

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

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Abstract

Individual differences in the metabolic activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons (PAHs) may influence cancer risk. This has been investigated in many studies using genotyping approaches, but the results to date have been inconsistent. We propose that carcinogen metabolite phenotyping would be a more reliable way to determine the role of host metabolism in PAH-related cancer. Many PAHs are metabolically activated by formation of bay-region diol epoxides. Phenanthrene, generally considered to be noncarcinogenic, is the simplest PAH with a bay region and is metabolized to diol epoxides by the same enzymes and with the same stereochemistry as the prototypic carcinogenic PAH, benzo[*a*]pyrene. The major end product of this metabolic activation pathway is *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans*, *anti*-PheT). We have developed a method for the analysis of *trans*, *anti*-PheT in human urine. *r*-1,*t*-2,4,*c*-3-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans*, *syn*-PheT) was used as internal standard. After hydrolysis by β -glucuronidase and sulfatase, solid phase extraction, and high-performance liquid chromatography collection, the sample was silylated and analyzed by gas chromatography-negative ion chemical ionization-mass spectrometry-selected ion monitoring at *m/z* 372. The resulting chromatograms were remarkably clean and *trans*, *anti*-PheT was readily detected in all human urine samples. Levels of *trans*, *anti*-PheT were 791 ± 363 pmol/mg creatinine ($n = 20$) in psoriasis patients treated with a PAH-containing ointment, 25.7 ± 16.8 pmol/mg creatinine ($n = 32$) in coke oven workers exposed to PAH, 4.58 ± 2.95 pmol/mg creatinine ($n = 31$) in

smokers, and 1.51 ± 1.15 pmol/mg creatinine ($n = 30$) in nonsmokers. Levels of *trans*, *anti*-PheT correlated with levels of 1-hydroxypyrene in the urine of coke oven workers, smokers, and nonsmokers. Thus, *trans*, *anti*-PheT appears to be an excellent biomarker of PAH uptake. Levels of *trans*, *anti*-PheT were 8,000–19,000 times higher than those of the corresponding metabolite of benzo[*a*]pyrene. The results of this study demonstrate that *trans*, *anti*-PheT can be detected in human urine. We propose that measurement of this metabolite of phenanthrene may be important as part of a carcinogen metabolite-phenotyping approach to determine individual response to PAH exposure.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are well-established environmental carcinogens that are likely to play a significant role in the etiology of human cancer. Mixtures containing PAH such as coke oven emissions, coal tars, and soots cause cancers of the skin and lung in occupationally exposed individuals (1–3). A substantial amount of convincing evidence implicates PAHs as important causative factors for lung cancer in smokers (4, 5). PAH may also be involved in the etiology of other tobacco smoke-related cancers (6).

PAHs require metabolic activation to express their carcinogenicity (7). Many PAHs are metabolically activated by the formation of bay-region diol epoxides such as anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE), illustrated for the prototypic PAH carcinogen benzo[*a*]pyrene (BaP) in Fig. 1 (8, 9). These diol epoxides bind to DNA, causing permanent mutations (10–13). The presence of BPDE-DNA adducts in human tissues has been conclusively established (14). Detoxification pathways including glutathione conjugation, glucuronidation, sulfation, and phenol formation compete with metabolic activation (4, 7). It is hypothesized that the balance between metabolic activation and detoxification affects cancer risk. Many studies have examined this hypothesis by investigating the relationship to cancer of variants in genes such as *GST-M1*, *CYP1A1*, and *MPO* (15–26). Such variants may affect the flux of activated or detoxified PAH metabolites. The results of these studies have generally shown fairly modest and inconsistent effects.

We propose that carcinogen metabolite phenotyping, *e.g.*, actual measurement of carcinogen metabolites resulting from activation and detoxification pathways, would give a more accurate picture of an exposed individual's response. Phenotyping would integrate all genetic, environmental, and other host properties that influence carcinogen metabolism. Therefore, we developed a method to quantify *r*-7,*t*-8,9,*c*-10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*trans*, *anti*-BaP-tetraol) in human urine (27). BPDE reacts mainly with H₂O

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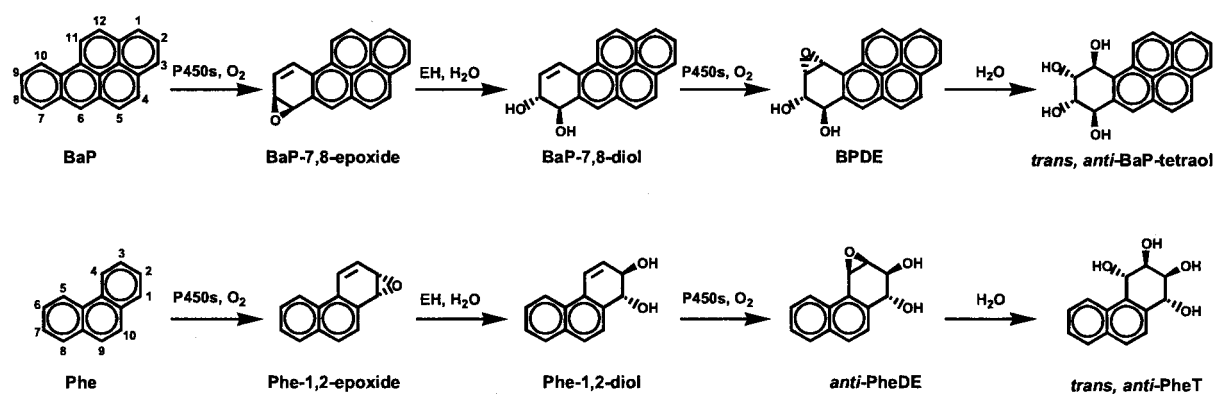


Fig. 1. Metabolism of benzo[*a*]pyrene (BaP) and phenanthrene (Phe) to *r*-7,*t*-8,9,*c*-10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*trans, anti*-BaP-tetraol) and *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans, anti*-PheT), via the corresponding diol epoxides anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) and *anti*-1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrophenanthrene (*anti*-PheDE). P450, cytochrome P450; EH, epoxide hydrolase.

in vivo; thus, the measurement of *trans, anti*-BaP-tetraol could establish how much BaP was metabolically activated by this pathway in a given individual. Although our method was quantitative, levels of *trans, anti*-BaP-tetraol in human urine were so low that this approach would not be feasible for large studies because multiple purification steps were necessary. Phenanthrene (Phe) is the simplest PAH with a bay region, a feature closely associated with carcinogenicity because bay-region diol epoxides are frequently ultimate carcinogens of PAH. Phe, however, is generally considered to be noncarcinogenic, probably because it has only three aromatic rings (1, 28). But Phe metabolism through the diol epoxide pathway closely mimics BaP metabolism by this route as illustrated in Fig. 1. Previous studies have shown that the same intermediates are formed with the same stereochemistry and that some of the same cytochrome P450 (P450) enzymes are involved, at least in the initial oxidation step (9, 29). Therefore, we proposed that 1,2,3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrenes (PheT) could be surrogate measures of carcinogenic PAH metabolic activation by the bay-region diol epoxide pathway. In this article, we report the results of our analyses of human urine for these compounds.

Materials and Methods

Apparatus. High-performance liquid chromatography (HPLC) was carried out with a Waters Associates (Milford, MA) system equipped with a model 996 photodiode array detector or a Shimadzu model RF 10AXL fluorescence detector (Columbia, MD). A 4.6 × 150-mm Prosphere 300 PAH, 5- μ m column (Alltech, Deerfield, IL) was eluted with an H₂O/methanol gradient at 1 ml/min as follows (percentage H₂O, time): 85%, 0–13 min; 85–77%, 13–16 min; 77–0%, 16–21 min. Gas chromatography-negative ion chemical ionization-mass spectrometry (GC-NICI-MS) was carried out with a Hewlett Packard (Agilent, Wilmington, DE) Model 5973 instrument equipped with a 0.25 mm (inside diameter) × 30 m, 0.15- μ m film thickness, DB-17MS column (Agilent), and a 0.53 mm (inside diameter) × 2 m deactivated fused silica precolumn. For confirmation of analyte identity, a 0.32 mm (inside diameter) × 30 m, 0.25- μ m film thickness, DB-5 column (Agilent) was used. Removal of solvents was carried out with an SVC-200 Speedvac (Thermo Savant, Holbrook, NY).

Materials. *syn*-1,2-Dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrophenanthrene; *anti*-1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrophenanthrene (*anti*-PheDE); *r*-1,*t*-2,3,*c*-4-tetrahydroxy-

1,2,3,4-tetrahydrophenanthrene (*trans, anti*-PheT); *r*-1,*t*-2,3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene; *r*-1,*t*-2,4,*c*-3-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans, syn*-PheT); and *r*-1,*t*-2,*c*-3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene were synthesized as described previously (30, 31).

β -Glucuronidase and arylsulfatase (from *Helix pomatia*, catalog no. 127-698) were obtained from Roche Diagnostics Corp., Indianapolis, IN. bis-Trimethylsilyltrifluoroacetamide containing 1% trimethylchlorosilane was purchased from Regis Technologies, Inc., Morton Grove, IL. C-18 Sep-pak Vac RC cartridges (500 mg) were procured from Waters Corp, Milford, MA.

Urine Samples. Urine samples from psoriasis patients, receiving Goekerman coal tar therapy, were obtained from Dr. Regina Santella, Columbia University, New York, NY. Urine samples from coke oven workers at one of the largest steel plants in Taiwan were provided by Dr. Ming-Tsang Wu, Kaohsiung Medical University, Kaohsiung, Taiwan. Both of these were the same samples analyzed previously for *trans, anti*-BaP-tetraol (27). Urine samples from smokers were obtained from ongoing studies examining the effects of reduction in smoking on levels of carcinogen metabolites. All of the samples were from current smokers and were obtained at baseline, before reduction in smoking. Urine samples from nonsmokers were obtained at baseline, before exposure, from individuals involved in a study of the effects of environmental tobacco smoke exposure on carcinogen metabolites, and from laboratory personnel.

Analysis of Urine for PheT. A 2-ml urine sample was placed in a 15-ml centrifuge tube and *trans, syn*-PheT (1 ng) was added as internal standard. The pH was adjusted to 5 by the addition of 1.5 ml of sodium acetate buffer (0.5 M, pH 5). Then β -glucuronidase (3,500 units) and arylsulfatase (28,000 units) were added, and the mixture was incubated overnight with shaking at 37°C. A Sep-pak cartridge was prewashed with 10 ml of methanol, then with 10 ml of H₂O. The sample was applied slowly. The cartridge was eluted with 15 ml of 0.15 M NH₄OH. The eluants were discarded. The cartridge was then eluted with 12 ml of 25% methanol. This fraction contained the analyte and internal standard. Solvents were removed by overnight concentration on a Speedvac, the clear cover of which was covered with aluminum foil. The residue was transferred into a 12 × 32 mm (300 μ l) polypropylene vial (Waters) with three 65- μ l portions of methanol/H₂O (1:1). To the vial was added 2 μ g of 4-hydroxyacetophenone, as an HPLC retention time marker (9.6 min). HPLC eluant was collected from 6.5–14.5

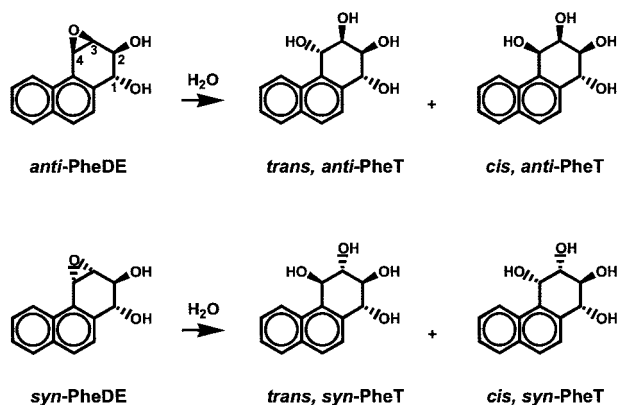


Fig. 2. Hydrolysis of *anti*-1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrophenanthrene (*anti*-PheDE) and *syn*-1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrophenanthrene (*syn*-PheDE) to 1,2,3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT) isomers: *trans*, *anti*-PheT, *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene; *cis*, *anti*-PheT, *r*-1,*t*-2,3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene; *trans*, *syn*-PheT, *r*-1,*t*-2,4,*c*-3-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene; *cis*, *syn*-PheT, *r*-1,*t*-2,*c*-3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene.

min for analysis of *trans*, *anti*-PheT. For the analysis of other PheT isomers, the material eluting from 6.5 to 22.5 min was collected. Retention times of the PheT were: *trans*, *syn*-PheT (12.1 min); *trans*, *anti*-PheT (9.7 min); *r*-1,*t*-2,3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (12.9 min), and *r*-1,*t*-2,*c*-3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (20 min). The collected HPLC fraction was concentrated to dryness overnight on the Speedvac, as above. The residue was transferred into a 1.8-ml, 12 × 32 mm Ekonal vial containing a 0.1-ml glass insert (Kimble, Vineland, NJ). The transfer was carried out with three 60- μ l portions of methanol. The solution was concentrated to dryness again. To the residue was added 10 μ l of acetonitrile, then 30 μ l of bis-trimethylsilyltrifluoroacetamide, and the mixture was heated at 60°C for 60 min in a gas chromatography (GC) oven with occasional mixing by tapping the side of the vial. Two μ l were injected on GC-NICI-MS using the splitless mode. The injection port temperature was 270°C. The oven temperature program was 80°C for 1 min, then 80–180°C at 20°C/min, then 180–250°C at 8°C/min, then hold for 2 min. The mass spectrometry (MS) transfer line temperature was 280°C. The flow rate was 1 ml/min He. NICI-MS conditions were as follows: collision gas, methane; source temperature, 150°C; quadrupole temperature, 106°C; electron multiplier voltage, 3000 v; emission current, 49.4 μ A; electron energy, 235 electron volts.

Analysis of Urine for 1-Hydroxypyrene. Urine samples from smokers and nonsmokers were analyzed by HPLC-fluorescence, using a modification of a published method (32). The details of the new method will be described separately.

Statistical Analysis. Because distributions of *trans*, *anti*-PheT, *trans*, *anti*-BaP-tetraol, and 1-hydroxypyrene were skewed, the values were transformed using the natural logarithm. The log-transformed data were near normality, as determined by graphical methods. The effect of exposure status on levels of the analytes was evaluated using ANOVA. Pairwise contrasts between exposure groups were constructed and tested using the *F* test. *P*s were adjusted using the method of Bonferroni (33), to maintain an overall level of significance of 0.05. Comparisons of *trans*, *anti*-PheT and 1-hydroxypyrene levels were carried out using Student's *t* test. Correlations among the analytes were examined using Pearson correlation coefficients.

Results

As illustrated in Fig. 2, there are four diastereomers of PheT that could be formed metabolically: two from *trans*- or *cis*-ring opening of *anti*-PheDE by attack of H₂O at the 4 position (*trans*, *anti*-PheT and *r*-1,*t*-2,3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene) and two from *trans*- or *cis*-ring opening of *syn*-1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrophenanthrene by similar attack of H₂O (*trans*, *syn*-PheT and *r*-1,*t*-2,*c*-3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene). We used *trans*, *syn*-PheT as internal standard.

The analytical scheme is summarized in Fig. 3. After the addition of *trans*, *syn*-PheT to a 2.0-ml urine sample, the mixture was incubated with β -glucuronidase and aryl sulfatase. This step was necessary because 90% of *trans*, *anti*-PheT in human urine is conjugated (data not shown). The samples were partially purified by solid phase extraction and HPLC, then were derivatized with bis-trimethylsilyltrifluoroacetamide to produce PheT-tetra(trimethylsilyl) ethers (TMS). A full scan NICI-MS of *trans*, *anti*-PheT-TMS showed a base peak at *m/z* 372 [*M* - [OSi(CH₃)₃ + Si(CH₃)₃]⁻ with little other fragmentation and no molecular ion (*m/z* 534). GC-NICI-MS-selected ion monitoring at *m/z* 372 proved to be highly sensitive for detection of *trans*, *anti*-PheT-TMS. A calibration curve demonstrating linearity when 0.2–20 pg (0.8–81 fmol) of *trans*, *anti*-PheT was derivatized is illustrated in Fig. 4. The detection limit was ~75 amol (40 fg of *trans*, *anti*-PheT-TMS) on column.

Typical GC-NICI-MS-selected ion monitoring traces of *trans*, *anti*-PheT-TMS in the urine of a smoker and a non-smoker are shown in Fig. 5. The indicated peaks are *trans*, *anti*-PheT-TMS and the silylated internal standard, *trans*, *syn*-PheT-TMS. The identity of *trans*, *anti*-PheT-TMS was confirmed by coinjection with a standard on two GC columns with stationary phases of differing polarity and by comparison of its tandem mass spectrometry (daughter ions of *m/z* 372) with that of a standard (Fig. 6). The daughter ion peak at *m/z* 281 corresponds to the loss of [HOSi(CH₃)₃ + H] from *m/z* 372, the peak at *m/z* 193 results from the loss of [(CH₃)₃SiO + HOSi(CH₃)₃] from *m/z* 372, and the peak at *m/z* 181 results

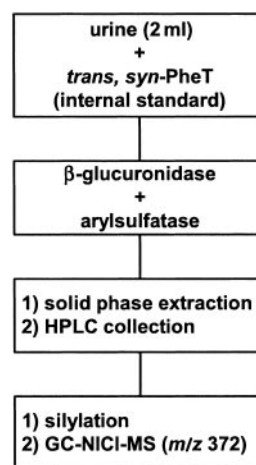


Fig. 3. Analytical scheme for quantitation of *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans*, *anti*-PheT) in human urine. *trans*, *syn*-PheT, *r*-1,*t*-2,4,*c*-3-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene; HPLC, high-performance liquid chromatography; GC-NICI-MS, gas chromatography-negative ion chemical ionization-mass spectrometry.

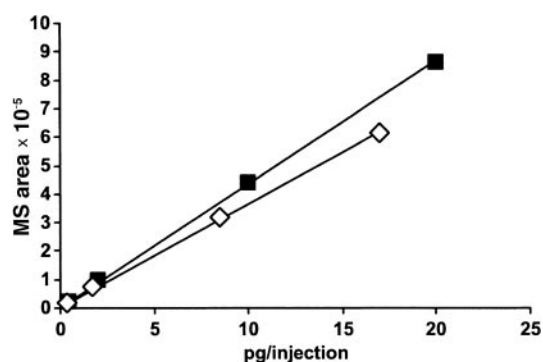


Fig. 4. Calibration curves for *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene tetramethylsilyl ethers (*trans*, *anti*-PheT-TMS; \diamond) and *r*-1,*t*-2,4,*c*-3-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene tetramethylsilyl ethers (*trans*, *syn*-PheT-TMS), internal standard (\blacksquare). MS, mass spectrometry.

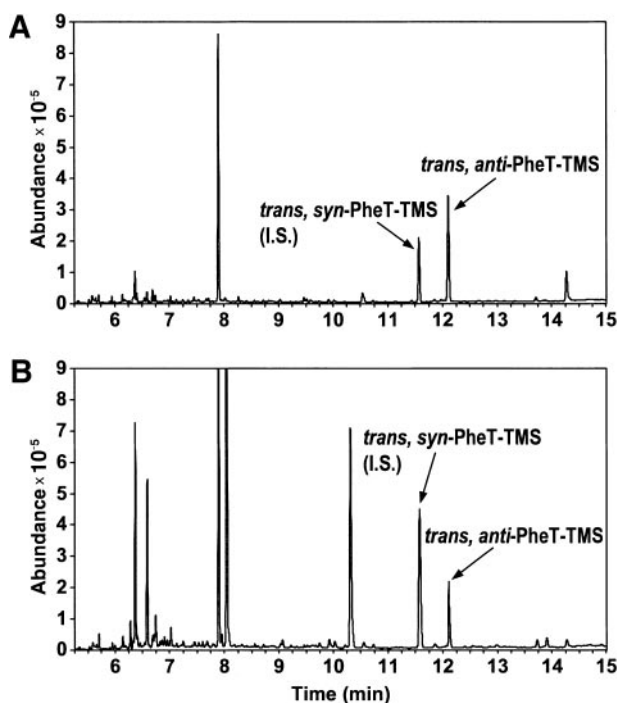


Fig. 5. Gas chromatography-negative ion chemical ionization-mass spectrometry-selected ion monitoring (GC-NICI-MS-SIM; m/z 372) chromatograms of *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans*, *anti*-PheT) in the urine of (A) a smoker and (B) a nonsmoker. I.S., internal standard; *trans*, *anti*-PheT-TMS, *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene tetramethylsilyl ethers; *trans*, *syn*-PheT-TMS, *r*-1,*t*-2,4,*c*-3-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene tetramethylsilyl ethers.

from the loss of $[(\text{CH}_3)_3\text{Si} + \text{CO}]$ from m/z 281 with the addition of hydrogen.

We also investigated the presence of other PheT isomers in human urine, using samples from three smokers and four nonsmokers. *trans*, *syn*-PheT was not added to these samples and a larger HPLC collection window was used. GC-NICI-MS-selected ion monitoring analysis at m/z 372 revealed the presence of small peaks corresponding in GC retention time to *r*-1,*t*-2,*c*-3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene-TMS (13.0 min, $8.9 \pm 2.7\%$ of total PheT), *trans*, *syn*-PheT-TMS (11.6 min, $6.0 \pm 4.2\%$ of total PheT), and *r*-1,*t*-2,3,4-

tetrahydroxy-1,2,3,4-tetrahydrophenanthrene-TMS (12.9 min, $6.1 \pm 3.3\%$ of total PheT). Because these peaks were small, we focused on the analysis of *trans*, *anti*-PheT only.

Precision was determined by dividing a urine sample, pooled from smokers, into six aliquots and analyzing each for *trans*, *anti*-PheT. The results were (mean \pm SD) 4.6 ± 0.75 pmol/ml (relative standard deviation = 16.2%). Accuracy was assessed by the standard addition method. Duplicate samples of another pooled urine sample from smokers, determined on replicate analysis to contain 1.58 ± 0.28 pmol/ml *trans*, *anti*-PheT ($n = 6$), were spiked with 3.5, 6.9, 13.8, or 27.6 pmol/ml *trans*, *anti*-PheT. Analysis produced the results summarized in Fig. 7. The added and measured levels of *trans*, *anti*-PheT were highly correlated ($r = 0.999$) and the y intercept was 1.83 pmol/ml, in agreement with the amount of *trans*, *anti*-PheT in the unspiked sample. Recoveries of internal standard were generally good, averaging $38.1 \pm 27.0\%$ ($n = 119$). The detection limit of the method was 0.2 fmol of *trans*, *anti*-PheT/ml urine.

The method was applied to the analysis of urine samples from psoriasis patients treated with a coal tar product, coke oven workers, smokers, and nonsmokers. The results are summarized in Fig. 8. Mean levels of *trans*, *anti*-PheT were highest

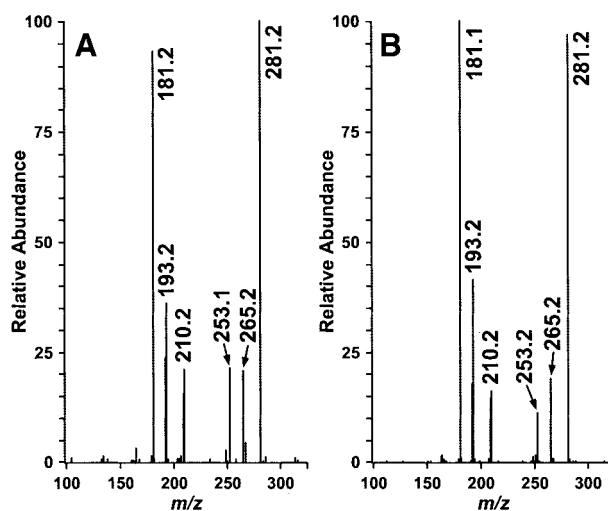


Fig. 6. Tandem mass spectrometry (MS/MS) spectra (daughter ions of m/z 372) of *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans*, *anti*-PheT): a, from a smoker's urine; b, standard.

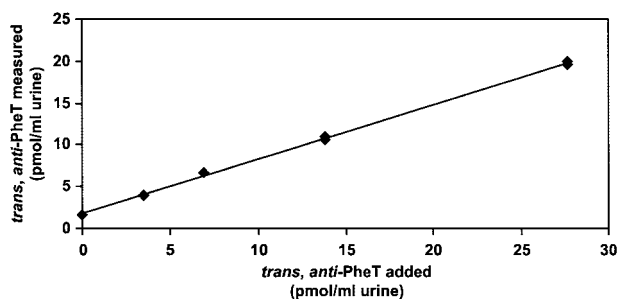


Fig. 7. Relationship between levels of *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans*, *anti*-PheT) added to pooled smokers' urine samples and levels measured. Points are the means of duplicate determinations that agreed within 4%. $r = 0.999$.

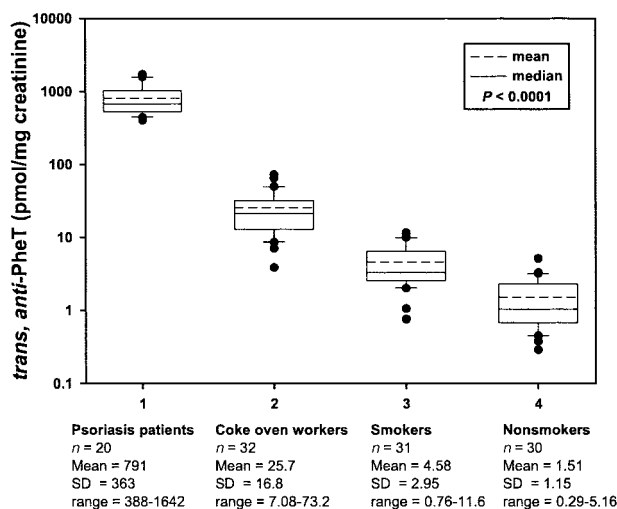


Fig. 8. Levels of *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans*, *anti*-PheT) in the urine of psoriasis patients, coke oven workers, smokers, and non-smokers. Each sample was analyzed once. Boxes, 25th to 75th percentile; lines outside boxes, 10th and 90th percentile; ●, outliers. Differences between all groups were significant, $P < 0.0001$.

Table 1 *r*-1,*t*-2,3,*c*-4-Tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans*, *anti*-PheT), *r*-7,*t*-8,9,*c*-10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*trans*, *anti*-BaP-tetraol), and 1-hydroxypyrene in urine

Group	Mean \pm SD (pmol/mg creatinine) ^a		
	<i>trans</i> , <i>anti</i> -PheT	<i>trans</i> , <i>anti</i> -BaP-tetraol ($\times 10^{-3}$)	1-hydroxypyrene
Psoriasis patients (<i>n</i> = 20)	791 \pm 363	41.4 \pm 40.9 ^b	9,390 \pm 10,600 ^c
Coke oven workers (<i>n</i> = 32)	25.7 \pm 16.8	3.15 \pm 3.04 ^b	100 \pm 62.0 ^d
Smokers (<i>n</i> = 31)	4.58 \pm 2.95	N.A. ^e	1.33 \pm 1.00
Nonsmokers (<i>n</i> = 30)	1.51 \pm 1.15	N.A.	0.58 \pm 0.53

^a Differences in each analyte level among the groups were statistically significant, $P < 0.0001$.

^b From Ref. 27.

^c From Ref. 34.

^d From Ref. 35.

^e N.A., not analyzed.

in psoriasis patients, followed by coke oven workers, smokers, and nonsmokers. All of the differences were significant ($P < 0.0001$).

Comparative levels of *trans*, *anti*-PheT, *trans*, *anti*-BaP-tetraol and 1-hydroxypyrene in the same urine samples are summarized in Table 1. The values for *trans*, *anti*-BaP-tetraol and some of the data for 1-hydroxypyrene are from previous studies (27, 34, 35). Differences in the levels of each analyte among all exposure groups were significant ($P < 0.0001$). Levels of *trans*, *anti*-PheT were 8,000–19,000 times higher than those of *trans*, *anti*-BaP-tetraol ($P < 0.0001$) in psoriasis patients and coke oven workers. Levels of *trans*, *anti*-PheT were about three times higher than levels of 1-hydroxypyrene in the urine of smokers and nonsmokers ($P < 0.001$). However, amounts of *trans*, *anti*-PheT were lower than those of 1-

hydroxypyrene in psoriasis patients and coke oven workers ($P < 0.001$).

The mean level of *trans*, *anti*-PheT (2.02 ± 1.25 pmol/mg creatinine, $n = 15$) was significantly higher ($P = 0.0045$) in female than in male nonsmokers (0.99 ± 0.78 pmol/mg creatinine, $n = 15$). Similarly, the mean amount of 1-hydroxypyrene (0.84 ± 0.65 pmol/mg creatinine, $n = 15$) was also higher in female than in male (0.33 ± 0.18 pmol/mg creatinine, $n = 15$) nonsmokers. No gender differences in the amounts of these analytes were found among smokers.

Correlations among amounts of *trans*, *anti*-PheT, *trans*, *anti*-BaP-tetraol and 1-hydroxypyrene in urine samples from individuals in different exposure groups are summarized in Table 2. Levels of *trans*, *anti*-PheT and 1-hydroxypyrene correlated significantly in the urine of coke oven workers, smokers, and nonsmokers. Levels of *trans*, *anti*-PheT correlated marginally, and levels of 1-hydroxypyrene correlated strongly, with levels of *trans*, *anti*-BaP-tetraol in the urine of coke oven workers. None of the analytes correlated with each other in the urine of psoriasis patients.

Levels of *trans*, *anti*-PheT and 1-hydroxypyrene correlated when all of the exposure groups were considered together ($r = 0.94$, $P < 0.0001$). Similarly, levels of *trans*, *anti*-PheT and *trans*, *anti*-BaP-tetraol also correlated when the data were analyzed in this way ($r = 0.83$, $P < 0.0001$).

Discussion

We have developed a relatively simple and sensitive method for analysis of *trans*, *anti*-PheT in human urine. The GC-NICI-MS-selected ion monitoring traces illustrated in Fig. 5 are extraordinarily clean, permitting ready quantitation of this analyte. The sensitivity of the method, with a detection limit of 0.2 fmol of *trans*, *anti*-PheT/ml urine, is outstanding. Currently, we are using 2-ml urine samples, but it is clear that this analysis could be performed on microliter samples of urine, likely diminishing the time required for preliminary purification steps. It seems probable, therefore, that measurement of this biomarker could be adapted to a high throughput format, and that ultimately it will be applicable in large epidemiological studies.

The metabolism of Phe to *anti*-PheDE requires three steps

Table 2 Correlations among levels of *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans*, *anti*-PheT), *r*-7,*t*-8,9,*c*-10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*trans*, *anti*-BaP-tetraol) and 1-hydroxypyrene in urine

Group	<i>trans</i> , <i>anti</i> -PheT/ 1-hydroxypyrene	<i>trans</i> , <i>anti</i> -PheT/ <i>trans</i> , <i>anti</i> -BaP-tetraol	1-hydroxypyrene/ <i>trans</i> , <i>anti</i> -BaP-tetraol
Psoriasis patients (<i>n</i> = 20)	$R = 0.22$	$R = 0.13$	$R = 0.37$
	$P = 0.35$	$P = 0.58$	$P = 0.11$
Coke oven workers (<i>n</i> = 32)	$R = 0.54$	$R = 0.33$	$R = 0.68$
	$P = 0.002$	$P = 0.06$	$P < 0.0001^a$
Smokers (<i>n</i> = 31)	$R = 0.35$	N.A. ^b	N.A.
	$P = 0.05$		
Nonsmokers (<i>n</i> = 30)	$R = 0.47$	N.A.	N.A.
	$P = 0.009$		

^a From Ref. 35.

^b N.A., not analyzed because levels of *trans*, *anti*-BaP-tetraol were not determined in these samples.

Table 3 Selected amounts of phenanthrene (Phe), pyrene, and benzo[*a*]pyrene (BaP) in various sources

	Phe	Pyrene	BaP
Coal tar ointment ($\mu\text{g/g}$) ^a	1600	700	140
Coke plant air samples ($\mu\text{g/m}^3$) ^b (personal air sampler)	15.1–170.3	1.5–48.9	0.9–15.8
Cigarette smoke (ng/cigarette) ^c	85–624	50–270	1–10

^a From Ref. 58.

^b From Ref. 38.

^c From Ref. 59.

(Fig. 1; Refs. 9, 36). P450s catalyze epoxidation, producing Phe-1,2-epoxide (29). Then, epoxide hydrolase catalyzes hydration of the epoxide, which produces Phe-1,2-diol. Finally, in a third step, oxidation of Phe-1,2-diol, catalyzed by P450s and other enzymes, produces *anti*-PheDE. Shou *et al.* (29) tested a variety of expressed human P450s and demonstrated that formation of Phe-1,2-epoxide (measured as Phe-1,2-diol after hydration by epoxide hydrolase) was catalyzed mainly by P450 1A2. Mouse P450s 1A1 and 1A2 were also efficient catalysts of this pathway. Bauer *et al.* (37) showed that human P450s 1A1 and 1A2 were most active in the catalysis of BaP-7,8-diol formation, which is the analogous pathway in the metabolic activation of BaP to BPDE (Fig. 1). Stereochemical features of Phe and BaP metabolism by this pathway are identical (9, 29). Epoxidation of Phe-1,2-diol by rat liver microsomes produces mainly *anti*-PheDE, which is analogous to the production of BPDE as the major product of BaP-7,8-diol oxidation (9). Hydration of *anti*-PheDE and BPDE proceeds mainly by *trans*-addition, giving *trans*, *anti*-PheT and *trans*, *anti*-BaP-tetraol, respectively (27, 31). Thus, there are considerable parallels in the metabolism of Phe and BaP via the bay-region diol epoxide activation pathway. Several other carcinogenic PAHs with bay regions are also metabolized with similar enzyme involvement and stereoselectivity (9). Therefore, *trans*, *anti*-PheT should be a valuable surrogate to assess the metabolic activation of BaP and other carcinogenic PAHs via diol epoxide formation. A potentially important feature of this metabolite as a biomarker is that it integrates all of the enzymatic, genetic, and environmental factors that impact on this metabolic activation pathway. In addition, the concentration of *trans*, *anti*-PheT in urine is 8,000–19,000 times as great as that of *trans*, *anti*-BaP-tetraol, making it a practical target for quantitation.

Jacob, Grimmer, and Dettbarn [Grimmer *et al.* (38) and Jacob *et al.* (39)] have quantified Phe-1,2-diol, the precursor to *anti*-PheDE and *trans*, *anti*-PheT, in the urine of coke oven workers, smokers, and nonsmokers. In coke oven workers, excretion of Phe-1,2-diol ranged from 12.2 to 234 pmol/mg creatinine (mean, 110 pmol/mg; based on 1.8 g of creatinine excreted in urine per day) for workers in different parts of the plant. Levels of Phe-1,2-diol averaged 1.1 pmol/mg creatinine in smokers and 1.3 pmol/mg creatinine in nonsmokers. These data are generally consistent with our mean values of 25.7 pmol/mg *trans*, *anti*-PheT in coke oven workers, 4.58 pmol/mg in smokers, and 1.51 pmol/mg in nonsmokers. Similar metabolite levels in human urine have been reported for other Phe dihydrodiols and for Phe phenols (38–40).

Selected data on levels of Phe, pyrene, and BaP in various sources relevant to this study are summarized in Table 3. In general, levels of Phe in a coal tar ointment, coke plant air samples, and cigarette smoke are greater than those of pyrene, which in turn are greater than those in BaP. We do not know the doses of Phe, pyrene, and BaP experienced by the subjects in

this study, but it is likely that the relative amounts are consistent with the data in Table 3. The relative metabolite levels of *trans*, *anti*-PheT and 1-hydroxypyrene are not consistent with exposure sources in the psoriasis patients and coke oven workers, because urinary 1-hydroxypyrene levels clearly exceeded the levels of *trans*, *anti*-PheT in both cases. In smokers, levels of *trans*, *anti*-PheT were higher than those of 1-hydroxypyrene, which is consistent with the somewhat higher levels of Phe than of pyrene in cigarette smoke. In all cases, *trans*, *anti*-BaP-tetraol levels were lowest among the three analytes in urine, consistent with the somewhat lower levels of BaP than Phe or pyrene in the sources. These comparisons should be viewed with considerable caution because there are major differences in the metabolism of Phe, pyrene, and BaP. The metabolism of Phe and BaP is likely more complex than that of pyrene, and there are differences in the excretion of metabolites in urine versus in feces (7, 41, 42).

Levels of *trans*, *anti*-PheT correlated with those of 1-hydroxypyrene within each exposure group (Table 2) except psoriasis patients. Moreover, levels of *trans*, *anti*-PheT correlated strongly with amounts of 1-hydroxypyrene when all of the exposures were combined. We have previously observed that levels of urinary *trans*, *anti*-BaP-tetraol and 1-hydroxypyrene correlated in the urine of coke oven workers (35). However, in this study, we observed only a moderate correlation between levels of *trans*, *anti*-PheT and *trans*, *anti*-BaP-tetraol in coke oven workers and found none in psoriasis patients. One major reason for this lack of correlation could be differences in the excretion of Phe versus BaP metabolites. In rats and guinea pigs dosed with [¹⁴C]Phe, 90 and 95%, respectively, of the excreted dose was found in urine, whereas at least 70–80% of [¹⁴C]BaP administered to mice and rats was excreted in the feces (41, 43). Individual differences in the excretion of BaP metabolites in urine versus feces could markedly affect the correlation between *trans*, *anti*-PheT and *trans*, *anti*-BaP-tetraol. In addition, quantitation of *trans*, *anti*-BaP-tetraol, the amounts of which are extremely low in urine, may be less reliable than quantitation of *trans*, *anti*-PheT. Nevertheless, the same relative metabolite levels in urine were observed for *trans*, *anti*-PheT and *trans*, *anti*-BaP-tetraol, with psoriasis patients having the highest amounts, followed by coke oven workers and smokers (27), and the two metabolites correlated when all exposures were combined. Collectively, our data strongly indicate that *trans*, *anti*-PheT, like 1-hydroxypyrene, may be a useful and practical biomarker for PAH uptake.

Despite its potential utility as an uptake marker, our main interest is application of *trans*, *anti*-PheT as a biomarker of risk. The two major factors that will affect its levels are exposure to Phe and conversion of Phe to *trans*, *anti*-PheT through the diol epoxide metabolic activation pathway. Thus, levels of *trans*, *anti*-PheT will integrate both exposure and metabolic activation. If exposure is highly variable in a given individual, then the utility of this biomarker as an indicator of metabolic activation will be compromised. Longitudinal studies are required to determine the reproducibility of *trans*, *anti*-PheT levels in individuals with a particular exposure pattern such as smokers. If levels of this metabolite are variable in an individual, then it will be necessary to construct an activation:detoxification ratio, which will decrease or eliminate the effects of exposure. Thus, levels of Phe phenols, which can be considered as detoxification products, could be used in the denominator of this carcinogen metabolite phenotyping ratio. Considerable methodology for the analysis of Phe metabolites in human urine is already available (44), and we expect that the development of a more complete Phe metabolite profile should be feasible.

Measurement of PAH diol epoxide-DNA adducts in humans may be a more direct approach to examining the relationship between carcinogen-DNA damage and cancer. Highly sensitive analytical methods have been developed for the quantitation of PAH-DNA adducts, but each has limitations (18, 45–49). Immunoassays for PAH-DNA adducts have been widely applied, but cross-reactivity of the antibodies complicates interpretation of the results (47). ^{32}P Postlabelling is extremely sensitive, but quantitation is difficult because there is presently limited characterization of the actual DNA adducts being measured (48). Structure-specific methods such as HPLC-fluorescence and GC-NICI-MS are available for the analysis of BPDE-DNA adducts in human tissues and blood cells, but the adducts are frequently undetectable, complicating their use in epidemiological studies (14). Urinary metabolites such as *trans, anti*-PheT are practical biomarkers because they can be quantified with certainty (50). We do not know whether Phe metabolite ratios in urine will reflect PAH-DNA adduct levels in target tissues. However, it should be noted that BPDE and perhaps other PAH diol epoxides can be transported in the blood, that BPDE-DNA adducts are ubiquitous and persistent in tissues of BaP-treated rodents, and that BaP metabolism in the liver is the main source of DNA adducts in other tissues (51–53). These data suggest that urinary PAH metabolites, which are likely formed mainly in the liver, could reflect DNA adduct levels in multiple tissues.

A limitation of the present methodology is the use of *trans, syn*-PheT as internal standard. Small amounts of *trans, syn*-PheT (about 6% of *trans, anti*-PheT) are apparently present in human urine. This could interfere with an accurate determination of recoveries and may explain in part our relatively high relative standard deviation. Other factors may also detract from the utility of *trans, syn*-PheT as an internal standard. We are presently synthesizing deuterium-labeled *trans, anti*-PheT, which should be a better internal standard than *trans, syn*-PheT.

We observed higher levels of *trans, anti*-PheT and 1-hydroxypyrene in female than in male nonsmokers. This observation, which is limited by the small number of subjects ($n = 15, 16$), and was not reproduced in smokers, requires further confirmation. Previous studies of 1-hydroxypyrene levels in urine have not reported consistent gender differences (54–57).

In conclusion, we have developed a simple and sensitive method for the analysis of *trans, anti*-PheT in human urine. This biomarker can be envisaged as an essential component of a carcinogen metabolite-phenotyping formula that can be applied to test the hypothesis that individual differences in PAH metabolism may influence cancer risk.

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References

- International Agency for Research on Cancer. Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental, and Experimental Data. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 32, pp. 33–91. Lyon, France: IARC, 1983.
- International Agency for Research on Cancer. Polynuclear Aromatic Compounds, Part 3. Industrial Exposures in Aluminum Production, Coal Gasification, Coke Production, and Iron and Steel Founding. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 34, pp. 65–131. Lyon, France: IARC, 1984.
- International Agency for Research on Cancer. Polynuclear Aromatic Compounds, Part 4. Bitumens, Coal-Tars and Derived Products, Shale Oils and Soots.

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 35, pp. 83–241. Lyon, France: IARC, 1985.

- Hecht, S. S. Tobacco smoke carcinogens and lung cancer. *J. Natl. Cancer Inst. (Bethesda)*, *91*: 1194–1210, 1999.
- Pfeifer, G. P., Denissenko, M. F., Olivier, M., Tretyakova, N., Hecht, S. S., and Hainaut, P. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene*, *21*: 7435–7451, 2002.
- Hoffmann, D., and Hecht, S. S. Advances in tobacco carcinogenesis. In: C. S. Cooper and P. L. Grover (eds.), *Handbook of Experimental Pharmacology*, Ed. 94/1, pp. 63–102. Heidelberg: Springer-Verlag, 1990.
- Cooper, C. S., Grover, P. L., and Sims, P. The metabolism and activation of benzo[a]pyrene. *Prog. Drug Metab.*, *7*: 295–396, 1983.
- Conney, A. H., Chang, R. L., Jerina, D. M., and Wei, S. J. C. Studies on the metabolism of benzo[a]pyrene and dose-dependent differences in the mutagenic profile of its ultimate carcinogenic metabolite. *Drug Metab. Rev.*, *26*: 125–163, 1994.
- Thakker, D. R., Yagi, H., Levin, W., Wood, A. W., Conney, A. H., and Jerina, D. M. Polycyclic aromatic hydrocarbons: metabolic activation to ultimate carcinogens. In: M. W. Anders (ed.), *Bioactivation of Foreign Compounds*, pp. 177–242. New York: Academic Press, Inc., 1985.
- Szeliga, J., and Dipple, A. DNA adduct formation by polycyclic aromatic hydrocarbon dihydrodiol epoxides. *Chem. Res. Toxicol.*, *11*: 1–11, 1998.
- Geacintov, N. E., Cosman, M., Hingerty, B. E., Amin, S., Broyde, S., and Patel, D. J. NMR solution structures of stereoisomeric covalent polycyclic aromatic carcinogen-DNA adducts: principles, patterns, and diversity. *Chem. Res. Toxicol.*, *10*: 112–146, 1997.
- Seo, K. Y., Jelinsky, S. A., and Loechler, E. L. Factors that influence the mutagenic patterns of DNA adducts from chemical carcinogens. *Mutat. Res.*, *463*: 215–246, 2000.
- Ramos, L. A., Ponten, I., Dipple, A., Kumar, S., Yagi, H., Sayer, J. M., Kroth, H., Kalena, G., and Jerina, D. M. Site-specific mutagenesis in *Escherichia coli* by N^2 -deoxyguanosine adducts derived from the highly carcinogenic fjord-region benzo[c]phenanthrene 3,4-diol 1,2-epoxides. *Chem. Res. Toxicol.*, *15*: 1619–1626, 2002.
- Boysen, G., and Hecht, S. S. Analysis of DNA and protein adducts of benzo[a]pyrene in human tissues using structure-specific methods. *Mutat. Res.*, *543*: 17–30, 2003.
- Taningher, M., Malacarne, D., Izzotti, A., Ugolini, D., and Parodi, S. Drug metabolism polymorphisms as modulators of cancer susceptibility. *Mutat. Res.*, *436*: 227–261, 1999.
- Benhamou, S., Lee, W. J., Alexandrie, A. K., Boffetta, P., Bouchardy, C., Butkiewicz, D., Brockmoller, J., Clapper, M. L., Daly, A., Dolzan, V., Ford, J., Gaspari, L., Haugen, A., Hirvonen, A., Husgafvel-Pursiainen, K., Ingelman-Sundberg, M., Kalina, I., Kihara, M., Kremers, P., Le Marchand, L., London, S. J., Nazar-Stewart, V., Onon-Kihara, M., Rannug, A., Romkes, M., Ryberg, D., Seidegard, J., Shields, P., Strange, R. C., Stucker, I., To-Figueras, J., Brennan, P., and Taioli, E. Meta- and pooled analyses of the effects of glutathione S-transferase M1 polymorphisms and smoking on lung cancer risk. *Carcinogenesis (Lond.)*, *23*: 1343–1350, 2002.
- Caporaso, N. E. Why have we failed to find the low penetrance genetic constituents of common cancers? *Cancer Epidemiol. Biomark. Prev.*, *11*: 1544–1549, 2002.
- Alexandrov, K., Cascorbi, I., Rojas, M., Bouvier, G., Kriek, E., and Bartsch, H. *CYP1A1* and *GSTM1* genotypes affect benzo[a]pyrene DNA adducts in smokers' lung: comparison with aromatic/hydrophobic adduct formation. *Carcinogenesis (Lond.)*, *23*: 1969–1977, 2002.
- Feyler, A., Voho, A., Bouchardy, C., Kuokkanen, K., Dayer, P., Hirvonen, A., and Benhamou, S. Point: myeloperoxidase $^{-463}\text{G} \rightarrow \text{A}$ polymorphism and lung cancer risk. *Cancer Epidemiol. Biomark. Prev.*, *11*: 1550–1554, 2002.
- Xu, L. L., Liu, G., Miller, D. P., Zhou, W., Lynch, T. J., Wain, J. C., Su, L., and Christiani, D. C. Counterpoint: the myeloperoxidase $^{-463}\text{G} \rightarrow \text{A}$ polymorphism does not decrease lung cancer susceptibility in Caucasians. *Cancer Epidemiol. Biomark. Prev.*, *11*: 1555–1559, 2002.
- Spivack, S. D., Fasco, M. J., Walker, V. E., and Kaminsky, L. S. The molecular epidemiology of lung cancer. *Crit. Rev. Toxicol.*, *27*: 319–365, 1997.
- Bartsch, H., Nair, U., Risch, A., Rojas, M., Wikman, H., and Alexandrov, K. Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. *Cancer Epidemiol. Biomark. Prev.*, *9*: 3–28, 2000.
- Houlston, R. S. *CYP1A1* polymorphisms and lung cancer risk: a meta-analysis. *Pharmacogenetics*, *10*: 105–114, 2000.
- McWilliams, J. E., Sanderson, B. J. S., Harris, E. L., Richert-Boe, K. E., and Henner, W. D. Glutathione S-transferase M1 (*GSTM1*) deficiency and lung cancer risk. *Cancer Epidemiol. Biomark. Prev.*, *4*: 589–594, 1995.

25. Rebbeck, T. R. Molecular epidemiology of the human glutathione *S*-transferase genotypes *GSTM1* and *GSTT1* in cancer susceptibility. *Cancer Epidemiol. Biomark. Prev.*, *6*: 733–743, 1997.
26. Houlston, R. S. Glutathione *S*-transferase M1 status and lung cancer risk: a meta-analysis. *Cancer Epidemiol. Biomark. Prev.*, *8*: 675–682, 1999.
27. Simpson, C. D., Wu, M. T., Christiani, D. C., Santella, R. M., Carmella, S. G., and Hecht, S. S. Determination of *r*-7,*t*-8,9,*c*-10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene in human urine by gas chromatography-negative ion chemical ionization-mass spectrometry. *Chem. Res. Toxicol.*, *13*: 271–280, 2000.
28. LaVoie, E. J., and Rice, J. E. Structure-activity relationships among tricyclic polynuclear aromatic hydrocarbons. In: S. K. Yang and B. D. Silverman (eds.), *Polycyclic Aromatic Hydrocarbon Carcinogenesis: Structure-Activity Relationships*, Vol. 1, pp. 151–175. Boca Raton, FL: CRC Press, 1988.
29. Shou, M., Korzekwa, K. R., Krausz, K. W., Crespi, C. L., Gonzalez, F. J., and Gelboin, H. V. Regio- and stereo-selective metabolism of phenanthrene by twelve cDNA-expressed human, rodent, and rabbit cytochromes P-450. *Cancer Lett.*, *83*: 305–313, 1994.
30. Lehr, R. E., Schaefer-Ridder, M., and Jerina, D. M. Synthesis and properties of the vicinal *trans* dihydrodiols of anthracene, phenanthrene, and benzo[*a*]anthracene. *J. Organ. Chem.*, *42*: 736–744, 1977.
31. Whalen, D. L., Ross, A. M., Yagi, H., Karle, J. M., and Jerina, D. M. Stereoelectronic factors in the solvolysis of bay-region diol epoxides of polycyclic aromatic hydrocarbons. *J. Am. Chem. Soc.*, *100*: 5218–5221, 1978.
32. Jongeneelen, F. J., Anzion, R. B. M., Leijdekkers, C. H. M., Bos, R. P., and Henderson, P. T. 1-Hydroxypyrene in human urine after exposure to coal tar and a coal tar derived product. *Int. Arch. Occup. Environ. Health*, *57*: 47–55, 1985.
33. Bland, J. M., and Altman, D. G. Multiple significance tests: the Bonferroni method. *BMJ*, *310*: 170, 1995.
34. Santella, R. M., Nunes, M. G., Blaskovic, R., Perera, F. P., Tang, D., Beachman, A., Lin, J. H., and DeLeo, V. A. Quantitation of polycyclic aromatic hydrocarbons, 1-hydroxypyrene, and mutagenicity in urine of coal tar-treated psoriasis patients and untreated volunteers. *Cancer Epidemiol. Biomark. Prev.*, *3*: 137–140, 1994.
35. Wu, M. T., Simpson, C. D., Christiani, D. C., and Hecht, S. S. Relationship of exposure to coke-oven emissions and urinary metabolites of benzo[*a*]pyrene and pyrene in coke-oven workers. *Cancer Epidemiol. Biomark. Prev.*, *11*: 311–314, 2002.
36. Baird, W. M., and Ralston, S. L. Carcinogenic polycyclic aromatic hydrocarbons. In: G. T. Bowden and S. M. Fischer (eds.), *Comprehensive Toxicology: Chemical Carcinogens and Anticarcinogens*, Vol. 12, pp. 171–200. New York: Elsevier Science Ltd, 1997.
37. Bauer, E., Guo, Z., Ueng, Y. F., Bell, L. C., Zeldin, D., and Guengerich, F. P. Oxidation of benzo[*a*]pyrene by recombinant human cytochrome P450 enzymes. *Chem. Res. Toxicol.*, *8*: 136–142, 1995.
38. Grimmer, G., Dettbarn, G., and Jacob, J. Biomonitoring of polycyclic aromatic hydrocarbons in highly exposed coke plant workers by measurement of urinary phenanthrene and pyrene metabolites (phenols and dihydrodiols). *Int. Arch. Occup. Environ. Health*, *65*: 189–199, 1993.
39. Jacob, J., Grimmer, G., and Dettbarn, G. Profile of urinary phenanthrene metabolites in smokers and non-smokers. *Biomarkers*, *4*: 319–327, 1999.
40. Angerer, J., Mannschreck, C., and Gundel, J. Biological monitoring and biochemical effect monitoring of exposure to polycyclic aromatic hydrocarbons. *Int. Arch. Occup. Environ. Health*, *70*: 365–377, 1997.
41. Chu, I., Ng, K. M., Benoit, F. M., and Moir, D. Comparative metabolism of phenanthrene in the rat and guinea pig. *J. Environ. Sci. Health B*, *27*: 729–749, 1992.
42. Bouchard, M., Krishnan, K., and Viau, C. Kinetics of tissue distribution and elimination of pyrene and 1-hydroxypyrene following intravenous administration of [¹⁴C]pyrene in rats. *Toxicol. Sci.*, *46*: 11–20, 1998.
43. Kotin, P., Falk, H. L., and Busser, R. Distribution, retention, and elimination of C14–3,4-benzopyrene after administration to mice and rats. *J. Natl. Cancer Inst. (Bethesda)*, *23*: 541–555, 1959.
44. Jacob, J., and Seidel, A. Biomonitoring of polycyclic aromatic hydrocarbons in human urine. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, *778*: 31–47, 2002.
45. Phillips, D. H., Farmer, P. B., Beland, F. A., Nath, R. G., Poirier, M. C., Reddy, M. V., and Turteltaub, K. W. Methods of DNA adduct determination and their application to testing compounds for genotoxicity. *Environ. Mol. Mutagen.*, *35*: 222–233, 2000.
46. Kriek, E., Rojas, M., Alexandrov, K., and Bartsch, H. Polycyclic aromatic hydrocarbon-DNA adducts in humans: relevance as biomarkers for exposure and cancer risk. *Mutat. Res.*, *400*: 215–231, 1998.
47. Poirier, M. C., Santella, R. M., and Weston, A. Carcinogen macromolecular adducts and their measurement. *Carcinogenesis (Lond.)*, *21*: 353–359, 2000.
48. Gupta, R. C. ³²P-Postlabeling for the detection of DNA adducts. In: G. P. Pfeifer (ed.), *Technologies for the Detection of DNA Damage and Mutations*, pp. 45–61. New York: Plenum Press, 1996.
49. Phillips, D. H. Smoking-related DNA and protein adducts in human tissues. *Carcinogenesis (Lond.)*, *23*: 1979–2004, 2002.
50. Hecht, S. S. Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. *Carcinogenesis (Lond.)*, *23*: 907–922, 2002.
51. Stowers, S. J., and Anderson, M. W. Formation and persistence of benzo[*a*]pyrene metabolite-DNA adducts. *Environ. Health Perspect.*, *62*: 31–39, 1985.
52. Ginsberg, G. L., and Atherholt, T. B. DNA adduct formation in mouse tissues in relation to serum levels of benzo[*a*]pyrene-diol-epoxides after injection of benzo[*a*]pyrene or the diol-epoxide. *Cancer Res.*, *50*: 1189–1194, 1990.
53. Wall, K. L., Gao, W. S., te Koppele, J. M., Kwei, G. Y., Kauffman, F. C., and Thurman, R. G. The liver plays a central role in the mechanism of chemical carcinogenesis due to polycyclic aromatic hydrocarbons. *Carcinogenesis (Lond.)*, *12*: 783–786, 1991.
54. Jongeneelen, F. J. Biological monitoring of environmental exposure to polycyclic aromatic hydrocarbons: 1-hydroxypyrene in urine of people. *Toxicol. Lett.*, *72*: 205–211, 1994.
55. Levin, J. O. First international workshop on hydroxypyrene as a biomarker for PAH exposure in man—summary and conclusions. *Sci. Total Environ.*, *163*: 165–168, 1995.
56. Roggi, C., Minoia, C., Sciarra, G. F., Apostoli, P., Maccarini, L., Magnaghi, S., Cenni, A., Fonte, A., Nidasio, G. F., and Micoli, G. Urinary 1-hydroxypyrene as a marker of exposure to pyrene: an epidemiological survey on a general population group. *Sci. Total Environ.*, *199*: 247–254, 1997.
57. Jongeneelen, F. J. Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. *Ann. Occup. Hyg.*, *45*: 3–13, 2001.
58. Bowman, E. D., Rothman, N., Hackl, C., Santella, R. M., and Weston, A. Interindividual variation in the levels of certain urinary polycyclic aromatic hydrocarbon metabolites following medicinal exposure to coal tar ointment. *Biomarkers*, *2*: 321–327, 1997.
59. Swauger, J. E., Steichen, T. J., Murphy, P. A., and Kinsler, S. An analysis of the mainstream smoke chemistry of samples of the U. S. cigarette market acquired between 1995 and 2000. *Regul. Toxicol. Pharmacol.*, *35*: 142–156, 2002.