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Longitudinal Study of [D₁₀]Phenanthrene Metabolism by the Diol Epoxide Pathway in Smokers

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Abstract

The extent of metabolism of [D₁₀]phenanthrene to [D₁₀]1,2,3,4-tetrahydroxy-1,2,3,4-tetradeuterophenanthrene ([D₁₀]PheT) could be a biomarker of human metabolic activation of carcinogenic polycyclic aromatic hydrocarbons, leading to identification of smokers particularly susceptible to lung cancer. The longitudinal stability of [D₁₀]PheT was evaluated in 24 cigarette smokers given 7 – 8 oral doses of [D₁₀]phenanthrene (10 µg) over 5.5 months. [D₁₀]PheT in 6 h urine was quantified after each dose. The overall coefficient of variation for 24 subjects was (mean ± S.D.) 27.4 ± 8.83%. Thus, a single administration of [D₁₀]phenanthrene is likely sufficient to determine a smoker's ability to metabolize it to [D₁₀]PheT.

Keywords

[D₁₀]phenanthrene; [D₁₀]1, 2, 3, 4-tetrahydroxy-1, 2, 3, 4-tetradeuterophenanthrene ([D₁₀]PheT); urinary metabolites; carcinogenic polycyclic aromatic hydrocarbons; cigarette smokers

Introduction

Lung cancer is the leading cause of cancer death in the world, responsible for 1.37 million deaths in 2008 (<http://www.who.int/mediacentre/factsheets/fs297/en/index.html>). Ninety percent of this incomprehensible death toll is caused by cigarette smoking in countries with prolonged use (International Agency for Research on Cancer 2004). Approximately 1 billion men and a quarter of a billion women in the world are smokers (Shafey et al. 2009). It does not seem likely that the worldwide tobacco problem will significantly diminish in the near future. It is crucial that we prevent people from starting to smoke. For those who have started, it is necessary to find better paths to cessation. About 11–24% of lifelong smokers will develop lung cancer (International Agency for Research on Cancer 2004). If the highly susceptible individuals could be identified at a young age, greater medical surveillance and more intensive smoking cessation strategies than usual might be applied, increasing the probability of successful lung cancer prevention.

Differences in carcinogen metabolism may influence lung cancer susceptibility. Cigarette smoke contains over 70 established carcinogens, many of which require metabolic activation to exert their carcinogenic effects, and there are competing detoxification pathways (Hecht 2012; International Agency for Research on Cancer 2004). Arguably, at any given dose,

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Declaration of Interest.

The authors report no declarations of interest.

individuals who metabolically activate cigarette smoke carcinogens more extensively will be at higher risk for lung cancer than those who do not, because the flux of potentially DNA damaging intermediates in their lung cells will be higher (Hecht 1999; Hecht 2003). This basic hypothesis has been examined in many studies of genetic polymorphisms in carcinogen metabolizing genes, but the results are modest to date (United States Department of Health and Human Services 2010). Our approach to this problem is different. We are exploring carcinogen metabolite phenotyping to identify potentially those smokers who are at highest risk for lung cancer.

Polycyclic aromatic hydrocarbons (PAH) are among the most important carcinogens in cigarette smoke (Hecht 2003; Hecht 2011). A large body of evidence implicates PAH as significant causes of lung cancer in smokers. Multiple carcinogenic PAH in cigarette smoke are powerful carcinogens which induce tumors of the lung and other tissues (International Agency for Research on Cancer 2004; International Agency for Research on Cancer 2010). Metabolic activation is an absolute requirement for PAH carcinogenicity. The most consistent and convincing data support the bay region diol epoxide metabolic activation pathway, illustrated for the classic carcinogen benzo[*a*]pyrene (BaP) in Figure 1 (Luch & Baird 2005). The ultimate carcinogen formed in this pathway, the bay region diol epoxide BPDE, easily reacts with DNA to produce adducts that have been identified in lung tissue DNA of smokers and may cause the mutations that are observed in the *TP53* tumor suppressor gene and *KRAS* oncogene in lung tumors of smokers (Conney 1982; Denissenko et al. 1996; Geacintov et al. 1997; Pfeifer & Besaratinia 2009; Rojas et al. 1998; United States Department of Health and Human Services 2010). BPDE also reacts with H₂O to produce BaP-tetraol, the end product of the BaP metabolic activation pathway (Zhong et al. 2010). Measurement of BaP-tetraol in human urine therefore provides an indicator of the extent of metabolic activation of BaP, which is not readily apparent by considering the complex polymorphisms in the genes involved in BaP metabolism (International Agency for Research on Cancer 2010). Smokers with higher levels of BaP-tetraol in their urine may logically be at higher risk for lung cancer.

Phenanthrene, like BaP, is a component of all PAH mixtures but phenanthrene is not carcinogenic (International Agency for Research on Cancer 2010). Nevertheless, phenanthrene is metabolized by essentially the same diol epoxide pathway as BaP, illustrated in Figure 1 (Nordquist et al. 1981; Shou et al. 1994; Thakker et al. 1985). The end product of this metabolic sequence, *1,2,3,4*-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT), is readily measured in urine because its concentration is about 10,000 times higher than that of BaP-tetraol (Hecht et al. 2003; Hecht et al. 2006; Hecht et al. 2010; Hochalter et al. 2011). Levels of PheT and BaP-tetraol in human urine correlate (Hochalter et al. 2011). Thus, measurement of PheT in human urine is an indicator of PAH exposure plus metabolic activation, and may therefore be a biomarker of lung cancer susceptibility in smokers. Consistent with this concept, PheT levels were significantly associated with lung cancer in a nested case control study of smokers in the Shanghai prospective cohort (Yuan et al. 2011), but a smaller study of serum PheT carried out in the U.S. did not show an association (Church et al. 2009).

Although PheT is a potential biomarker of lung cancer risk in smokers, its utility could be diminished by the fact that dietary and ambient air exposure to phenanthrene also produce the same metabolite, even in non-smokers (Hecht et al. 2005). Thus, PheT is present in the urine of all humans tested so far, whether or not they are smokers, although levels in smokers are higher than in non-smokers (Hecht et al. 2003; Hecht et al. 2005). Dietary exposure to phenanthrene is not likely to be related to lung cancer in smokers. Therefore, as an approach to the development of a biomarker that can uniquely measure PAH metabolic activation in smokers, we have proposed the use of [*D*₁₀]phenanthrene (Figure 2) (Zhong et

al. 2011a; Zhong et al. 2011b). There is no exposure to [D₁₀]phenanthrene from environmental sources or from cigarette smoke. There is no primary deuterium isotope effect in the metabolism of [D₁₀]phenanthrene to [D₁₀]PheT. Thus, administration of [D₁₀]phenanthrene to smokers followed by measurement of [D₁₀]PheT in urine could potentially identify those smokers most capable of metabolically activating PAH by the diol epoxide pathway and who are therefore potentially at higher risk for lung cancer upon exposure to PAH in cigarette smoke. In earlier studies, we have shown that oral administration of [D₁₀]phenanthrene and measurement of [D₁₀]PheT in the 6 h urine of a smoker correlates strongly with levels of this metabolite in 48 h urine, and that oral dosing of [D₁₀]phenanthrene leads to approximately the same amount of [D₁₀]PheT in urine as administration of [D₁₀]phenanthrene in the smoke of a cigarette (Zhong et al. 2011b). These results demonstrated that oral administration of [D₁₀]phenanthrene to smokers followed by analysis of their 6 h urine was a practical and effective way to assess inter-individual differences in the metabolism of [D₁₀]phenanthrene to [D₁₀]PheT. In the study reported here, we further explored this biomarker by assessing the longitudinal reproducibility of [D₁₀]PheT levels in smokers given multiple doses of [D₁₀]phenanthrene. The results would inform us whether a single oral dose of [D₁₀]phenanthrene is sufficient to assess percent conversion to [D₁₀]PheT in a given individual. Previous longitudinal studies have assessed the consistency of unlabelled PheT in urine and plasma of smokers (Church et al. 2010; Hecht et al. 2005).

Methods

Chemicals

[D₁₀]Phenanthrene (>99% chemical purity, containing 2% non-deuterated phenanthrene) was purchased from Cambridge Isotope Laboratories and was re-purified by HPLC in the University of Minnesota Molecular and Cellular Therapeutics GMP facility as previously described (Zhong et al. 2011a). PheT was kindly provided by Haruhiko Yagi and the late Donald M. Jerina, National Institutes of Health. [¹³C₆]PheT and [D₁₀]PheT were prepared as described (Carmella, Yoder, & Hecht 2006; Hecht, Villalta, & Hochalter 2008).

Study Design

This study was approved by the U.S. Food and Drug Administration (IND 72,537) and the University of Minnesota Institutional Review Board Human Subjects Committee. Study participants were recruited by a member of the research staff of the University of Minnesota Tobacco Research Programs using posted flyers, newspaper advertisements, and the program website. Inclusion criteria were as follows: current smoker of at least 10 cigarettes per day for the past year and in good physical and mental health with no unstable medical condition as determined by medical history and investigator assessment; and in stable mental health (not currently experiencing unstable or untreated psychiatric diagnosis including substance abuse as determined by the DSM-IV criteria). Female subjects who were pregnant or nursing were excluded. Subjects provided written consent prior to enrollment.

Subjects meeting these criteria were invited to the research clinic for an orientation visit and to participate in screening, including a pregnancy test for females. They completed a questionnaire including information on age, gender, medical history, medication use, and smoking history.

This was a single center, non-randomized, open label study. Subjects were asked to fast starting from midnight before each 6–10 a.m. visit to the research clinic. At each visit, the subject received an oral dose of 10 µg (53.2 nmol) of [D₁₀]phenanthrene in 5 ml of 20%

ethanol-80% water solution. Each dose was freshly prepared by adding 4 ml of H₂O to a solution of 10 µg [D₁₀]phenanthrene in 1 ml of ethanol. The subjects were allowed to eat a light breakfast at the research clinic one h after consuming the dose. The breakfast consisted of approximately 60% carbohydrate, 25% fat and 15% protein for a total of about 550 calories. They collected all of their urine for 6 h after consuming the dose. The volume of each urine collection was measured and aliquots were frozen at -20 °C until analysis.

The schedule of dosing for this longitudinal study was as follows: dose 1 as described in the previous paragraph, with all subsequent doses following the same protocol. Urine was collected at weeks 0, 2, 4, 6, 10, 14, 18 and 22. Thus, 8 doses were administered over a period of 5.5 months. Some subjects missed one dose, as noted in "Results."

Analysis of [D₁₀]PheT in urine

An aliquot of urine (100 µl), collected from subjects to whom [D₁₀]phenanthrene had been administered, was placed in a 1.5 ml polypropylene tube and mixed with 150 µl of H₂O, 45 µl of 2.5 M NaOAc buffer, pH 5, β-glucuronidase (1050 units) and arylsulfatase (8400 units) from *Helix pomatia* (Roche), and 100 fmol of [¹³C₆]PheT internal standard in 20 µl isopropyl alcohol. (Previous studies have shown that PheT is excreted mainly as glucuronide conjugates (Hecht et al. 2003).) The mixture was incubated overnight with shaking at 37 °C. The sample was then partially purified by loading onto a 30 mg Oasis MCX mixed mode cation exchange sorbent cartridge (Waters Co., Milford MA) that had been activated with 1 ml of CH₃OH and 1 ml of H₂O. The cartridge was washed with 0.4 ml of 20% CH₃OH in H₂O, 0.4 ml of 20% CH₃OH containing 2% formic acid, and then 0.4 ml of 20% CH₃OH, and the analyte was eluted with 0.25 ml of 50% CH₃OH in H₂O, collected in a 1 ml silanized vial, and the solvents were removed on a Speedvac. The residue was dissolved in 200 µl of H₂O with sonication and loaded on a 30 mg Oasis MAX mixed mode anion exchange sorbent cartridge (Waters) that had been activated with 2 ml of CH₃OH and 1 ml of H₂O. The cartridge was washed with 0.4 ml of 20% CH₃OH in H₂O, 0.4 ml of 20% CH₃OH containing 2% ammonium hydroxide, and then 0.4 ml of 20% CH₃OH, and the analyte was eluted with 0.25 ml of 50% CH₃OH in H₂O, collected in a 1 ml silanized vial, and the solvents were removed on a Speedvac. The residue was dissolved in 100 µl of CH₃OH with sonication, transferred to an insert vial, concentrated to dryness, and dissolved in 10 µl of bis(trimethylsilyl)trifluoroacetamide) plus 1% trimethylchlorosilane (Regis Technologies, Morton Grove, IL) with sonication and mixing. The samples were heated at 60 °C for 60 min or allowed to stand at room temperature for 2 days to effect complete derivatization, and 1 µl was analyzed by GC-NICI-MS/MS. Recovery of analytes was 40%.

Gas chromatography-negative ion chemical ionization-tandem mass spectrometry (GC-NICI-MS/MS) was carried out with a TSQ Quantum instrument (Thermo Scientific, San Jose, CA). [D₁₀]PheT, [¹³C₆]PheT, and PheT were detected as their trimethylsilyl derivatives. The GC was equipped with a 0.25 mm i.d. × 0.15 µm film thickness × 30 m DB17-MS column (Agilent Technologies, Palo Alto, CA) with a 0.53 mm i.d. × 3 m deactivated fused silica pre-column and a Hi-Temp 0.25 mm i.d. deactivated fused silica MS transfer line (Agilent). The oven temperature program was 80 °C for 1 min, then 80–200 °C at 35 °C/min, then 200–215 °C at 3 °C/min, then 215–320 °C at 35 °C/min, and then hold for 4 min. The carrier gas was He at a constant flow rate of 1.2 ml/min, the split/splitless injection port (operated in the splitless mode) was kept at 250 °C, and the MS transfer line was kept at 320 °C. The NICI-MS/MS conditions were as follows: CI gas, argon at 1.1 ml/min; source temperature, 200 °C; and emission current 200 µA. Selected reaction monitoring (SRM) with a collision energy of 12 eV, electron energy of -70 eV, scan width of *m/z* 0.02, Q1 peak width of 0.4 units and Ar collision gas at 1.0 mTorr was used to detect [D₁₀]PheT, [¹³C₆]PheT, and PheT at, *m/z* 382 → *m/z* 220, *m/z* 378 → *m/z* 216, *m/z* 372 → *m/z* 210, respectively.

Statistical analysis

Descriptive statistics including median, range, mean, standard deviation, 95% confidence interval and coefficient of variation (CV) were calculated for each subject. CV was calculated as standard deviation/mean expressed as a percentage. The mean CV was calculated as the average of the CVs of all the subjects. The intra-class correlation coefficient $\rho_I = \sigma_b^2 / \sigma_b^2 + \sigma_w^2$ where σ_w^2 and σ_b^2 are the within- and between-subject variance, respectively, was also calculated through a general linear mixed model. The model included week as a continuous fixed effect and random intercepts as a random effect.

SAS 9.2 (SAS Institute Inc., Cary, NC, USA) was used for all the analyses.

Results

The method used in this study for the analysis of [D₁₀]PheT in urine was different from those reported previously, in which we used phenylboronic acid solid-phase extraction cartridges or HPLC for sample preparation prior to GC-NICI-MS/MS analysis (Hecht et al. 2010; Zhong et al. 2011a). We have recently experienced some problems with analyte enrichment using phenylboronic acid solid-phase extraction cartridges, potentially leading to low recoveries and inconsistent results. The method reported here is also faster and more convenient than the HPLC enrichment method.

A typical chromatogram obtained upon analysis of a 6 h urine sample from an individual to whom [D₁₀]phenanthrene had been administered is illustrated in Figure 3.

The chromatogram shows clear quantifiable peaks for PheT (present in all human urine samples), the internal standard [¹³C₆]PheT, and [D₁₀]PheT. Since [D₁₀]PheT and [¹³C₆]PheT have different retention times, signal suppression is a potential problem, because urine matrices from different individuals could have differential effects on these two peaks. The solid-phase extraction protocol, Q1 peak width of 0.4 units (as opposed to 0.7 units), and scan width of m/z 0.02 (as opposed to m/z 0.1) described in Methods were *all* necessary to avoid signal suppression (data not shown). To evaluate possible signal suppression under these conditions, 100 μ l urine samples from 10 smokers and 5 non-smokers were processed by solid-phase extraction as described in “Methods”, and then a constant amount of [D₁₀]PheT (38.5 fmol) was added to each sample. Analysis of these samples gave a value of 38.5 ± 1.0 fmol (CV = 2.65%), demonstrating that signal suppression was not a problem under the conditions described in “Methods.” Overall inter-day precision of the method was determined by including, with each set of analyses, a positive control urine sample to which [D₁₀]PheT had been added. The inter-day analytical CV was 6.0%.

Demographic and smoking data for the 24 subjects who completed the study are summarized in Table 1. There were 14 males and 10 females; 10 blacks, 12 whites, and 2 subjects of mixed ethnicity. The subjects were 38.7 ± 12.4 years old (range 18–56 years) and they smoked 15.9 ± 5.7 cigarettes per day (range 10 – 30).

Levels of [D₁₀]PheT in the 6 h urine samples from the 24 subjects to whom either 7 or 8 doses of [D₁₀]phenanthrene had been administered are summarized in Table 2. The overall median and mean amounts of [D₁₀]PheT were 3733 ± 1715 pmol per 6 h and 3775 ± 1670 pmol per 6 h. Table 2 also presents data on the minimum and maximum levels of [D₁₀]PheT in the urine of each subject, the 95% lower and upper confidence limits of the mean values, and the CV for each subject of the [D₁₀]PheT levels in their 6 h urine over the 7 or 8 doses of [D₁₀]phenanthrene. The CV values are the critical parameters in this study since they indicate the amount of variation of [D₁₀]PheT levels after the 7 or 8 doses of [D₁₀]phenanthrene in each subject. The overall CV was $27.4 \pm 8.83\%$, with a range of 6.33–

43.4%. Only three of the 24 subjects had CV values greater than 35%. The overall CV for naturally occurring PheT in the urine of our subjects was 48.2%. The intra-class correlation coefficients for [D₁₀]PheT were 67.2% on the original scale and 70.3% on the log scale.

Discussion

The results of this study, with a mean CV of 27.4% for [D₁₀]PheT in the 6 h urine of the 24 subjects given 7 or 8 doses of [D₁₀]phenanthrene, strongly support the use of a single determination of urinary [D₁₀]PheT to represent an individual smoker's ability to metabolize [D₁₀]phenanthrene by the diol epoxide pathway. These results have important practical implications pertinent to the design of clinical studies investigating the relationship of [D₁₀]PheT levels to lung cancer susceptibility in smokers, as we are proposing, because they demonstrate that a relatively simple single administration clinical protocol can be adopted. We already have established that an oral dose of [D₁₀]phenanthrene, with collection of 6 h urine, can provide the same information as collection of 24 h urine, and, more importantly, the same information as incorporation of [D₁₀]phenanthrene into a cigarette, which requires a far more labor-intensive approach (Zhong et al. 2011b).

The mean CV of 27.4% is substantially less than the 48.2% CV observed for PheT in the same subjects and further supports the use of [D₁₀]phenanthrene to assess metabolism by the diol epoxide pathway. The higher CV for PheT is comparable to the value of 40.5% which we observed previously in a longitudinal study of urinary PheT in smokers and to the value of 47% which we observed for plasma PheT in smokers (Church et al. 2010; Hecht et al. 2005). These higher CV values are due to variable human exposure to phenanthrene from cigarette smoking, diet, and environmental sources.

The mean CV of 27.4% for [D₁₀]PheT observed here can be compared to those of urinary cotinine (a nicotine metabolite) and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL, a tobacco-specific lung carcinogen metabolite) of 36% and 30%, respectively, as determined in a previous longitudinal study (Church et al. 2010). The intra-class correlation coefficient of 70.1% determined here also compares favorably to values of 58% and 76% for urinary cotinine and total NNAL determined previously (Church et al. 2010). Single measurements of urinary cotinine and total NNAL have been widely used in studies of nicotine and carcinogen uptake by smokers (Hecht, Yuan, & Hatsukami 2010).

The mean percentage of the dose of [D₁₀]phenanthrene excreted as [D₁₀]PheT in the 6 h urine of the subjects in this study was 7.0 %. In our previous study of [D₁₀]phenanthrene, the mean percentage of dose excreted in the 6 h urine was 3.3% (Wang et al. 2012). Other urinary metabolites of phenanthrene in humans have been identified as phenols and mercapturic acids. In one study, levels of deuterated phenanthrene phenols ranged from 0.6–7.2% of the dose in smokers who received an oral dose of [D₁₀]phenanthrene (Zhong et al. 2011b) while a second investigation demonstrated approximately equal amounts of PheT and phenanthrene phenols in the urine of more than 300 smokers (Hecht et al. 2006). Mercapturic acids resulting from glutathione conjugation of phenanthrene diol epoxides and phenanthrene-9,10-epoxide appear to be relatively minor metabolites in human urine (Hecht, Villalta, & Hochalter 2008; Upadhyaya et al. 2006). Based on studies in laboratory animals, most phenanthrene metabolites are excreted in the urine, but there still appears to be a considerable amount of uncharacterized phenanthrene metabolites in human urine (Chu et al. 1992).

The longitudinal consistency of [D₁₀]PheT formation in the subjects in this study is encouraging for our proposed use of [D₁₀]phenanthrene to investigate inter-individual differences in PAH metabolism by the diol epoxide pathway. We hypothesize that the

conversion of [D₁₀]phenanthrene to its diol epoxide is the critical metabolic pathway indicating potential DNA adduct formation and carcinogenicity. This pathway arguably differs among individuals. We expect that the diol epoxide is rapidly hydrolyzed by cellular H₂O and excreted in the urine as our measured parameter [D₁₀]PheT. One strength of this approach is that it is not confounded by differences in environmental exposure to PAH, which can be substantial. For example, recent studies have shown that PAH exposure, and therefore levels of PAH metabolites in urine, are far greater in subjects from Shanghai and Poland than in subjects from the U.S., independent of smoking status (Kensler et al. 2012; St Helen et al. 2012). Diet and environmental pollution are significant contributors to PAH exposure in smokers and non-smokers (International Agency for Research on Cancer 2010). Dietary PAH exposure is not likely to influence lung cancer susceptibility in smokers, but urinary PAH metabolite levels will be affected by this exposure. This is not a problem when using [D₁₀]phenanthrene to assess metabolism. Another strength of our approach is that the use of a phenotypic biomarker encompasses all genetic, enzymatic and metabolic differences among subjects.

There were certain limitations to this study. The number of subjects was relatively small and the overall time period was only 5.5 months. These parameters, which were dictated by available resources, precluded a more detailed investigation of the possible effects of gender, age, ethnicity, or extent of smoking on longitudinal stability, but based on the data we did obtain, there were no significant differences.

In summary, we have demonstrated that levels of urinary [D₁₀]PheT in smokers to whom [D₁₀]phenanthrene has been administered 7 or 8 times over a 5.5 month period are relatively constant. These results indicate that a single dose of [D₁₀]phenanthrene can be used to estimate a smoker's ability to metabolize it to [D₁₀]PheT, a proposed biomarker of carcinogenic polycyclic aromatic hydrocarbon metabolic activation.

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Figure 1.

Metabolism of BaP to BaP-tetraol and phenanthrene to PheT. Specific enantiomers of BaP-tetraol and PheT are shown. The opposite enantiomers are also formed via BaP-9,10-diol-7,8-epoxide and phenanthrene-3,4-diol-1,2-epoxide, respectively. In the case of phenanthrene, the 3,4-diol-1,2-epoxide pathway predominates in smokers. In this study, [D₁₀]PheT was quantified without regard to specific enantiomer composition. We have previously shown a high correlation between levels of the minor PheT enantiomer illustrated and the sum of both PheT enantiomers, as measured here, in smokers' urine (Hochalter et al. 2011).

Figure 2. Structures of [D₁₀]phenanthrene and [D₁₀]PheT. A specific enantiomer of the latter is illustrated (see legend of Figure 1).

Figure 3. GC-NICI-MS/MS chromatograms obtained upon analysis of a 6 h urine sample from a subject to whom 10 μ g of [D₁₀]phenanthrene had been administered. A, PheT which is observed in all human urine samples resulting from exposure to phenanthrene; B, internal standard [¹³C₆]PheT; C, [D₁₀]PheT from metabolism of [D₁₀]phenanthrene. Analytes were detected as their trimethylsilyl derivatives.

Table 1

Demographic and smoking date for 24 study subjects

Subject	Sex	Ethnicity	Age	Cigarettes per Day
1	M	B	41	20
2	F	B	43	12
3	F	B	43	30
4	M	W	42	20
5	M	W	28	15
6	M	B	52	10
7	M	B	51	15
8	M	W	44	20
9	F	W	18	12
10	M	W	39	20
11	M	W	26	15
12	M	W	30	10
13	M	B	33	11
14	F	W	51	20
15	F	W	22	10
16	F	W	55	10
17	F	W	20	18
18	M	B	56	18
19	M	B	25	15
20	M	B	37	15
21	F	Mixed	41	15
22	F	W	53	30
23	M	B	56	10
24	F	Mixed	22	10
14M/10F		10B/12W /2 mixed	(mean $\pm$ S.D.)	(mean $\pm$ S.D.)
			38.7 $\pm$ 12.4	15.9 $\pm$ 5.7
			(range) 18–56	(range) 10–30

Table 2

Levels of [D₁₀]PheT (pmol/ 6 h urine) in 24 subjects who took 7 or 8 oral doses of [D₁₀]phenanthrene (53.2 nmol) over a period of 5.5 months

Subject	Number of doses	Median	Mean	S.D. ^a	Minimum	Maximum	95% LCLM ^b	95% UCLM ^c	CV (%) ^d
1	8	2507	2350	619.4	1299	3126	1833	2868	26.4
2	7	5450	5389	1430	3923	8010	4067	6711	26.5
3	8	4542	4760	1027	3689	6330	3902	5619	21.6
4	8	4463	4700	1445	2598	6968	3492	5909	30.8
5	7	3319	3723	1099	2261	5250	2706	4740	29.5
6	7	1228	1406	609.6	619.9	2181	841.7	1969	43.4
7	8	1033	1101	388.8	596.5	1796	776.2	1426	35.3
8	8	5338	4819	1945	823.0	7036	3192	6445	40.4
9	8	6664	6313	2073	2016	8950	4580	8046	32.8
10	8	5346	5038	1608	1808	7037	3693	6383	31.9
11	7	3748	3786	664.1	3054	5111	3172	4400	17.5
12	8	3826	3847	505.9	3058	4526	3424	4270	13.2
13	8	1207	1243	331.2	746.3	1838	966.2	1520	26.6
14	8	2652	2625	457.6	2090	3494	2243	3008	17.4
15	8	2195	2237	725.5	1285	3159	1630	2843	32.4
16	8	3239	3244	205.3	2868	3524	3072	3416	6.33
17	7	3059	3016	807.0	2026	4409	2270	3763	26.8
18	8	7232	7706	1619	5870	10960	6353	9059	21.0
19	8	5263	5043	1123	3400	6750	4104	5981	22.3
20	8	4773	4969	1206	3214	6820	3960	5977	24.3
21	7	1688	1878	632.9	1179	2939	1293	2464	33.7
22	7	4325	4173	1462	1765	6498	2822	5525	35.0
23	7	1987	2489	984.8	1240	3788	1578	3400	39.6
24	8	4516	4753	1123	2845	6158	3815	5692	23.6

^aS.D., standard deviation

^b95% LCLM, 95% lower confidence level of the mean

^c95% UCLM, 95% upper confidence level of the mean

d_{CV} , coefficient of variation