an intravenous bolus dose.

The pharmacokinetic results are shown in Table 2. The AUC ranged from 6560 to 92200 fmol-min/mL with a terminal elimination half-life of 290–681 min. The apparent clearance ranged from 577 – 8110 mL/min.

Discussion

This study is unique. It is the first to investigate human metabolism of a PAH specifically delivered by inhalation in cigarette smoke, without interference by other sources of exposure

such as air pollution or the diet. The use of cigarettes containing [D_{10}]Phe made this possible.

Our results clearly demonstrate that the formation of Phe diol epoxides occurs rapidly in smokers. The biomarker [D_{10}]PheT, which results from hydrolysis of [D_{10}]Phe diol epoxides, was readily detected in all plasma samples after subjects smoked a cigarette containing [D_{10}]Phe, and its levels were maximal 15–30 min after the subjects finished smoking the cigarette. These results are significant because PAH diol epoxides react readily with DNA, induce mutations, and are considered to be ultimate carcinogens of multiple PAH in cigarette smoke (6). In fact, the concentration-time profiles looked similar to what one might have expected if an intravenous bolus dose had been administered, indicating how rapidly exposure to [D_{10}]PheT develops. This type of profile lacks both an absorptive and a distributive phase, and the highest concentration is at the first time of blood sampling. This concentration-time profile is even more surprising considering that it is the profile of the *metabolite* rather than the parent compound. The pharmacokinetic interpretation of this profile is that the multi-stage metabolic processing of [D_{10}]Phe to its diol epoxide occurs extremely rapidly and supports the concept of the lung as an important “first-pass” metabolizing organ for compounds absorbed from cigarette smoke.

While previous studies indicate that PAH diol epoxides are formed in humans and that their levels may be higher in smokers than in non-smokers, their rates of formation in smokers were unknown (12,15,21). As shown in Scheme 1, diol epoxide formation requires three steps catalyzed by P450s and epoxide hydrolase. Multiple competing reactions such as glutathione-*S*-transferase-catalyzed detoxification of the initially formed epoxides, or glucuronidation of the diol metabolites could retard or even prevent diol epoxide formation (8). Our results suggest that these competing reactions have relatively little impact on rates of diol epoxide formation.

One striking finding is the large variability in the exposure of the subjects to [D_{10}]Phe diol epoxides. Subjects 2 and 4 had the lowest exposures (as measured by the AUC $_{0\text{-}\infty}$) which resulted in very high apparent clearances, indicating that the mechanisms which remove [D_{10}]PheT from the body of these subjects are relatively efficient. On the other hand, Subjects 1 and 6 had very high AUCs, indicating that these subjects have greater overall exposure to [D_{10}]Phe diol epoxides. It is unlikely that this wide range of exposure is due to differences in dosing, considering the sophisticated and well-monitored means by which the cigarettes were smoked. It is much more likely that the difference in exposures is due to the subjects’ relative abilities to form and eliminate the metabolites of Phe.

Stable isotope labeling has been used previously in studies of nicotine and cotinine metabolism in tobacco users (28,29). These studies have defined pharmacokinetic aspects of nicotine metabolism and have probed the utility of metabolic ratios in predicting nicotine dependence in smokers. A deuterium labeled tobacco-specific nitrosamine has also been added to cigarettes to investigate its metabolic products in smokers (30). However, no previous studies have examined PAH metabolism in smokers using a deuterated probe compound.

The results reported here should serve as a stark warning to those who are considering starting to smoke cigarettes: PAH diol epoxide formation occurs immediately and is not a theoretical long term effect. PAH diol epoxides are DNA damaging compounds which can cause genetic damage and initiate the carcinogenic process.

This study has some limitations. We used [D_{10}]Phe as a representative PAH because Phe is generally considered non-carcinogenic. Addition of a carcinogenic PAH to a cigarette would be potentially hazardous and possibly unethical. Although there are similarities in the P450s

that catalyze diol epoxide formation from Phe and BaP – P450s 1A1, 1A2, and 1B1 – as well as similarities in their detoxification by glutathione-*S*-transferases (19,31,32), there are also some potential differences in the metabolism and toxicokinetics of Phe versus other PAH that could affect the interpretation of our results. First, metabolism of Phe by the angular ring diol epoxide pathway occurs mainly via Phe reverse diol epoxide (**5**) in humans and to a much smaller extent by Phe bay region diol epoxide (**2**)(Scheme 1)(15), although our unpublished data indicate that measurements of PheT and diol epoxide **2** specifically are highly correlated. It is the bay region diol epoxide which, strictly speaking, would represent the potentially carcinogenic pathway. Second, the rates of tissue distribution and metabolism of Phe and higher molecular weight carcinogenic PAH such as BaP might be quite different, as highly lipophilic PAH such as BaP diffuse slowly into tissues (6). Thus, the results for Phe might not be generalizable to higher molecular weight PAH.

In summary, we demonstrate that the formation of Phe diol epoxides, as represented by the biomarker PheT, is rapid in smokers. These results provide the first direct evidence for human metabolic activation of a PAH delivered in cigarette smoke and indicate the potential for immediate genetic damage in a cigarette smoker.

Acknowledgments

This study was supported by grant no. CA-92025 from the National Cancer Institute. Gas chromatography-mass spectrometry was carried out in core facilities of the Masonic Cancer Center, University of Minnesota, supported in part by National Cancer Institute grant CA-77598. We thank Bob Carlson for editorial assistance.

References

1. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 83. IARC; Lyon, FR: 2004. Tobacco Smoke and Involuntary Smoking; p. 33-1187.
2. Secretan B, Straif K, Baan R, Grosse Y, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Cogliano V. A review of human carcinogens--Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. Lancet Oncol 2009;10:1033–1034. [PubMed: 19891056]
3. Denissenko MF, Pao A, Tang M, Pfeifer GP. Preferential formation of benzo[*a*]pyrene adducts at lung cancer mutational hot spots in P53. Science 1996;274:430–432. [PubMed: 8832894]
4. Hecht SS. Tobacco carcinogens, their biomarkers, and tobacco-induced cancer. Nature Rev Cancer 2003;3:733–744. [PubMed: 14570033]
5. Pfeifer GP, Denissenko MF, Olivier M, Tretyakova N, Hecht SS, Hainaut P. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. Oncogene 2002;21:7435–7451. [PubMed: 12379884]
6. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 92. IARC; Lyon, FR: 2007. Some Non-Heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures; p. 35-818.
7. Dipple, A.; Moschel, RC.; Bigger, CAH. Polynuclear aromatic hydrocarbons. In: Searle, CE., editor. Chemical Carcinogens, Second Edition, ACS Monograph 182. Vol. 1. American Chemical Society; Washington, D.C: 1984. p. 41-163.
8. Cooper CS, Grover PL, Sims P. The metabolism and activation of benzo[*a*]pyrene. Prog Drug Metab 1983;7:295–396.
9. Conney AH. Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G.H.A. Clowes Memorial Lecture. Cancer Res 1982;42:4875–4917. [PubMed: 6814745]
10. Luch, A.; Baird, WM. Metabolic activation and detoxification of polycyclic aromatic hydrocarbons. In: Luch, A., editor. The carcinogenic effects of polycyclic aromatic hydrocarbons. Imperial College Press; London: 2005. p. 19-96.

11. Phillips DH. Smoking-related DNA and protein adducts in human tissues. Carcinogenesis 2002;23:1979–2004. [PubMed: 12507921]
12. Boysen G, Hecht SS. Analysis of DNA and protein adducts of benzo[*a*]pyrene in human tissues using structure-specific methods. Mutation Res 2003;543:17–30. [PubMed: 12510015]
13. Tretyakova N, Matter B, Jones R, Shallop A. Formation of benzo[*a*]pyrene diol epoxide-DNA adducts at specific guanines within *K-ras* and *p53* gene sequences: stable isotope-labeling mass spectrometry approach. Biochemistry 2002;41:9535–9544. [PubMed: 12135376]
14. Alexandrov K, Rojas M, Geneste O, Castegnaro M, Camus AM, Petruzzelli S, Giuntini C, Bartsch H. An improved fluorometric assay for dosimetry of benzo(a)pyrene diol- epoxide-DNA adducts in smokers' lung: comparisons with total bulky adducts and aryl hydrocarbon hydroxylase activity. Cancer Res 1992;52:6248–6253. [PubMed: 1423269]
15. Hecht SS, Carmella SG, Villalta PW, Hochalter JB. Analysis of phenanthrene and benzo[*a*]pyrene tetraol enantiomers in human urine: relevance to the bay region diol epoxide hypothesis of benzo[*a*]pyrene carcinogenesis and to biomarker studies. Chem Res Toxicol 2010;23:900–908. [PubMed: 20369855]
16. Shou M, Korzekwa KR, Krausz KW, Crespi CL, Gonzalez FJ, Gelboin HV. Regio- and stereo-selective metabolism of phenanthrene by twelve cDNA-expressed human, rodent, and rabbit cytochromes P-450. Cancer Lett 1994;83:305–313. [PubMed: 8062229]
17. Nordqvist M, Thakker DR, Vyas KP, Yagi H, Levin W, Ryan DE, Thomas PE, Conney AH, Jerina DM. Metabolism of chrysene and phenanthrene to bay-region diol epoxides by rat liver enzymes. Mol Pharmacol 1981;19:168–178. [PubMed: 7207460]
18. Thakker, DR.; Yagi, H.; Levin, W.; Wood, AW.; Conney, AH.; Jerina, DM. Polycyclic aromatic hydrocarbons: metabolic activation to ultimate carcinogens. In: Anders, MW., editor. Bioactivation of Foreign Compounds. Academic Press, Inc; New York: 1985. p. 177-242.
19. Schober W, Pusch G, Oeder S, Reindl H, Behrendt H, Buters JT. Metabolic activation of phenanthrene by human and mouse cytochromes P450 and pharmacokinetics in CYP1A2 knockout mice. Chem Biol Interact 2010;183:57–66. [PubMed: 19766613]
20. Hecht SS, Chen M, Yagi H, Jerina DM, Carmella SG. *r*-1,*t*-2,3,*c*-4-Tetrahydroxy-1,2,3,4-tetrahydrophenanthrene in human urine: a potential biomarker for assessing polycyclic aromatic hydrocarbon metabolic activation. Cancer Epidemiol Biomarkers & Prev 2003;12:1501–1508.
21. Hecht SS, Chen M, Yoder A, Jensen J, Hatsukami D, Le C, Carmella SG. Longitudinal study of urinary phenanthrene metabolite ratios: effect of smoking on the diol epoxide pathway. Cancer Epidemiol Biomarkers & Prev 2005;14:2969–2974.
22. Hecht SS, Carmella SG, Yoder A, Chen M, Li Z, Le C, Jensen J, Hatsukami DK. Comparison of polymorphisms in genes involved in polycyclic aromatic hydrocarbon metabolism with urinary phenanthrene metabolite ratios in smokers. Cancer Epidemiol Biomarkers & Prev 2006;15:1805–1811.
23. Carmella SG, Yoder A, Hecht SS. Combined analysis of *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in smokers' plasma. Cancer Epidemiol Biomarkers & Prev 2006;15:1490–1494.
24. Jacob J, Seidel A. Biomonitoring of polycyclic aromatic hydrocarbons in human urine. J Chromatog B 2002;778:31–47.
25. Hecht SS. Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. Carcinogenesis 2002;23:907–922. [PubMed: 12082012]
26. Hecht SS, Villalta PW, Hochalter JB. Analysis of phenanthrene diol epoxide mercapturic acid detoxification products in human urine: relevance to molecular epidemiology studies of glutathione-*S*-transferase polymorphisms. Carcinogenesis 2008;29:937–943. [PubMed: 18477646]
27. Ding YS, Trommel JS, Yan XJ, Ashley D, Watson CH. Determination of 14 polycyclic aromatic hydrocarbons in mainstream smoke from domestic cigarettes. Environ Sci Technol 2005;39:471–478. [PubMed: 15707046]
28. Hukkanen J, Jacob P III, Benowitz NL. Metabolism and disposition kinetics of nicotine. Pharmacol Rev 2005;57:79–115. [PubMed: 15734728]

29. Dempsey D, Tutka P, Jacob P III, Allen F, Schoedel K, Tyndale RF, Benowitz NL. Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. Clin Pharmacol Ther 2004;76:64–72. [PubMed: 15229465]

30. Stepanov I, Upadhyaya P, Feuer R, Jensen J, Hatsukami DK, Hecht SS. Extensive metabolic activation of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in smokers. Cancer Epidemiol Biomarkers & Prev 2008;17:1764–1773.

31. Shou M, Korzekwa KR, Crespi CL, Gonzalez FJ, Gelboin HV. The role of 12 *c*DNA-expressed human, rodent, and rabbit cytochromes P450 in the metabolism of benzo[*a*]pyrene and benzo[*a*]pyrene *trans*-7,8-dihydrodiol. Mol Carcinogenesis 1994;10:159–168.

32. Upadhyaya P, Hochalter JB, Balbo S, McIntee EJ, Hecht SS. Preferential glutathione conjugation of a reverse diol epoxide compared with a bay region diol epoxide of benzo[a]pyrene in human hepatocytes. Drug Metab Dispos 2010;38:1397–1402. [PubMed: 20547966]

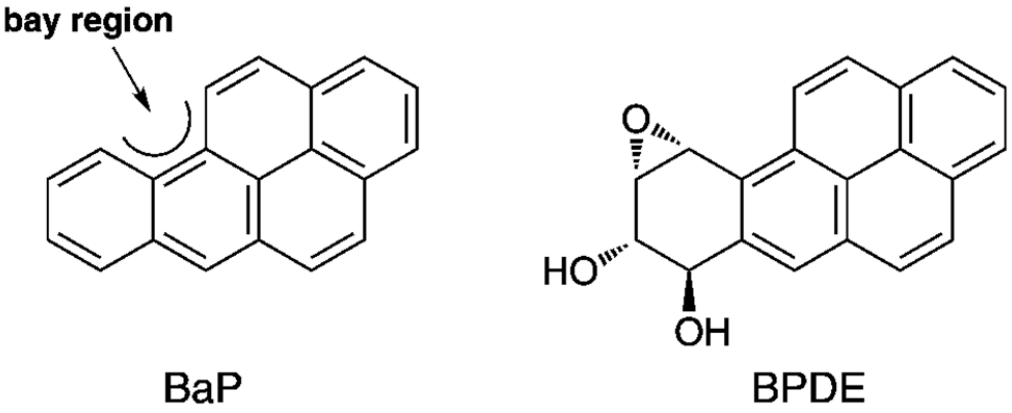


Figure 1.
Structures of BaP and BPDE

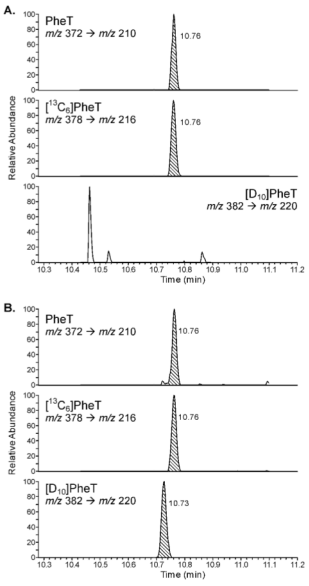


Figure 2.

GC-NICI-MS/MS-SRM analysis of samples containing Phe and $[D_{10}]$Phe metabolites isolated from plasma of smokers: (A) 30 min prior to smoking a cigarette to which $[D_{10}]$Phe had been added; and (B) 15 min after smoking that cigarette. The transitions illustrated were monitored to analyze for PheT and $[D_{10}]$PheT in plasma, and internal standard $[^{13}C_6]$PheT which was added to all plasma samples. Shaded peaks correspond to the target analytes. PheT is present in all samples due to exposure of the smoker to Phe from cigarette smoke and environmental or dietary sources. $[D_{10}]$PheT is present only after smoking the cigarette containing $[D_{10}]$Phe. The internal standard for the analysis is $[^{13}C_6]$PheT.

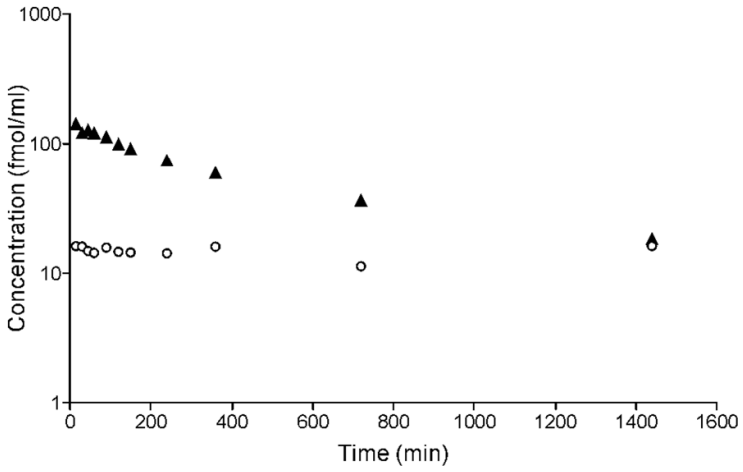
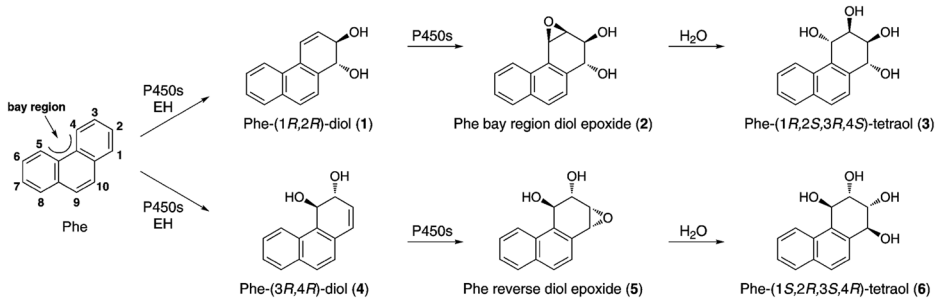


Figure 3.
Levels of [D_{10}]PheT (closed triangles) and PheT (open circles) in the plasma of subject 1 at various intervals after smoking a cigarette containing [D_{10}]Phe (N = 11).



Scheme 1.

Formation of diol epoxides and tetraols in the metabolism of Phe. Three steps are required for diol epoxide formation, catalyzed sequentially by cytochrome P450s, epoxide hydrolase (EH), and cytochrome P450s. All metabolites have the absolute stereochemistry indicated. Compounds **3 + 6** comprise PheT, measured in this study.

Table 1

Levels of [D_{10}]PheT in plasma of 12 subjects at various times after smoking a cigarette containing [D_{10}]Phe

	[D_{10}]PheT (fmol/mL plasma)											
Time point (min)	**Subject 1**	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**	**Subject 6**	**Subject 7**	**Subject 8**	**Subject 9**	**Subject 10**	**Subject 11**	**Subject 12**
−30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	142.0	11.0	70.2	48.1	76.1	203.5	50.3	62.1	134.5	44.9	67.6	44.9
30	121.0	8.8	64.4	62.4	76.6	190.4	39.5	56.5	142.3	48.6	70.8	38.4
45	127.3	9.4	67.3	42.0	64.5	127.9	37.4	56.9	122.7	36.0	60.4	39.8
60	120.4	8.3	63.3	36.8	61.1	158.0	43.8	51.0	98.3	40.9	72.8	38.9
90	110.9	7.9	55.5	36.2	51.7	136.8	39.4	40.7	88.5	49.1	52.2	37.5
120	99.0	8.5	57.7	34.0	53.2	116.7	42.6	50.8	64.1	43.5	54.3	32.8
150	91.2	7.5	57.1	33.4	48.5	105.7	48.0	35.9	63.1	43.3	42.8	31.4
240	73.8	6.2	50.6	24.4	N/A[a]	89.5	37.9	32.9	51.4	36.0	41.0	27.5
360	59.3	5.8	44.5	20.1	27.5	66.6	20.3	19.6	38.3	27.0	39.5	26.9
540 or 720	36.4	3.1	30.1	8.6	12.6	43.5	23.2	16.9	12.1	20.6[b]	29.8[b]	18.8[b]
1440	18.4	1.2	14.9	3.8	ND[c]	16.1	5.8	4.1	3.2	3.4	3.3	4.9

[a] N/A, Not available

[b] 540 min

[c] ND, not detected, detection limit 0.05 fmol/mL.

Table 2

Pharmacokinetics parameters of [D_{10}]PheT in 12 subjects after smoking a cigarette containing 10 μg (53.2 nmol) [D_{10}]Phe

	AUC (min*fmol/mL)	Terminal half-life (min)	CL/F (mL/min)
Subject 1	85900	651	619
Subject 2	6560	489	8110
Subject 3	63500	681	837
Subject 4	22800	402	2330
Subject 5	29000	290	1840
Subject 6	92200	523	577
Subject 7	35700	483	1490
Subject 8	29700	385	1790
Subject 9	40200	295	1320
Subject 10	30500	354	1750
Subject 11	39600	295	1340
Subject 12	29200	447	1830
Mean	**42100**	**441**	**1990**
S.D.	25600	132	2000