A Urinary Metabolite of Phenanthrene as a Biomarker of Polycyclic Aromatic Hydrocarbon Metabolic Activation in Workers Exposed to Residual Oil Fly Ash

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Abstract

Residual oil fly ash is a chemically complex combustion product containing a significant component of potentially carcinogenic transition metals and polycyclic aromatic hydrocarbons (PAH). Various biomarkers of PAH exposure have been investigated previously, most notably 1-hydroxypyrene (1-OHP), in urine. In this study, we assessed the utility of *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans, anti*-PheT), a metabolite of phenanthrene, to detect occupational PAH exposure. Urine samples collected across the workweek were analyzed for 1-OHP and *trans, anti*-PheT in boilermakers (n = 20) exposed to residual oil fly ash. Median baseline urinary *trans, anti*-PheT concentrations were 0.50 μg/g creatinine in current tobacco smokers and 0.39 μg/g creatinine in nonsmokers. Median baseline urinary 1-OHP concentrations in smokers and nonsmokers were 0.31 and 0.13 μg/g creatinine, respectively. To study further the effect of smoking exposure on the urinary PAH markers, urinary cotinine was used. Although urinary *trans, anti*-PheT and 1-OHP concentrations were correlated (Spearman r = 0.63; P < 0.001) for all subjects, the regression coefficient between log-transformed *trans, anti*-PheT and log 1-OHP was statistically significant only for subjects with low levels of urinary cotinine or for nonsmokers. Each 1-unit increase in log 1-OHP was associated with a 0.77-unit increase (95% confidence interval, 0.45-1.09) in log *trans, anti*-PheT in subjects with low levels of urinary cotinine (P < 0.001). In these subjects, dichotomized occupational exposure status was a significant predictor of log *trans, anti*-PheT (P = 0.02) but not of log 1-OHP (P = 0.2). In conclusion, we found that urinary *trans, anti*-PheT was detected in levels comparable with 1-OHP in occupationally exposed workers, particularly nonsmokers. This study shows that urinary *trans, anti*-PheT may be an effective biomarker of uptake and metabolic activation of PAHs. (Cancer Epidemiol Biomarkers Prev 2005;14(3):687–92)

Introduction

Residual oil fly ash (ROFA) is a chemically complex air pollutant resulting from the combustion of residual fuel oil in electric power-generating boilers. Primarily composed of fine particles with a mass median aerodynamic diameter ≤2.5 μm ($PM_{2.5}$; ref. 1), the chemical composition of ROFA includes sulfates, silicates, and carbon- and nitrogen-containing compounds (2). In addition, ROFA contains a significant component of bioavailable transition metals, including vanadium, iron, and nickel (3). Polycyclic aromatic hydrocarbons (PAH) also are an important constituent of fly ash produced from the incomplete combustion of fuel oil (4). ROFA has been found to be associated with severe respiratory symptoms (5), airway inflammation (6, 7), and reduced pulmonary function (8, 9) in occupationally exposed workers. Previous epidemiologic and toxicologic researches have focused mainly on the metal component of ROFA, with a particular interest in vanadium. However, with the recent development of improved biomarkers of PAH exposure, the role of PAHs in the toxicity of ROFA can be investigated further.

PAHs are a group of organic compounds, generally occurring as a complex mixture in combustion products (4). More than 100 different PAHs have been identified. One of the most notable compounds is benzo[*a*]pyrene (BaP), a probable carcinogen to humans as defined by the IARC (10). Various biomarkers in urine and blood have been used to determine PAH exposure (11-14), with urinary 1-hydroxypyrene (1-OHP) emerging as the most commonly used biomarker. 1-OHP is the principal metabolite of the four-ring PAH pyrene, representing 90% of the urinary excretion of pyrene in humans (15). The measurement of urinary 1-OHP has been used in various epidemiologic studies investigating PAH exposure from tobacco smoke, traffic air pollution, and occupational sources (16-19).

Although urinary 1-OHP has been established as the primary biomarker for determining exposure to PAHs, it may not be the most relevant marker to express carcinogenic PAH exposure. The most potent PAH carcinogens require metabolic activation to form reactive bay region diol epoxides, which can bind covalently to DNA (20). Pyrene, the parent compound of 1-OHP, does not have a bay region and is not carcinogenic. Phenanthrene, with its three aromatic rings, is the simplest PAH with a bay region. Although phenanthrene is generally considered to be noncarcinogenic, its metabolism to *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans, anti*-PheT) through the diol epoxide pathway closely

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mimics the metabolism of the probable carcinogen BaP to *r*-7, *t*-8,9,*c*-10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*trans, anti*-BaP-tetraol; ref. 21). Therefore, *trans, anti*-PheT can be regarded as a biomarker of uptake plus metabolic activation of PAHs.

This study evaluates the utility of *trans, anti*-PheT as a biomarker of occupational PAH exposure in a group of boilermakers performing maintenance and repairs on oil-fired boilers. Occupational PAH exposure resulted mainly from the ROFA that coated the surfaces of the boilers and accumulated in the ash pit. We studied the association between urinary 1-OHP and *trans, anti*-PheT and whether smoking or occupational exposure affected the relationship between the two biomarkers. In addition, we investigated the individual effects of smoking and occupational exposure on 1-OHP and *trans, anti*-PheT concentrations in cross-workweek urine samples.

Materials and Methods

Study Population. The study was approved by the Institutional Review Board of the Harvard School of Public Health (Boston, MA). Written informed consent was obtained from each subject. The study population consisted of 20 boilermakers working at a power plant during an overhaul of oil-fired boilers. The overhaul entailed removing and replacing the interior wall panels and water-circulating tubing of the boilers and repairing the ash pit. Most subjects did not consistently wear respirators; therefore, their exposure to ROFA was primarily through inhalation. The workers were prohibited from smoking inside the power plant but were permitted to smoke during their breaks and lunch hour. The subjects were monitored during a 5-day work period in June 1999. Self-administered questionnaires were used to obtain information on medical history, including respiratory symptoms and diseases, smoking history, and occupational history.

Urine Sample Collection. The collection of urine samples began on the first day of the workweek before the workshift. Urine samples were collected before and after the workshift each day during the 5-day sampling period. After samples were collected in sterile 120 mL urine collection cups, they were aliquoted into 15 mL polypropylene tubes and stored at −20°C until ready for analysis.

Urine Analysis for Creatinine. A polypropylene tube containing 5 mL frozen urine was sent to ESA Laboratories, Inc. (Chelmsford, MA) for creatinine analysis. The creatinine level in the urine sample was measured with a Shimadzu model UV-1601 spectrophotometer using the Jaffé reaction (22).

Urine Analysis for Cotinine. Urinary analysis of cotinine was done at ESA Laboratories. Urinary cotinine was determined using reverse-phase high-performance liquid chromatography with UV spectrophotometry detection (23).

Urine Analysis for 1-OHP. The analytic procedure to determine urinary 1-OHP levels using reverse-phase high-performance liquid chromatography with fluorescence detection has been described previously (16, 24, 25). Reported levels of 1-OHP are the sum of the free compounds and the sulfate and glucuronide conjugates. The urinary 1-OHP concentrations were adjusted for the urinary creatinine concentrations (μg 1-OHP/g creatinine) to control for the variability in urine dilution.

Urine Analysis for *Trans, Anti*-PheT. Urine samples collected before the workshift on the first and last days of work during the monitoring period were analyzed for *trans, anti*-PheT. Urinary analysis of *trans, anti*-PheT was done at the University of Minnesota Cancer Center. The analysis of *trans, anti*-PheT by gas chromatography-negative ion chemical ionization-mass spectrometry has been described previously (21). Briefly, 1 mL urine was placed in a 15 mL centrifuge tube containing 1 ng of the internal standard, [D_{10}]*trans, anti*-PheT. After adjusting the pH of the sample to 5, β-glucuronidase and arylsulfatase were added and the mixture was incubated overnight while shaking at 37°C. The sample was applied to a prewashed Sep-Pak cartridge and first eluted with 15 mL of 0.15 mol/L NH_4OH. After discarding the eluted solutes, the cartridge was then washed with 12 mL of 25% methanol. The solvents were evaporated overnight on a SpeedVac and the residue was transferred into a 300 μL polypropylene vial with three 65 μL portions of methanol/H_2O. Reverse-phase high-performance liquid chromatography was used to obtain a subfraction containing *trans, anti*-PheT. High-performance liquid chromatography eluent was collected from 6.5 to 14.5 minutes for the analysis of *trans, anti*-PheT and the collected high-performance liquid chromatography fraction was concentrated to dryness overnight. The residue was transferred into a 1.8 mL Ekonical vial using three 60 μL portions of methanol and concentrated to dryness again. Acetonitrile (10 μL) and bis-trimethylsilyltrifluoroacetamide (30 μL) were added to derivatize the residue to produce PheT-tetratrimethylsilyl ether. The concentration of *trans, anti*-PheT-tetratrimethylsilyl ether in the sample was determined by gas chromatography-negative ion chemical ionization-mass spectrometry–selected ion monitoring at m/z 372. The reported levels of *trans, anti*-PheT are actually the sum of the free compounds and the sulfate and glucuronide conjugates. The urinary *trans, anti*-PheT concentration was adjusted to the urinary concentration of creatinine (μg *trans, anti*-PheT/g creatinine) to control for the variability in urine dilution.

Particulate Exposure Assessment. The determination of occupational $PM_{2.5}$ exposure using personal exposure monitors has been described previously (26). Briefly, subjects were randomly selected to wear personal exposure monitors during their workshift. A personal exposure monitor (model 200, MSP Corp., Minneapolis, MN) with a 2.5 μm impactor cut size was used to collect the air sample on a polytetrafluoroethylene membrane filter (Gelman Laboratories, Ann Arbor, MI). The mass collected on the filter was divided by the air volume sampled to calculate the gravimetric $PM_{2.5}$ concentration. $PM_{2.5}$ concentrations were standardized to 8-hour time-weighted averages.

Statistical Analysis. Statistical analyses were done using SAS version 6.12 (SAS Institute, Inc., Cary, NC). Smoking status was determined using information provided in the self-administered questionnaires. Study population characteristics between current smokers and nonsmokers were compared using two-sample t tests and Wilcoxon rank-sum tests with exact Ps.

The mean (SD) and median pre-exposure and post-exposure concentrations of 1-OHP and *trans, anti*-PheT were calculated after stratifying by smoking status. Pre-exposure data represent urine samples collected before the workshift on the first day of work. Post-exposure data were from urine samples collected before the workshift on the last day of work during the 5-day monitoring period. Nonparametric Spearman rank correlation coefficient was calculated to determine the relationship between urinary 1-OHP and *trans, anti*-PheT concentrations. Linear mixed models were constructed to investigate further the association between urinary 1-OHP and *trans, anti*-PheT. Log-transformed *trans, anti*-PheT was regressed on log 1-OHP in the linear mixed models. 1-OHP and *trans, anti*-PheT concentrations were log transformed to improve normality and stabilize the variance. A generalized autoregressive covariance structure was used because it resulted in the best Akaike's Information Criterion compared with models with other covariance structures (27). Restricted maximum likelihood was used to estimate

the covariance variables. Interaction terms were included to study the effect of occupational exposure (pre-exposure = 0, post-exposure = 1) and smoking (creatinine-adjusted urinary cotinine concentrations, μg/g creatinine) on the association between log *trans, anti*-PheT and log 1-OHP.

The univariate and multivariate predictors of log 1-OHP and log *trans, anti*-PheT also were investigated using mixed models. The effects of occupational exposure and smoking on log 1-OHP and log *trans, anti*-PheT were investigated. In addition, effect modification by smoking was investigated by including an interaction term between occupational exposure status and urinary cotinine levels in the model. The level of significance for all analyses was set at 0.05.

Results

Description of Study Population. Population demographic data are summarized in Table 1. The study population consisted of 20 men, 10 (50%) of whom were current smokers. Their ages ranged from 18 to 59 years, with 2 weeks to 40 years of work experience as a boilermaker. The baseline median urinary creatinine-adjusted cotinine level was 13 μg/g creatinine (range, 5-23 μg/g creatinine) for nonsmokers. Among the smokers, there was a wide range of urinary cotinine levels from 14 to 1,723 μg/g creatinine (median, 285 μg/g creatinine).

During the overhaul in 1999, the boilermakers generally worked 10-hour shifts. Personal exposure samples were collected over a mean sampling time of 8.8 hours (SD, 1.2 hours). The median occupational $PM_{2.5}$ 8-hour time-weighted average was 0.36 mg/m^3 (range, 0.10-3.14 mg/m^3) for nonsmokers and 0.46 mg/m^3 (range, 0.11-2.28 mg/m^3) for smokers. The median occupational $PM_{2.5}$ concentrations were not significantly different between nonsmokers and smokers ($P = 0.7$).

Analysis of Urinary 1-OHP and *Trans, Anti*-PheT Concentrations. A summary of the urinary 1-OHP and *trans, anti*-PheT concentrations is shown in Table 2. The median pre-exposure 1-OHP concentration in smokers [0.31 μg/g creatinine (Q_{25}-Q_{75}, 0.21-0.75)] was significantly greater ($P = 0.02$) than that in nonsmokers [0.13 μg/g creatinine (Q_{25}-Q_{75}, 0.11-0.28)]. The median pre-exposure urinary *trans, anti*-PheT concentrations were 0.50 μg/g creatinine (Q_{25}-Q_{75}, 0.26-1.09) in smokers and 0.39 μg/g creatinine (Q_{25}-Q_{75}, 0.22-0.69) in nonsmokers. Unlike 1-OHP, the median pre-exposure *trans, anti*-PheT concentrations in nonsmokers and smokers were not significantly different ($P = 0.4$).

Table 1. Study population characteristics

	Nonsmokers (n = 10)	Current smokers (n = 10)	P*
Age (y)			
Mean ± SD	46.5 ± 10.8	44.3 ± 13.6	0.7
Range	18 - 55	23 - 59	
Years as boilermaker			
Mean ± SD	23.5 ± 9.4	20.0 ± 16.0	0.6
Range	1 - 34	0.04 - 40	
Urinary cotinine concentration (μg/g creatinine)			
Mean ± SD	13 ± 6	555 ± 665	0.03
Median	13	285	<0.001
Range	5 - 23	14 - 1,723	
Occupational $PM_{2.5}$ 8-hour time-weighted average (mg/m^3)			
No. samples	13	17	
Mean ± SD	0.81 ± 0.86	0.66 ± 0.64	0.6
Median	0.36	0.46	0.7
Range	0.10 - 3.14	0.11 - 2.28	

*Two-sample t tests and Wilcoxon rank-sum tests with exact Ps used to compare characteristics in nonsmokers and smokers.

Across the workweek, marginally significant changes in 1-OHP and *trans, anti*-PheT concentrations were observed in nonsmokers but not in smokers. Nonsmokers had a 0.11 μg/g creatinine (Q_{25}-Q_{75}, 0.03-0.42) increase in urinary 1-OHP concentrations ($P = 0.08$) and 0.18 μg/g creatinine (Q_{25}-Q_{75}, −0.08 to 0.96) increase in *trans, anti*-PheT concentrations ($P = 0.06$) after occupational exposure to ROFA.

Association between Urinary 1-OHP and *Trans, Anti*-PheT Concentrations. Urinary 1-OHP and *trans, anti*-PheT concentrations were significantly correlated, with a Spearman correlation coefficient of 0.63 ($P < 0.001$). The strong correlation between 1-OHP and *trans, anti*-PheT persisted even after stratifying by smoking status (nonsmokers, $r = 0.69$; smokers, $r = 0.60$) and occupational exposure status (pre-exposure, $r = 0.51$; post-exposure, $r = 0.68$).

The association between urinary 1-OHP and *trans, anti*-PheT concentrations was examined further using linear mixed models (Table 3). Urinary 1-OHP and *trans, anti*-PheT concentrations were log transformed to improve normality and stabilize the variance. In the crude analysis, each unit increase in log 1-OHP was significantly associated with a 0.68 [95% confidence interval (95% CI), 0.41-0.95] increase in log *trans, anti*-PheT. Adjusting for age, exposure status, and urinary cotinine level did not change the regression coefficient significantly [0.66 (95% CI, 0.36-0.95)].

To investigate whether exposure or smoking status affected the relationship between log 1-OHP and log *trans, anti*-PheT, interaction terms were included in the adjusted model. Exposure status was not found to modify the relationship between log 1-OHP and log *trans, anti*-PheT. In both pre-exposure and post-exposure samples, the association between log 1-OHP and log *trans, anti*-PheT was statistically significant ($P \leq 0.01$).

The association between log 1-OHP and log *trans, anti*-PheT was found to be modified by urinary cotinine level. In subjects who had low levels of urinary cotinine (10th percentile = 8 μg/g creatinine), the association between log 1-OHP and *trans, anti*-PheT was statistically significant [0.77 (95% CI, 0.45-1.09)]. In contrast, in subjects with high levels of urinary cotinine (90th percentile = 1,360 μg/g creatinine), there was a much weaker association between log 1-OHP and *trans, anti*-PheT [0.14 (95% CI, −0.59 - 0.86)].

Predictors of Urinary 1-OHP and *Trans, Anti*-PheT Concentrations. Predictors of 1-OHP and *trans, anti*-PheT concentrations in urine were investigated using mixed models (Table 4). Occupational exposure to high levels of particulates was not found to predict log 1-OHP in both univariate and multivariate models ($P > 0.1$). As a biomarker of smoking exposure, urinary cotinine concentration was used to indicate smoking dose in smokers. Urinary cotinine level also was not found to be a significant predictor of log 1-OHP.

In contrast, occupational exposure was found to be a significant predictor of log *trans, anti*-PheT even after adjusting for urinary cotinine concentration ($P = 0.05$). Urine samples collected near the end of the workweek had a log *trans, anti*-PheT value that was 0.43 (95% CI, 0.001-0.86) greater than that collected before the workweek began. However, the significant association between occupational exposure and log *trans, anti*-PheT was found to be modified by smoking. At low urinary cotinine levels (10th percentile), the association between exposure status and log *trans, anti*-PheT was statistically significant ($P = 0.02$). Occupational exposure to ROFA across the workweek was associated with an increase in log *trans, anti*-PheT by 0.58 (95% CI, 0.11-1.05) in those with low levels of urinary cotinine. However, at high urinary cotinine levels (90th percentile), occupational exposure was not a significant predictor of log *trans, anti*-PheT.

Table 2. Summary of 1-OHP and *trans, anti*-PheT concentrations (μg/g creatinine) in urine

	Nonsmokers			Current smokers		
	Pre-exposure	Post-exposure	Δ	Pre-exposure	Post-exposure	Δ
1-OHP						
No. samples	10	8	8	9	10	9
Mean ± SD	0.20 ± 0.16	0.39 ± 0.36	0.21 ± 0.30	0.51 ± 0.45	0.73 ± 0.68	0.16 ± 0.84
Median	0.13	0.26	0.11	0.31	0.65	−0.01
Q_{25} - Q_{75}	0.11 - 0.28	0.12 - 0.56	0.03 - 0.42	0.21 - 0.75	0.17 - 0.91	−0.10 - 0.10
Trans, anti-PheT						
No. samples	10	10	10	10	10	10
Mean ± SD	0.48 ± 0.33	1.02 ± 0.95	0.55 ± 0.84	0.67 ± 0.52	1.26 ± 1.89	0.59 ± 1.88
Median	0.39	0.52	0.18	0.50	0.74	0.08
Q_{25} - Q_{75}	0.22 - 0.69	0.44 - 1.40	−0.08 - 0.96	0.26 - 1.09	0.48 - 0.92	−0.26 - 0.34

Discussion

PAHs are ubiquitous in the general environment, released into the ambient air by tobacco smoke, vehicle exhausts, and other incomplete combustion sources. However, the highest exposure to PAHs typically occurs in the occupational setting, including coking plants, energy-generating power plants, asphalt production plants, and chemical production plants. In the present study, we investigated the utility of urinary *trans, anti*-PheT as a biomarker of occupational PAH exposure in ROFA-exposed boilermakers. We found that urinary *trans, anti*-PheT concentrations were well correlated with the more established marker of PAH exposure (1-OHP). In addition, dichotomized occupational exposure status was found to be a significant predictor of log-transformed *trans, anti*-PheT concentrations from cross-workweek urine samples among nonsmokers.

Metabolites of BaP, one of the most notable PAH carcinogens, also have been analyzed in the urine samples of other occupationally exposed groups. BaP is metabolized by cytochrome *P*450 enzymes to form arene oxides that undergo hydration and oxidation to form 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene enantiomers, which bind to DNA and are ultimate carcinogens of BaP. A study by Wu et al. investigating PAH exposure in coke-oven workers measured urinary *trans, anti*-BaP-tetraol produced by 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene hydration (14). Although *trans, anti*-BaP-tetraol was detected in the urine of these highly exposed coke-oven workers, the concentrations were too low to make the analysis feasible for routine monitoring. In the coke-oven workers, the urinary *trans, anti*-BaP-tetraol concentration was 0.4 nmol/mol creatinine compared with 9.7 μmol/mol creatinine of 1-OHP.

Ideally, metabolites of probable carcinogens, such as *trans, anti*-BaP-tetraol, should be used to determine an individual's uptake and metabolic response. Although phenanthrene is not considered to be carcinogenic, it undergoes a similar metabolic pathway as BaP, forming a diol epoxide that undergoes hydration to produce *trans, anti*-PheT. Therefore, *trans, anti*-PheT may serve as a surrogate marker for both uptake and metabolic activation of carcinogenic PAHs. Unlike *trans, anti*-BaP-tetraol, *trans, anti*-PheT is detectable in concentrations comparable with 1-OHP in urine. In our study, the baseline median urinary *trans, anti*-PheT concentrations were 0.50 μg/g creatinine in current tobacco smokers and 0.39 μg/g creatinine in nonsmokers. The urinary *trans, anti*-PheT concentrations were 2- to 3-fold greater than the urinary 1-OHP concentrations (0.31 μg/g creatinine in smokers and 0.13 μg/g creatinine in nonsmokers).

The urinary *trans, anti*-PheT concentrations were found to be moderately to highly correlated with the urinary 1-OHP concentrations in our boilermakers (r = 0.63). The adjusted linear mixed models indicated that a 1-unit increase in log 1-OHP was associated with a 0.66-unit increase (95% CI, 0.36-0.95) in log *trans, anti*-PheT. Dichotomized occupational exposure status was not found to modify the association between log *trans, anti*-PheT and log 1-OHP (P = 0.7). However, the results suggested that the relationship between log *trans, anti*-PheT and log 1-OHP might be affected by smoking exposure. For subjects with low urinary cotinine levels, each unit increase in log 1-OHP was associated with a 0.77-unit increase (95% CI, 0.45-1.09) in log *trans, anti*-PheT. In contrast, there was no significant association between log 1-OHP and log *trans, anti*-PheT in subjects with high levels of urinary cotinine (P = 0.7). In a previous study, the correlation between urinary 1-OHP and *trans, anti*-PheT concentrations also was greater in nonsmokers (r = 0.47; P = 0.009) than in smokers (r = 0.35; P = 0.05; ref. 21). Although phenanthrene and pyrene, the parent compounds of 1-OHP and *trans, anti*-PheT, are both present in tobacco smoke (28), these results indicate that their uptake by smokers varies.

Dichotomized occupational exposure status was found to be a significant predictor of urinary *trans, anti*-PheT concentrations in subjects with low levels of urinary cotinine (P = 0.02). In subjects with low urinary cotinine levels, the log *trans, anti*-PheT was 0.58 (95% CI, 0.11-1.05) greater in the cross-workweek, post-exposure urine samples compared with the pre-workweek samples. In subjects with high urinary cotinine levels, occupational exposure was not a significant predictor of log *trans, anti*-PheT. The crude cross-workweek changes in urinary *trans, anti*-PheT levels, as indicated in Table 2, were 0.18 μg/g creatinine in nonsmokers and a smaller 0.08 μg/g creatinine in smokers. At the higher baseline level of 0.50 μg/g creatinine, the additional PAH exposure from ROFA did not contribute significantly to urinary *trans, anti*-PheT levels in smokers (P = 0.7).

Table 3. Regression coefficients (95% CI) for log-transformed *trans, anti*-PheT concentrations regressed on log-transformed 1-OHP concentrations

	Coefficient* (95% CI)	*P*
Crude association	0.68 (0.41 - 0.95)	<0.001
Adjusted association †	0.66 (0.36 - 0.95)	<0.001
Effect modification by exposure †		
Pre-exposure	0.58 (0.17 - 0.99)	0.01
Post-exposure	0.70 (0.35 - 1.06)	<0.001
Effect modification by cotinine level †		
Urinary cotinine at 10th percentile	0.77 (0.45 - 1.09)	<0.001
Urinary cotinine at 90th percentile	0.14 (−0.59 - 0.86)	0.7

*Regression coefficients express the change in log *trans, anti*-PheT concentration with each 1-unit increase in log 1-OHP.

†Models were adjusted for age, occupational exposure status, and urinary cotinine levels.

Table 4. Univariate and multivariate predictors of log-transformed 1-OHP and *trans, anti*-PheT concentrations

	Log 1-OHP		Log *trans, anti*-PheT	
	Coefficient (95% CI)*	*P*	Coefficient (95% CI)*	*P*
Univariate model				
Occupational exposure	0.35 (−0.14 - 0.85)	0.1	0.46 (0.04 - 0.88)	0.03
Urinary cotinine concentration	0.04 (−0.02 - 0.10)	0.2	0.04 (−0.02 - 0.09)	0.2
Multivariate model†				
Occupational exposure	0.30 (−0.20 - 0.80)	0.2	0.43 (0.001 - 0.86)	0.05
Urinary cotinine concentration	0.04 (−0.01 - 0.10)	0.1	0.03 (−0.02 - 0.09)	0.2
Multivariate model with interaction term†				
Exposure, cotinine at 10th percentile	0.36 (−0.22 - 0.95)	0.2	0.58 (0.11 - 1.05)	0.02
Exposure, cotinine at 90th percentile	0.11 (−0.93 - 1.16)	0.8	−0.13 (−1.04 - 0.77)	0.8

NOTE: Mixed effect models were used to calculate the coefficients (95% CI) of the predictors.
*The exposure coefficient is expressed as pre-exposure = 0 and post-exposure = 1. The smoking coefficient is expressed as the change per 100 μg/g creatinine incremental change in cotinine level.
†Multivariate models with interaction term are adjusted for age.

Other previous studies found a significant increase in urinary 1-OHP levels in various occupational populations, including remediation site workers (29), fire fighters (30), coke-oven workers (19), and asphalt pavers (31). Our previous study from the same boilermaker population also observed a significant increase in 1-OHP across the workweek in non-smokers (16). In contrast to these studies, we did not find a significant increase in urinary 1-OHP even after stratifying by smoking exposure. The lack of a significant increase may be attributable to several factors. First, in our previous study, 1-OHP analysis was done on 166 urine samples from the 20 subjects. Urine samples were collected before and after the workshift each day during the 5-day sampling period. However, because we only had two urine samples from across the workweek analyzed for *trans, anti*-PheT (n = 40), we limited the 1-OHP analysis to cross-workweek samples as well (n = 37). Second, the definition of exposure status differed between the two studies. In our previous study, we defined dichotomized exposure status by the workday, with pre-workshift samples from each day being coded as nonexposed and post-workshift samples being coded as exposed. In the present study, the two cross-workweek, pre-workshift urine samples were analyzed, with the sample collected on the first day of the workweek coded as nonexposed and the latter one coded as exposed. We chose to analyze the pre-workshift sample from the end of the workweek to control for potential circadian variability. Finally, the previous study dichotomized smoking status, whereas we used urinary cotinine levels as a marker of smoking exposure. Urinary cotinine levels were used in this study to provide a better measure of smoking exposure in our subjects.

One of the major limitations of our study was our lack of data on dietary sources of PAH exposure. Due to the ubiquitous presence of PAHs in the environment, they are detected in uncooked food and further generated in the cooking process (32). In our future work, we would like to investigate the contribution of dietary sources on urinary *trans, anti*-PheT and 1-OHP concentrations in our occupationally exposed population.

Consistent with previous work, our study found a moderate to high correlation between urinary 1-OHP and *trans, anti*-PheT concentrations, indicating that *trans, anti*-PheT is a good uptake biomarker for PAHs. However, *trans, anti*-PheT was not developed for this sole purpose. As a biomarker of phenanthrene uptake plus metabolic activation, *trans, anti*-PheT has the potential to be used to identify those individuals who might be particularly sensitive to the carcinogenic effects of PAH. To investigate the capability of *trans, anti*-PheT to perform carcinogen metabolite phenotyping, we would need to adjust for differences in PAH exposure. In this study, we were unable to determine the occupational PAH exposure of the individual subjects due to missing personal exposure data. Experiments directed toward this goal are currently in progress. Further, as phenanthrene is not carcinogenic per se, to confirm that *trans, anti*-PheT can serve as a biomarker for the metabolic activation of potentially carcinogenic PAHs, its values should be correlated to metabolites of probable carcinogens. The analytic method for one such metabolite, *trans, anti*-BaP-tetraol, has been established (14). However, as the very low concentrations of *trans, anti*-BaP-tetraol make analysis difficult even for highly exposed occupational populations, comparisons of the two metabolites were not done in this study. Nevertheless, *trans, anti*-PheT may be a more useful biomarker than 1-OHP because it represents the end product of a pathway closely associated with the carcinogenic effects of PAH.

In conclusion, we found that *trans, anti*-PheT was detectable in urine at moderate levels and well correlated with urinary 1-OHP concentrations in workers exposed to PAHs from ROFA. Further, our results suggest that urinary *trans, anti*-PheT concentrations may be a more sensitive marker of occupational PAH exposure than urinary 1-OHP in non-smokers. This study shows that *trans, anti*-PheT may be a feasible and effective biomarker for the metabolic activation of potentially carcinogenic PAHs, such as BaP.

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A Urinary Metabolite of Phenanthrene as a Biomarker of Polycyclic Aromatic Hydrocarbon Metabolic Activation in Workers Exposed to Residual Oil Fly Ash

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