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An assessment of a urinary biomarker for total human environmental exposure to benzo[*a*]pyrene

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Abstract Urinary banzo[*a*]pyrene (BaP) metabolite levels were compared to human environmental exposure to BaP through inhalation and dietary ingestion to assess the predictive validity of the exposure biomarker. These measurements were made for 14 adult volunteers over 14 consecutive days, once during summer/fall, again during winter periods. Based on personal air monitoring, median potential inhalation doses of 11.0 and 2.3 ng/day were estimated for the winter and summer/fall studies, respectively. A median potential ingested dose of 176 ng/day, estimated from "duplicate plate" sampling, exceeded inhalation by 6- and 122-fold for the winter and summer/fall studies, respectively. "Total" urinary BaP metabolites were measured using a published "reverse metabolism" (BaP) method of analysis. Median rates of urinary BaP metabolite elimination for the winter and summer/fall studies were 121 and 129 ng/day, respectively. The changes in inhaled and ingested potential doses were regressed on the change in urinary metabolite elimination from week 1 to week 2 to test the predictive validity of the biomarker measurement. The regression was statistically significant ($r = 0.620$, $p = 0.015$, $n = 25$) when body weight was included and two extreme values were removed. Consistent with the exposure measurements showing diet as the dominant route of exposure, most of the variation in urinary metabolite elimination was explained by the ingested dose. It is concluded that the measurement of urinary BaP by "reverse metabolism" is qualitative and of marginal predictive validity as an exposure biomarker due to the method's low recoveries and the large unexplained variance.

Key words Benzo[*a*]pyrene · Polycyclic aromatic hydrocarbons · Biomarker · Reverse metabolism · Human exposure

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Introduction

Due to the widespread presence of polycyclic aromatic hydrocarbons (PAHs) in our environment and their health effects, a thorough understanding of the extent and dynamics of human exposure is warranted. Biomarkers can provide a powerful and practical tool in the risk-based assessment of exposure in that the measurement is biologically relevant, it provides direct and absolute evidence that an individual has been exposed, and it is integrated over time and routes. Such a measurement can enhance an assessment of exposure

by capturing host (e.g., physiology, gender, age, diet, activity) and contaminant (substrate, solubility, stability, particle size) influences on contact rate and absorption, thereby reducing uncertainties in the exposure continuum from source to effect. In some cases, biomarker measurements are more predictive of health effect and therefore an important parameter in estimating risk (Perera 1986; Fowle 1984; Ehrenberg 1988; Needleman and Gatsonis 1990). By itself, however, a biomarker measurement provides no information about the route or source of an exposure. This information is derived from measurements of the concentration of the contaminant in the media and the individual's contact with the media or reconstruction of the exposure from the biomarker using pharmacokinetic models.

The ability of a biomarker measurement to provide an integrated measurement is especially valuable for multimedia environmental contaminants such as PAHs, due to the economical, methodological, and practical difficulties of collecting and analyzing samples from multiple media. Research concerning biomarkers of exposure has been identified as central to our evolving understanding of human exposure to environmental contaminants (National Research Council 1989; Ott 1990; Lioy 1990).

The value of biomarker measurement in interpreting exposure is directly related to the reliability and validity of the measurement. The validity of a biomarker measurement takes on broad dimensions of complexity and importance due to the intricacies of sampling and analysis from dynamic biological matrices and processes. Schulte (1989) defines and describes a useful approach for considering these parameters in the context of the human exposure continuum.

As with much of the biomarker research, PAH research has evolved from occupational hygiene. These studies consider high exposure situations and are usually presumed to be single medium in nature (e.g., air). For PAHs such a presumption can lead to serious misclassification of exposure due to the prevalence of PAH contamination of food. Several investigators have examined the predictive relationship of urinary metabolites by reverse metabolism (BaP) and high-end exposure resulting from smoking or from occupational exposure (Becher and Bjorseth 1983; Venier et al. 1985; Haugen et al. 1986; Michels and Einbrodt 1979; Hutcheon et al. 1983). Results from these studies are equivocal in showing that the biomarker correctly classifies individuals by exposure group. It is the purpose of this study to evaluate this "occupational" biomarker within the context of total environmental exposure.

Material and methods

Experimental design

This study was conducted as a component of the Total Human Environmental Exposure Study (THEES). Various aspects of this multifaceted study have been reported elsewhere (Lioy et al. 1988, 1990; Waldman et al. 1990, 1991; Greenberg et al. 1990; Butler et al. 1993; Freeman et al. 1991). The potential dose estimates described herein are based on the personal air and duplicate plate concentrations reported by Waldman et al. (1991) and Greenberg et al. (1990). The dose values derived here differ from those of Waldman et al., however, in that individual factors (age, weight, sex, and activity level) were considered in estimating ventilation rate and dietary mass ingestion in an attempt to derive a more precise estimate of dose.

Biomarker measurements were obtained in two 14-day study periods occurring in the winter (January 8–22) and fall (September 16–30) of 1988. Personal air (24-h), "duplicate plate" dietary, and urine samples were collected for each individual.

Study participants were recruited in the town of Phillipsburg (population 16 500) in western New Jersey. As a purposeful study, sample collection was not statistically based (National Research Council 1991). Phillipsburg was of interest due to high ambient benzo[*a*]pyrene (BaP) levels (annual mean: 0.81 ng/m^3; highest daily: 7.8 ng/m^3) measured during a survey (1982) in which 27 sites across the state of New Jersey were sampled (Harkov and Greenberg 1985). The community is further described by Lioy et al. (1988) and Waldman et al. (1991).

Fourteen persons participated during these two periods from seven and eight residences, respectively. With ten persons volunteering for both study periods, a total of 24 different people (13 males and 11 females; median age 41 years, ranging from 21 to 70 years) participated in the biomarker study. Occupations varied among the study participants: five persons were retired and 16 individuals were employed outside the home. There were not any smokers nor was anyone employed in an occupation characterized by high PAH exposures such as a foundry or smokehouse.

Although PAHs constitute a large chemical class (organic hydrocarbons with $\geqslant$ two fused benzene rings) with constituents in gas and/or particulate phases, a single particle-bound analyte was selected as a surrogate due to logistical constraints relating to sampling and analytical methodologies and costs. BaP was selected because: (1) as a carcinogen and a ubiquitous environmental contaminant, human exposure is a public health concern; (2) BaP's occurrence, chemistry, metabolism, and health effects have been well characterized; (3) analytical and sampling methods exist for air, water, food, and urine.

Analytical methods

BaP in personal air

Filters were analyzed according to U.S. EPA methods employing thin-layer chromatographic (TLC) separation and plate scanning spectrofluorimetry (Swanson et al. 1978; Swanson and Walling 1981). Quality control for the air sample analysis was conducted using U.S. National Bureau of Standards SRM 1647 urban particulate certified 2.9 $\pm$ 0.5 μg BaP/g particulate. Analysis of this standard in triplicate showed the precision to be 20%. All samples were extracted and analyzed in duplicate by splitting the 25-mm sample filter. This duplicate analysis yielded a mean percent difference of 8% with 6.5% relative standard deviation. Recovery was determined to be (mean $\pm$ SD) 104.3% $\pm$ 14.7% from the analysis ($n = 9$) of spiked filters. The limit of detection (4 times noise) for the personal air filters was determined as 0.5 ng/filter. Of 15 field blank filters

analyzed, 14 were non detectable and one was reported as 0.09 ng/filter. All field samples were above the limit of detection.

BaP in food

Samples were analyzed according to methods developed by Howard et al. (1986) and Xia et al. (1985) and applied by Greenberg et al. (1990). The method essentially consists of the digestion of a 100-g sample homogenate in ethanol and KOH, liquid extraction using isooctane, and clean-up with a 30 g florisil column. BaP detection and quantification were conducted by TLC/fluorescence as described for the air samples. The limit of detection for this method was 5 ng/kg. Recovery was evaluated from spiked samples and was reported to be 84% $\pm$ 10% ($n = 7$). The method precision yielded 14% average deviation from the mean based on duplicate analyses ($n = 16$).

BaP metabolites in urine measured by "reverse metabolism"

Urinary BaP metabolites were analyzed by the "reverse metabolism" method as applied previously in human studies by Becher and Bjorseth (1983) and based on chemistry developed by Konieczny and Harvey (1979). This method involves the reduction of the oxidized BaP metabolites back to the parent compound (thus the name "reverse metabolism"), resulting in increased yields (for BaP a 13-fold increase compared with the method without the reduction step) and simplified analysis, i.e., a single analyte (Becher and Bjorseth 1983).

The primary components of the analytical procedure included: (1) sample preparation involving acid hydrolysis of conjugates; (2) sample clean-up and concentration with liquid and solid (C_{18} cartridge) extraction; (3) chemical reduction with HI; (4) separation with TLC; and (5) detection with TLC plate scanning spectrofluorometry. Due to its high sensitivity and specificity, the U.S. EPA TLC/fluorescence procedure (Swanson et al. 1978) replaced the gas chromatography (GC) technique used by Becher and Bjorseth (1983). This modification was designed to economically accommodate the large number of samples generated by this study.

A comprehensive description of the method was provided by Ouyang (1989), who reported the method detection limit to be 0.5 ng/100 ml for BaP. Precision and recovery results differed between the two sampling periods due to a modification in the analytical procedure (in the later study period red phosphorous was added during the reductive step) to improve recoveries. Precision (relative deviation from the mean) for each of the sampling periods was reported to be 30% and 14% ($n = 5$) while recoveries of 8% and 20%, respectively, were reported (Ouyang 1989). Recovery was determined using radiolabeled BaP metabolites (obtained from the urine of ^{3}H-BaP treated mice supplied by Professor Regina Santella, Columbia University), $n = 5$, and the analysis of a standard BaP solution. All results reported have been corrected for recovery.

Sampling

Personal air

The implementation of 24-hour community-based air sampling required that pumps designed for 8-h occupational sampling be redesigned for decreased noise levels and prolonged operation periods (Buckley et al. 1988). Integrated samples (24-h) were collected on glass fiber 25-mm filters for 14 consecutive days at a flow rate of 4 l/min with MS&TM PM-10 personal impactor.

Diet

Dietary sampling was conducted by the "duplicate plate" method, which required participants to set aside a sample representing a one-fourth portion of their meals and snacks. All food items were sampled except liquids (such as milk, coffee, and ice cream). These samples were placed in an aluminium container, sealed, frozen in the household freezer, and collected daily. Participants were asked to keep a daily diary of the food and beverages consumed, including approximate amounts and method of preparation. Meals from only one individual in each household were requested although two persons may have been participating. The diet for this designated person was assumed to apply to the second participant within the household. This assumption seemed reasonable since meals were shared by the family members.

Urine

The winter period sample collection protocol included the collection of all voids while at home. Participants were asked to record the time and date of each collected void and the time of the last void before the first collected sample (e.g., before leaving work in the afternoon) to determine the sample duration. Each void was collected separately and samples were stored frozen and later aggregated in the laboratory according to the 24-h sample period of the diet and personal air exposure samples. The urine samples were aggregated to lag the exposure samples by 2 h to compensate for the time from exposure to elimination.

To encourage more consistent collection, during the summer/fall collection period the sampling protocol was altered so that only the last and first voids of the day (including any intermediate nighttime voids) were collected except on weekends, when all samples while at home were requested. Urinary elimination of BaP was reported as ng/day. The ng BaP/day elimination was estimated from the day's partial measurement by assuming that the elimination rate over the measured portion of the day was the same as that over the remaining portion.

Potential dose

The inhaled and ingested potential doses are derived from personal exposure measurements (air: ng BaP/m^3; diet: ng BaP/kg) coupled with dose factors (i.e., volume of air breathed and amount of food ingested each day). These values do not account for bioavailability stemming from lung deposition or gastrointestinal absorption but rather represent intake (i.e., amount inhaled/ingested), which is the potential amount that could be absorbed if it were 100% bioavailable (Federal Register 1992).

Inhalation

The daily inhalation volume is coupled with personal air concentration to give the inhalation *potential* dose as shown in Eq. 1 (Federal Register 1992; Lioy 1990):

$$PD_{inh} = C_{PA} \times V, \quad (1)$$

where PD_{inh} = potential inhalation dose (ng/day), C_{PA} = 24-h personal air BaP concentration (ng/m^3); and V = daily ventilation volume (m^3/day).

The estimation of the daily ventilation volume was based on two sources of information: first, demographic data regarding ventilation rates (m^3/h) at various levels of activity (resting, light, moderate, and heavy) for adult females and males (U.S. EPA 1989); and second, the

level and duration of activity of the participants as determined from self-reported daily activity questionnaires. From these data, the daily inhalation volume was calculated as shown in Eq. 2.

$$V_i = \sum(R_{ij} \times t_{ij}), \tag{2}$$

where V_i = daily ventilation volume for person i (m^3/day); R_{ij} = rate of ventilation for person i at activity level j (m^3/h); and t_{ij} = time occupied by person i at activity level j (h).

Ingestion

The amount of food and beverages ingested along with the concentration of BaP in these dietary items determines the potential ingested dose.

$$PD_{ing} = C_F \times M_F \tag{3}$$

where PD_{ing} = daily potential ingested does (ng/day); C_F = concentration of BaP in 24-h composite duplicate plate (ng/kg); M_F = amount of food ingested in 24 h (kg/day)

The study was designed using the "duplicate plate" method of sample collection to estimate the amount of BaP ingested from proportional samples (25%) provided by the participants. However, when this method was used to estimate the dietary mass ingested by scaling the amount set aside (4 ×), the calculated amount was about one-half of the amount suggested by EPA reference values (U.S. EPA 1983). Furthermore, no significant correlations ($P \leq 0.10$) were detected when the "duplicate plate" amount was compared to variables expected to be predictive of consumption, i.e., sex, body weight, surface area, and activity (Buckley 1991). The susceptibility of the duplicate plate sample collection to biases has been reported by other investigators (Guthrie 1981; Smiciklas-Wright and Guthrie 1984). An additional limitation of the "duplicate plate" method in this study was that the dietary exposure of two individuals within a household was represented by a single person's sample (i.e., one dietary sample was used to represent the ingested exposure for the second individual within the household designated for personal sampling). Therefore, an alternate approach was developed to estimate the amount ingested.

This method, referred to here as the "mass kcal method", incorporated reference values for daily mass ingested scaled by individual factors of level of activity and body weight. The U.S. EPA (1983) provides an estimate of daily food ingested (excluding beverages) in g/day classifications of age and sex: 2590 and 2149 g/day for males, and 1777 and 1711 for females, in age range of 25–60 years and > 60 years, respectively (ICRP 1974). The reported individual activity level (Freeman et al. 1991) together with standard energy consumption rates (ICRP 1974) was used to estimate daily energy consumption (kcal/day) (note that a dietary "calorie" is equal to a thermochemical kilocalorie). Both the ICRP and the daily activity questionnaires employed identical categories of activity: resting, light, moderate, and heavy. The "resting" level was used to classify sleeping or napping. The kcal expended during this activity was determined as the basal metabolism (BM) by the multiple linear regression predictive models based on sex, age, height, and weight reported by ICRP (1974). The rate of energy expenditure for the nonresting levels of activity is given by ICRP (1974) for males and females, respectively: "light" 3.8 and 2.6 (e.g., office work, light housework); "moderate" 6.7 and 4.7 (e.g., light industry, heavy housework); and "heavy" 9.6 and 6.8 (e.g., commercial fishing, foundry work) (ICRP 1974). An estimate of the total energy expended in a day was given by summing the product of the rate of energy expended and duration for respective activities. The basal metabolism calculation which includes body weight as a parameter in its estimation was employed to account for the effect of body weight on energy expenditure. A proportioning factor consisting of the individual basal metabolism to that of a reference was employed to ultimately determine the total daily energy expenditure as shown in Eq. 4:

$$E_i = \frac{BM_i}{BM_{ref}} \sum (A_{ij} \times t_{ij}), \tag{4}$$

where E_i = total energy expended (kcal/day) for individual i; BM_i = basal metabolism for person i (kcal/day); BM_{ref} = basal metabolism for person i of reference weight (kcal/day); A_{ij} = rate of energy expenditure for person i at activity level j where j represents resting, light, moderate, and heavy levels of activity (kcal/h); and t_{ij} = duration of activity j for person i(h).

Equation 4 provides a person-specific estimate of the total daily energy expenditure based on age, sex, level of activity, and body weight. This value is used to provide a person-specific estimate of daily food mass ingested by proportioning of EPA's reference daily mass ingestion as shown in Eq. 5. The reference energy expenditure (kcal/day) is given by ICRP (1974) by age and sex:

$$M_i = M_{ref} \times \frac{E_i}{E_{ref}}, \tag{5}$$

where M_i = mass ingested for person i (g/day); M_{ref} = EPA reference mass ingested (g/day); E_i = energy expenditure for person i (kcal/day); and E_{ref} = reference energy expenditure (kcal/day).

Having estimated the amount ingested, the ingested potential dose is readily determined as shown in Eq. 3 as the product of the amount ingested and the measured BaP concentration for a sample.

Data analysis

The relationship between the potential dose (inhalation and diet) and the associated urinary BaP metabolite elimination was examined using multiple linear regression to compare the variation of urinary BaP elimination with dietary and inhalation exposure variables. Additional variables including body weight, body fat, and alcohol use were incorporated into the regression to investigate their contribution in explaining the variability in urinary BaP elimination. PC SAS (SAS Institute, Inc., Cary, N.C.) was used to perform the multiple linear regression procedures.

Correlation analysis and multiple linear regression were used to examine the relationship between the potential inhalation and ingestion doses and the urinary elimination values. Two sets of data were available. The first was for an individual – person I.D. (PID) 2011. For this individual, samples were analyzed on a daily basis over a 14-day period (summer/fall period). The individual's 14-day data set was unique since it contained a complete time series of *daily* BaP inhalation, ingestion, and urine elimination measurements which provided an analysis without interpersonal variability. PID 2011 was selected (post-sample collection) because of the reliability and regularity of the sample collection and the completeness of records. For this individual, the daily amount ingested was determined based on the actual sample mass (scaling the sample amount by a factor of 4 assuming that the participant set aside a one-quarter representative sample consistent with the sampling instructions). This individual's estimate of proportional mass ingested was believed to be reliable because of the conscientiousness with which she provided samples and because of her former career as a nutritionist.

The second set of data from the remaining subjects (both study periods) consisted of 7-day average values for both exposure and urinary elimination. The 7-day potential inhalation dose was determined as the arithmetic mean of the seven daily values. The ingestion dose was determined based on the analysis of the 7-day food composite and "mass kcal" amount ingested. The winter period urine elimination was similarly calculated as the arithmetic mean of seven daily values. Summer/fall period urine samples were analyzed as a 7-day composite and therefore represented an average weekly

elimination. All dietary samples were analyzed as a 7-day composite. A laboratory error in preparing one urine sample composite resulted in one 2-week composite rather than two 1-week composites so that a total of 55 person-weeks were available for analysis. In addition to the dose estimates, physical factors including body weight, surface area, age, and activity were examined for their influence on the BaP elimination in the urine.

Results

Potential dose estimates

The BaP inhaled dose was determined from personal air measurements for 14 and 18 persons over 14 days, giving a potential of 194 (12 days were sampled for one individual) and 252 person-days for the winter and summer/fall sampling periods, respectively. There was a total of 191 and 223 measurements resulting in a completion rate of 98% and 88%, respectively (samples collected < 9.5 h were rejected). The average personal air sampling duration was 22.5 and 20.6 h for each of the respective study periods. The distribution of BaP personal air concentrations is summarized in Table 1. The geometric mean and median are reported due to the skewed nature of the distributions (PC SAS gives positive skewness values of 2.8 and 2.3 and the Shapiro-Wilk Statistic "prob. < W" indicates a non-normal distribution).

The personal air concentration measurements were combined with estimated individual ventilation rates to give the potential inhalation dose (Eq. 1). The ventilation volume for the 20 individuals participating in both study periods varied from 10 m^3/day for an elderly retiree who was predominantly sedentary, to 38 m^3/day for a 43-year-old plant worker. The mean rate for both periods was 19 m^3/day. The mean and standard deviation for men and women was 22.5 (1.52) and 13.0 (1.42) m^3/day, respectively. Potential dose summary statistics are provided in Table 2.

BaP dietary ingestion was estimated for ten and nine individuals from eight households during the winter and summer/fall sampling periods, respectively. On average 14 meals/week (range of 3–21) were collected for each study participant (total of 521 meals). The average sample mass was 77 g/meal with a coefficient of variation of 44%. Combining the daily samples into 7-day composites resulted in a total of 38 samples available for analysis. The potential ingested BaP dose was calculated as the product of dietary concentration (ng/kg) and ingested food mass (kg/day) (Eq. 3). Dose estimations were expanded from the 38 persons providing samples to include the second individual within the household that was also participating in the personal air and urine sampling for a total of 56 persons (Table 3).

Table 1 Personal air BaP concentrations (ng/m^3)

Statistic	Study period		
	Winter	Winter[a]	Summer/fall
Geom. mean	0.66	0.63	0.11
Median	0.70	0.70	0.11
25% quartile	0.32	0.32	0.06
75% quartile	1.43	1.35	0.20
Range	0.05–101	0.05–5.67	0.01–1.07
Prob. < W	0.013		0.013
No. individuals	14	14	18
n[b]	191	189	223

[a] Results are determined excluding two extreme values (35 and 90 interquartile ranges from the median)
[b] Personal air samples with run times < 9.5 h were excluded

Table 2 Inhalation potential BaP dose (ng/day)

Statistic	Study period		
	Winter	Winter[a]	Summer/fall
Geom. mean	10.7	10.7	2.1
Median	11.0	11.3	2.3
25% quartile	4.8	4.9	1.1
75% quartile	25.1	23.7	4.0
Range	0.5–3200	0.5–126	0.1–21.5
Prob. < W	0.07[b]		0.01
No. individuals	14	14	18
n[c]	191	189	223

[a] Results are determined excluding two extreme values (35 and 90 interquartile ranges from the median)
[b] Test for normality is significant at the $p = 0.05$ level
[c] Personal air samples with run times < 9.5 h were excluded

Table 3 Dietary BaP concentration and potential ingested dose for the combined winter and summer/fall study periods

Statistic	Concentration (ng/kg)	Ingested dose (ng/day)
Minimum	5	9
25% quartile	28	63
Median	71	176
Geometric mean	74	179
75% quartile	146	415
Maximum	933	2195
n	38	56

Urinary BaP elimination

Urinary elimination was evaluated on a daily and weekly basis for the winter and summer/fall sampling periods, respectively. The winter sampling entailed the collection and analysis of daily urine voids for 14 persons over 15 days. Of 210 person-days potentially available, 197 were collected (94%). During the summer/fall sample collection period efficiency was less due to an altered sampling protocol which requested the

Table 4 BaP urinary concentration and elimination

	Winter[a]	Summer/fall[b]	PID 2011[a]
Concentraion (ng/l)			
Geom. mean	122	124	40
Median	125	121	50
25% quartile	52	79	26
75% quartile	150	205	68
Range	13–1996	34–339	10–93
n	197	27	14
Elimination rate (ng/day)			
Geom. mean	119	121	62
Median	121	129	73
25% quartile	55	71	40
75% quartile	261	257	100
Range	8–2212	26–327	15–225
n	197	27	14

[a] Reported values are for sample collection over a day's period
[b] Reported values are for a 7-day sample prepared by combining seven daily samples

last and first void of the day rather than all samples while at home. During the winter and summer/fall study periods, an average daily sample volume of 607 and 565 ml was collected over an average period of 15 and 12.4 h, respectively. Assuming a constant rate of output this elimination translates into a daily urine output of 971 and 1094 ml respectively. This output is within the range of reference values: 500–2000 ml (Diem and Lentner 1970) and 500–2900 ml (ICR 1974). The sample collection characteristics for PID 2011 did not differ greatly from these mean values, with an average urine collection period of 13 h/day and an average daily sample volume of 780 ml. The urinary BaP concentrations and rates of elimination are summarized in Table 4.

Comparison of potential dose with urinary metabolite elimination

Pearson and Spearman correlation and multiple linear regression analyses were used to identify the amount of variability in urinary BaP metabolite elimination that could be explained by the estimated potential doses (inhalation and ingestion), physiological variables (body weight, body fat, surface area), and alcohol use. This analysis was conducted for the *daily* data of a single individual, PID 2011 ($n = 14$), and for the average *weekly* data for the remainder of the study participants ($n = 55$).

The correlation analysis for the daily PID 2011 data was significant ($r_{sp} = 0.64$, $p = 0.035$) for the ingested potential dose variable based on rank when a 1-day lag was assumed in the urinary elimination of a day's dietary dose.

Seven-day average values were available for the remainder of the subjects. For this data set, the change (Δ; the difference from week 1 to week 2) in the potential dose was compared to the corresponding change in BaP metabolite elimination so that the effect of an increase/decrease in the potential dose could be directly related to the increase/decrease in metabolite elimination ($n = 27$). The changes (Δ) in inhalation and ingestion dose variables are plotted against the changes (Δ) in urine elimination in Fig. 1 and are characterized by considerable scatter. However, the plots suggest some trend in that the data are clustered in the II and III quadrants where a ± change in the dose corresponded to ± change in urine elimination.

The correlation of Δurine with Δinhalation was not significant ($p \geqslant 0.10$). A plot of these data shows an extreme value for inhaled dose for PID 1102. This value is attributed to recreational welding, which occurred on 2 days during week 2 for this individual. Even with this value removed, the correlation analysis remained non-significant ($p \geqslant 0.10$).

A scatter plot of Δ ingested dose versus Δurine similarly shows a data point (labeled PID 1042) that deviates from the points clustering at the axis intersection and the II/III quadrants. For this individual, an unexplained large decrease in the dietary dose (− 1096 ng/day) resulted in an increase in urine elimination (+ 661 ng/day), contrary to what would be

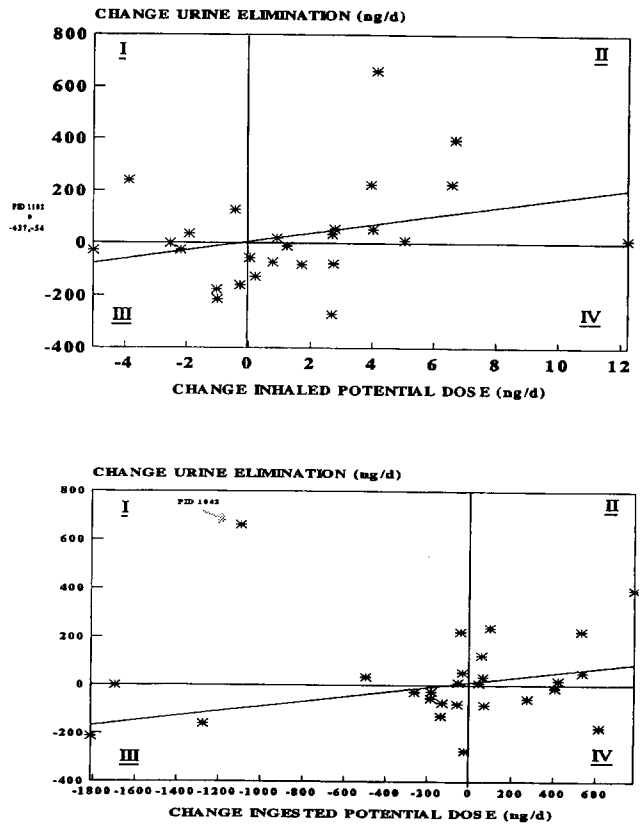

Fig. 1 Comparison of change in urinary BaP elimination with (**a**) change in inhaled dose and (**b**) change in ingested dose

Table 5 Correlation between change in BaP dose and change in urinary elimination

	Variables Regressed on Δurine			Data Excluded[a]
	ΔInhalation	ΔIngestion	Body wt.	
r	0.287	0.402	0.297	1102 and 1041
p	0.165	0.047	0.149	($n = 25$)
r	0.308	0.109	0.429	1102
p	0.126	0.598	0.029	($n = 26$)
r	0.089	0.402	0.297	1041
p	0.664	0.042	0.141	($n = 26$)
r	0.226	0.097	0.428	$n = 27$
p	0.911	0.631	0.026	

[a] Person I.D. (PID) whose data values were excluded from the analysis.

expected. There is no explanation for this "extreme" data point. If the value is removed from the data set, a significant correlation is observed ($r = 0.402$, $p = 0.042$, $n = 26$).

A correlation analysis was also conducted between Δurine and other individual factors such as body weight, surface area, body fat, and alcohol consumption. Of these variables, only body weight was significantly correlated with Δurine ($r = 0.428$, $p = 0.026$, $n = 27$) (Table 5).

The independent variables, Δdietary and Δinhalation doses, and body weight (excluding values for PID 1102 and 1041 discussed above) were examined in a multiple regression model:

$$\Delta U = b_0 + b_1(\Delta D) + b_2(\Delta I) + b_3(\mathrm{BW}), \quad (6)$$

where ΔU = change in weekly average rate of urinary BaP metabolite elimination from week 1 to 2 (ng/day); $b_{0,1,2,3}$ = regression coefficients; ΔD = change in weekly average BaP dietary dose from week 1 to week 2 (ng/day); ΔI = change in weekly average BaP dose due to inhalation from week 1 to week 2 (ng/day); and BW = body weight (lbs).

The multiple linear regression model obtained using these three variables was significant ($r^2 = 0.38$, $p = 0.015$, $n = 25$) and gave the following equation:

$$\Delta U = -235 + 0.11(\Delta D) + 7.2(\Delta I) + 1.3(\mathrm{BW})$$

Coefficient			
Stand. error:	0.04	7.4	0.53
p value:	0.02	0.34	0.02

The Δingested dose and body weight coefficients are statistically significant ($p < 0.05$) with relatively small standard errors. In contrast, the inhaled dose coefficient has a large standard error and is not significant. These results indicate that Δingested dose and body weight are primarily responsible for explaining the variability in urinary elimination. The influence of diet relative to inhalation is qualitatively consistent with the magnitude of the respective exposure measurements, i.e., diet accounted for > 85% of the total dose. No significant correlations ($p \geq 0.10$) were found among the three independent variables, indicating the absence of collinearity among the regressors.

Discussion

The current study investigated the amount of variability in urinary BaP elimination, as measured by "reverse metabolism", that could be explained by inhalation and ingestion potential dose and physical/activity variables. Such an investigation provides an assessment of the predictive validity of the biomarker for exposure. Since individual variability between the variables formed the basis of this analysis and no additional individual variance was gained by considering the adsorbed dose, potential dose was the appropriate estimate for this analysis. The analysis was not extended to mass balance considerations due to the low recoveries of the reverse metabolism method and unquantified fecal elimination.

Sampling was conducted to optimize the predictive relationship between the exposure and biomarker variables by (1) measuring both inhalation and ingestion exposure pathways; (2) the collection of long-term integrated urine samples (representing on average 1/2 of a day's total void); and (3) the quantification of metabolite elimination as a rate rather than creatinine corrected, thereby eliminating the added inter- and intrapersonal variability in creatinine elimination (Jackson 1966; Knuiman et al. 1986; Alessio et al. 1985).

In spite of these efforts, a large portion of the variability in urinary BaP elimination remained unexplained, as was evidenced by multiple linear regression analysis. This was true for two different data sets. The first data set included variability between individuals while the second consisted of the daily variation for a single individual. It was anticipated that stronger correlations would be achieved for the *intra*personal data set compared with the *inter*personal data set due to the added *inter*personal variability introduced by the latter. In fact, based on rank, a significant correlation ($r_{sp} = 0.64$, $p = 0.035$) was observed for the *intra*personal data set when a 1-day lag in BaP elimination was assumed. A 1-day lag in the appearance of urinary metabolites for the *intra*personal data set would have been possible under the conditions of the sampling protocols (dietary and urinary) if the dietary BaP consistently appeared in the breakfast and lunch meals and if an approximate biological $t_{1/2}$ of 4.4 h is assumed as indicated for 1-hydroxypyrene (Buckley and Lioy 1992).

For the *inter*personal data set, when the change in BaP metabolite elimination was regressed on the

change in the potential dose, although not statistically significant ($p = 0.10$), plots of the data suggested an appropriate trend. This model was significant ($r^2 = 0.38$; $p = 0.015$) when the changes in exposure and BaP elimination variables were considered and when two extreme values were excluded. Since there is no a priori justification for exclusion of these data, the statistical significance of the model is considered conditional. Additional research is required to more fully understand the sources of variability in the BaP elimination.

The results reported here are consistent with earlier studies that have shown that urinary BaP (by reverse metabolism) provides a poor classification variable in distinguishing between groups differentially exposed, e.g., exposed workers versus controls or smokers versus nonsmokers (Becher and Bjorseth 1983; Venier et al. 1985; Haugen et al. 1986; Michels and Einbrodt 1979). Haugen et al. (1986) reported no statistically significant differences between smokers and nonsmokers. Similarly, Venier et al. (1985) reported no statistically significant difference in urinary BaP or PAH elimination between exposed workers and controls (smoking and nonsmoking). Becher and Bjorseth (1983) did show a significant difference between non-occupationally exposed smokers and non-smokers although the difference for the occupational groups (exposed versus controls) was dampened. In these studies, only the inhalation route of exposure was considered in the classification of exposure. Such an approach is likely to result in misclassification of exposure due to the confounding influence of dietary PAH exposure, which can be of the same order of magnitude as smoking or occupational inhalation exposure, as reported here and in other corroborating studies (Lioy et al. 1988; Buckley and Lioy 1992; Santodonato et al. 1981; Greenberg et al. 1990; Howard and Fazio 1980; Masuda and Kuratsune 1971; Osborne and Crosby 1987; Lintas and DeMatthaeis 1979). However, since the current study accounted for the dietary exposure and a large fraction of the unexplained variability remained, this suggests that other important unidentified variables may be influencing the variability in the biomarker.

The median concentrations of BaP in urine (corrected for recovery) for the current study (winter 125 ng/l, summer/fall 121 ng/l) are generally consistent with results from previous studies employing the reverse metabolism procedure for comparable populations. Becher and Bjorseth (1984) reported a mean of 125 ng/l among non smoking controls (mean of four individuals). Venier et al. (1985) reported 0.03 μg/g creatinine (uncorrected for analytical recovery) for a non smoking control group ($n = 5$). This value translates to 54 ng/l, assuming a standard creatinine concentration of 1.8 g/l (Diem 1970). Grimmer et al. (1991) reported metabolite levels of 10 ng/l for the 3- and 9-hydroxylated BaP metabolites among eight control subjects (for occupational exposure). This order of magnitude consistency indicates general comparability between this and other studies where the reverse metabolism method of analysis has been employed.

Although the reverse metabolism method is advantageous in enhancing sensitivity and has been applied by several investigators, there are uncertainties associated with its conversion efficiency for specific metabolites. There have been a number of recent studies showing promising results for alternate methods of quantifying urinary PAH biomarkers (Ouyang et al. 1994; Weston et al. 1994). Recent studies have shown that the pyrene metabolite, 1-hydroxypyrene, may be a more promising biomarker of exposure since it has demonstrated predictive results, favorable sensitivity and recovery, and since there is a relative abundance of pyrene among environmental PAHs (Buckley and Lioy 1992; Tolos et al. 1990; Jongeneelen et al. 1988).

The measured air exposure levels giving *median* inhalation potential dose estimates of 2.3 and 11.0 ng/day (summer/fall and winter studies respectively) are consistent with previous studies. A year before the current study, Lioy et al. (1988) reported a mean of 20 ng/day for the winter season. Santodonato et al. (1981) estimated 9.4–43.5 ng/day based on literature data. Using the same personal air concentrations as the current study, Waldman et al. (1991) reported *mean* applied dose levels of 3.6 and 21 ng/day for the respective seasons. The results from this study differ from those reported by Waldman et al. due to differing summary statistics and the greater rigor in dose estimation required by this investigation for comparisons to body burden (e.g., Waldman et al. assumed a standard ventilation rate of 20 m^3/day).

Ingested BaP accounted for a larger and more variable source of exposure compared with inhalation. Potential dose estimates of 9–2195 ng/day, with a median value of 179 ng/day are comparable to duplicate diet results reported by Vaessen et al. (1986): 30 ng/day (range ND–35); Dennis et al. (1983) 250 ng/day; market basket results reported by De Vos et al. (1990): 120–290 ng/day; and estimates by Santodonato et al. (1981): 160–1600 ng/day. In contrast to these estimates in the 30–300 ng/day range, Hattemer-Frey and Travis (1991) estimate the long-term average daily potential dose of 2160 ng/day based on a Fugacity Food Chain model. The relatively high estimate for this latter study may stem from overestimation of the model's bioconcentration or biotransfer factors and limitations in the model's ability to account for food contamination during preparation.

The importance of dietary monitoring in classifying exposure to PAHs is substantiated by an assessment of the relative contribution of the exposure pathways. During the fall study period, ingestion exceeded inhalation for all person-weeks by a factor of 122 (median), ranging from 8–872, whereas during the winter period, due to higher BaP air levels, the median factor was only 6 (range: 0.6–189). These results indicate that

environmental or occupational epidemiological or exposure/biomarker studies that fail to consider dietary PAH exposure are likely to result in serious misclassification of exposure. In addition to food and air, there is emerging evidence to suggest that house dust may be an important medium to consider in evaluating exposure (Chuang et al. 1993).

Conclusions

Urinary BaP (metabolites by reverse metabolism) was found to have only marginal predictive validity for exposure as shown by the large fraction of its variability that was unexplained by exposure. The considerable unexplained variability may be due to factors not considered in this investigation or due to sampling and/or analytical errors. Additional research is required to reduce these sources of uncertainty and to identify additional factors that might explain the BaP elimination variability. A recommended approach for further investigation includes small-scale, controlled measurement intensive studies that afford accurate control or measurement of the exposure, dosimetry, and elimination variables (including feces) so that the kinetic, mass balance, and causal relationship between variables can be more fully explored and defined. Until this is done, urinary BaP by the reverse metabolism method has little practical utility as an exposure biomarker.

The concept of the "reverse metabolism" is advantageous in that sensitivity is enhanced and results are independent of metabolite profile. However, because analytical recoveries are low and not metabolite specific, this technique is more appropriately considered qualitative rather than quantitative. Further investigations of the chemistry and recoveries of this method are required to more fully understand its potential in evaluating human exposure to PAHs.

Diet is the dominant source for environmental exposure to BaP as shown by both the biomarker and the exposure assessment results. Epidemiology, exposure, or biomarker research (environmental or occupational) that fails to consider the dietary pathway of exposure is likely to result in serious misclassification of exposure.

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