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CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) IN AIRBORNE PARTICLES AND ASSESSMENT OF HUMAN EXPOSURE TO PAHS

A Dissertation
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CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) IN AIRBORNE PARTICLES AND ASSESSMENT OF HUMAN EXPOSURE TO PAHS

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To my husband, son and family

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My utmost appreciation goes to my family. I want to dedicate this to my wonderful husband Jon for his unconditional love, patience and for being my dearest friend. I would never be able to accomplish this without his support! I want to thank my son Kaidon – the best baby in the world who always makes me smile. Last but not least, I thank my dad, mom, sister and the rest of my family in China.

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LIST OF SYMBOLS AND ABBREVIATIONS

PAH:	Polycyclic aromatic hydrocarbon
Me-PAH:	Methylated polycyclic aromatic hydrocarbon
OH-PAH:	Monohydroxy Polycyclic aromatic hydrocarbon
1-PYR:	1-Hydroxypyrene
BAP:	Benzo[a]pyrene
PM:	Particulate matter
ASACA:	Assessment of Spatial Aerosol Composition in Atlanta
ASE:	Accelerated solvent extraction
GC/MS:	Gas chromatography/mass spectrometry
GC/HRMS:	Gas chromatography/high resolution mass spectrometry
LOD:	Limit of detection
CV:	Coefficient of variation

SUMMARY

Polycyclic aromatic hydrocarbons (PAH), a group of carcinogenic and mutagenic pollutants, are byproducts of incomplete combustions of organic materials such as wood, fossil fuels, meat, and tobacco, and are therefore distributed ubiquitously in the ambient air, soil and water environments. PAH in the atmosphere, especially in densely populated areas, is significantly affected by anthropogenic emissions, such as automobile exhaust, fossil fuel combustion, biomass burning, cigarette smoking, and industrial activities. Particulate matter (PM) air pollution, especially fine particles with aerodynamic diameters less than 2.5 μm ($PM_{2.5}$), has been linked to increased morbidity and mortality rates, as well as various adverse health effects such as respiratory and cardiovascular diseases. The association of $PM_{2.5}$ with toxic compounds such as PAHs could further increase the health effects. Human exposure to PAHs can occur through three routes, i.e. inhalation, ingestion and dermal absorption. For the general population, the main exposure routes are inhalation of polluted air or cigarette smoke and ingestion of food containing PAHs.

Atlanta is a rapidly growing city with high PM air pollutant. However, little is known on PAHs levels, especially $PM_{2.5}$-bound PAHs concentrations in this area. To characterize $PM_{2.5}$-bound PAHs in Atlanta, a method was developed to provide sensitive and reliable measurements of 28 PAHs and methyl-PAHs (Me-PAH) in archived daily $PM_{2.5}$ samples collected with low flow rate (16 L/min or 24 m^3 air/sample). Then $PM_{2.5}$ samples taken at three sampling sites (urban, suburban-highway and rural) in the metropolitan Atlanta area during 2003-2004 were analyzed and the levels, seasonal and spatial variation of PAHs were studied. Correlation analyses between PAHs and other air pollutants such as $PM_{2.5}$, OC, EC and potassium ion (K^+) were conducted and the results indicated that PAHs had common sources as other pollutants, but with a distinct seasonal

effect. Retene, a proposed biomass burning tracer, captured both the high leaves-grasses-bushes-branches burning season and the high wood burning months, suggesting that it might be a more general indicator of biomass burning than potassium, which is more specific to wood burning.

Assessment of human exposure to environmental chemicals such as PAHs can be accomplished either through environmental monitoring in which concentrations of PAHs in environmental samples (air, food, water, soil, etc.) are determined, or through biomonitoring in which internal levels of PAHs in human body (e.g. urinary hydroxy PAHs, or OH-PAHs) are measured as indicators for assessing overall exposure to PAHs. A lot of studies have been reported since the early 1990's to assess high occupational exposure to PAHs; however, limited information is available on non-occupational exposure of the general populations to PAHs in the environment. Even less information is available on the temporal, intra- and inter-subject variability of these biomarkers. A method was developed to measure hydroxyl PAH metabolites (OH-PAH) in urine samples. A study was carried out to study the variability of the urinary biomarker levels in a non-occupationally exposed non-smoking reference group. Levels of urinary PAH metabolites varied widely both within-subject and between-subjects and the within-day variance far exceeded the between-day variance. There were also considerable temporal correlations for these biomarkers. Sample size calculations were conducted and taking 24-hour voids would require the least number of subjects, which can be used for future epidemiological study design. Finally, a study was conducted to evaluate exposure to PAHs in an urban setting among a group of non-occupational exposed non-smokers employing both personal air sampling and urine biomonitoring. PAH levels varied largely in air samples taken at home, at work, and while driving or jogging. Monitoring urinary OH-PAH levels can capture both inhalation and dietary exposures. Total inhaled PAH was correlated with total excreted OH-PAHs, suggesting that by combining personal air

sampling and biomonitoring, exposure to environmental PAHs can be well characterized even for low-level exposure.

CHAPTER 1

INTRODUCTION

Particulate matter (PM) air pollution has been linked to increased morbidity and mortality rates, as well as various adverse health effects such as respiratory and cardiovascular diseases (Cakmak et al., 2007; Kappos et al., 2004; Lebowitz, 1996; Ostro et al., 2007; Pope, III et al., 2004). Particulate matter with aerodynamic diameters less than 2.5 μm ($PM_{2.5}$ or fine particulate matter) are of special concern because, when inhaled, they can penetrate effectively into the respiratory system and deposit deeply in the bronchioles and alveoli of the lungs. The association of fine particles with mutagenic and carcinogenic chemicals — such as polycyclic aromatic hydrocarbons (PAHs) — may contribute to these acute health effects or potentially result in long-term health risks.

Polycyclic aromatic hydrocarbons (PAHs) are a group of toxic pollutants formed during incomplete combustion of organic materials such as wood, fossil fuels, meat, and tobacco. They are found in automobile exhaust, wood smoke, cigarette smoke, and also found at high concentrations in certain food such as barbequed food (ATSDR, 1995; Bostrom et al., 2002; Guillen et al., 1997; IARC, 1983); thus this class of compounds is ubiquitously distributed in ambient air, food, soil, as well as in many occupational environments.

PAHs have generated considerable interest, not only because of their wide distribution in the environment, but also their carcinogenic and mutagenic potential (ATSDR, 1995; IARC, 1983). PAHs, such as benzo[a]pyrene (BAP), which is present in cigarette smoke and certain occupational environments, have been positively associated with lung cancer (Armstrong et al., 2004; Denissenko et al., 1996). In addition, PAHs have also been reported to have reproductive, developmental, hemato-, cardio-, neuro-, and immuno-toxicities in humans and laboratory animals (ATSDR, 1995; IARC, 1983).

Given their carcinogenic and mutagenic potential and their wide distribution resulting in exposure in the general population, PAHs have generated considerable interest; 16 PAHs have been included in U.S. EPA's list of 188 hazardous air pollutants (U.S.EPA, 1990).

In ambient air, PAHs are found in both gaseous and particle phases. PAHs with 2-3 aromatic rings exist almost exclusively in the gaseous phase, while compounds with 4 or more aromatic rings are primarily associated with the particulate fraction (Eiguren-Fernandez et al., 2004; Li et al., 2006; Re-Poppi and Santiago-Silva, 2005). In addition, it has been reported that PAH-associated particles exist predominantly as fine particles, i.e., in the $PM_{2.5}$ size range, especially in areas with heavy traffic (Cancio et al., 2004; Duan et al., 2005; Ohura et al., 2004). Because the carcinogenic PAHs (e.g. benzo[a]pyrene and benz[a]anthrene) are mostly associated with the particulate matter, many studies on PAHs in ambient air have been focused on PAHs bound to PM, particularly PM2.5 (Borlakoglu et al., 1993; Bourotte et al., 2005; Ohura et al., 2004; Papageorgopoulou et al., 1999; Sanderson et al., 2004; Sklorz et al., 2007).

Human exposure to PAHs can occur through inhalation of polluted air or cigarette smoke, ingestion of food containing PAHs, as well as dermal absorption from soil or material that contains PAHs. For the general population, ingestion and inhalation are the two major routes of exposure (ATSDR, 1995; Bostrom et al., 2002; Ramesh et al., 2004). For certain occupations, such as coal tar roofers and coke oven workers, dermal absorption can become the primary route of PAH exposure (ATSDR, 1995). After entering the human body, PAHs undergo a series of biotransformation processes. In Phase I metabolism, PAHs are oxidized by the cytochrome P450 enzymes to form reactive epoxide intermediates, followed by reduction or hydrolysis to hydroxylated derivatives (OH-PAHs). In Phase II metabolism, the OH-PAHs are conjugated to glucuronic acid and/or sulfate to increase the water solubility of the metabolite and to facilitate elimination through urine, bile, or feces (Grover, 1986; Pelkonen and Nebert, 1982). Urinary OH-PAHs have been used as biomarkers to assess human exposure to

PAHs, with 1-hydroxypyrene (1-PYR) as the most commonly used indicator in biomonitoring studies (Jacob and Seidel, 2002).

Assessment of human exposure to PAHs in the ambient air can be accomplished either through environmental monitoring in which concentrations of PAHs in air samples are used to assess human intakes, or through biomonitoring in which internal levels of PAHs in human body (e.g. urinary OH-PAHs) are measured as indicators for assessing overall exposure to PAHs. The objectives of this dissertation were, (i) to develop a method measuring PAHs in ambient PM samples; (ii) to characterize $PM_{2.5}$-bound PAHs in Atlanta; (iii) to develop a method measuring OH-PAHs in urine samples; (iv) to study variability of urinary OH-PAHs in a reference population; and (v) to assess non-occupational exposure to PAHs in ambient air through both personal air sampling and biomonitoring. .

Structure and Scopes of the Thesis

Chapter 2. Determination of Twenty-eight Polycyclic Aromatic Hydrocarbons in Air Particulate Matter using Direct Elution and Isotope Dilution GC/MS

Chapter 2 introduces a newly developed method for measuring 28 PAHs and their methylated derivatives (Me-PAHs) in PM samples using a novel direct elution extraction method followed by isotope dilution gas chromatography/ high resolution mass spectrometry (GC/HRMS).

Chapter 3. Characterization of $PM_{2.5}$-bound PAHs in Atlanta

Chapter 3 describes two studies characterizing $PM_{2.5}$-bound PAHs at three sampling sites (urban, suburban-highway and rural) in Atlanta, i.e. a pilot study reporting PAHs from $PM_{2.5}$ samples taken during December 2003 and June 2004 and a year-around study on samples taken in the whole year of 2004. In this chapter, discussions were

carried out on the concentrations, seasonal and site variations, specific source markers, and correlations of PAHs to other air pollutants.

Chapter 4. Measurement of Urinary Mono-Hydroxy Polycyclic Aromatic Hydrocarbons Using Automated Liquid-Liquid Extraction and Gas Chromatography/Isotope Dilution High Resolution Mass Spectrometry

Chapter 4 described the development of a method measuring hydroxylated PAH (OH-PAHs) metabolites in human urine samples using automated liquid-liquid extraction and GC/HRMS determination. Urinary OH-PAHs have been used as biomarkers for PAH exposure and this method has been used in biomonitoring studies assessing human exposure to PAHs in the environment.

Chapter 5. Variability of Urinary Polycyclic Aromatic Hydrocarbon Metabolite Levels in Adults

Chapter 5 studied variability of urinary OH-PAH metabolites in a group of non-occupationally exposed adult over a period of a week. Effect of creatinine adjustment was evaluated. Sample size requirements were calculated to reflect three sampling methods: spot urine, first morning void and 24-hour void sampling. Results from this variability study can be applied to the design of future epidemiological studies.

Chapter 6. Assessment of Non-occupational Exposure to Polycyclic Aromatic Hydrocarbons through Personal Air Sampling and Urinary Biomonitoring

Chapter 6 described a study aimed at assessing inhalation exposure to ambient PAHs in a group of non-smoking adults who were not non-occupationally exposed. Eight subjects collected personal air samples and all urinary excretions over 8 days, while following a recommended dietary plan. Exposure assessments were carried out using both information from personal air samples and urinary biomonitoring.

Chapter 7. Conclusion

Chapter 7 provides conclusions of the dissertation and gives recommendations on the future directions in this field.

CHAPTER 2

DETERMINATION OF TWENTY-EIGHT POLYCYCLIC AROMATIC HYDROCARBONS IN AIR PARTICULATE MATTER USING DIRECT ELUTION AND ISOTOPE DILUTION GAS CHROMATOGRAPHY/MASS SPECTROMETRY

(Li Z, Porter EN, Romanoff LC, Trinidad DA, Mulholland JA and Sjodin A. *International Journal of Environmental Analytical Chemistry*, submitted)

Abstract

We are reporting a method for measuring 28 polycyclic aromatic hydrocarbons (PAH) and their methylated derivatives (Me-PAHs) in air particulate matter (PM) samples using isotope dilution gas chromatography/high resolution mass spectrometry (GC/HRMS). In this method, PM samples collected on quartz filters were spiked with internal standards, loaded into solid phase extraction cartridges, and eluted by dichloromethane. The extracts were concentrated under a gentle stream of nitrogen, spiked with a recovery standard, and analyzed by GC/HRMS at 10,000 resolution. Sixteen ^{13}C-labeled PAHs and 2 deuterated Me-PAHs were used as internal standards to account for instrument variability and losses during sample preparation. Recoveries of labeled internal standards were in the range of 95-129% which was comparable to the commonly used sonication-assisted extraction and higher than accelerated solvent extraction (ASE). Furthermore, the proposed method is less time-consuming than the comparative methods and eliminates the need of a filtration step required after the sonication extraction method. Limits of detection ranged from 41 to 366 pg/sample for the 28 analytes. This method was used to analyze reference materials from the National

Institute of Standards and Technology (NIST). The results were consistent with those from ASE and sonication extraction, and were also in good agreement with the inter-laboratory comparison results. The proposed method was then used to measure PAHs on $PM_{2.5}$ samples collected at three sites (urban, suburban and rural) in Atlanta, USA, and the results were consistent with an earlier study measuring $PM_{2.5}$ samples using ASE.

2.1 Introduction

Particulate matter (PM) air pollution has been linked to increased morbidity and mortality rates, as well as adverse health effects such as respiratory and cardiovascular diseases (Ostro et al. 2007; Pope, III et al. 2004). Particles with aerodynamic diameters less than 2.5 μm ($PM_{2.5}$) are of special concern because, when inhaled, such particles can penetrate and deposit deep in the bronchioles and alveoli of the lungs. Further, the association of fine particles with mutagenic and carcinogenic chemicals — such as polycyclic aromatic hydrocarbons (PAHs) — may increase the health effects of such particulates.

PAHs are a group of carcinogenic and mutagenic pollutants formed during incomplete combustion of organic materials and are ubiquitously present in the ambient air, soil and water (ATSDR 1995; IARC 1983). PAHs, exemplified by benzo[a]pyrene, have been shown to be causative agents of lung, esophageal, gastric, colorectal, bladder, skin, prostate and cervical cancers in humans and animal models (ATSDR 1995). The general population is primarily exposed to PAHs through inhalation of polluted air or cigarette smoke and through ingestion of PAH-containing foods (Bostrom et al. 2002; Ramesh et al. 2004). PAHs have generated considerable interest due to their carcinogenic and mutagenic potential and the fact that humans are exposed to PAHs on a daily basis. This is exemplified by the inclusion of 16 PAHs (Figure 2.1) on the U.S. EPA's list of 188 hazardous air pollutants (U.S.EPA 1990).

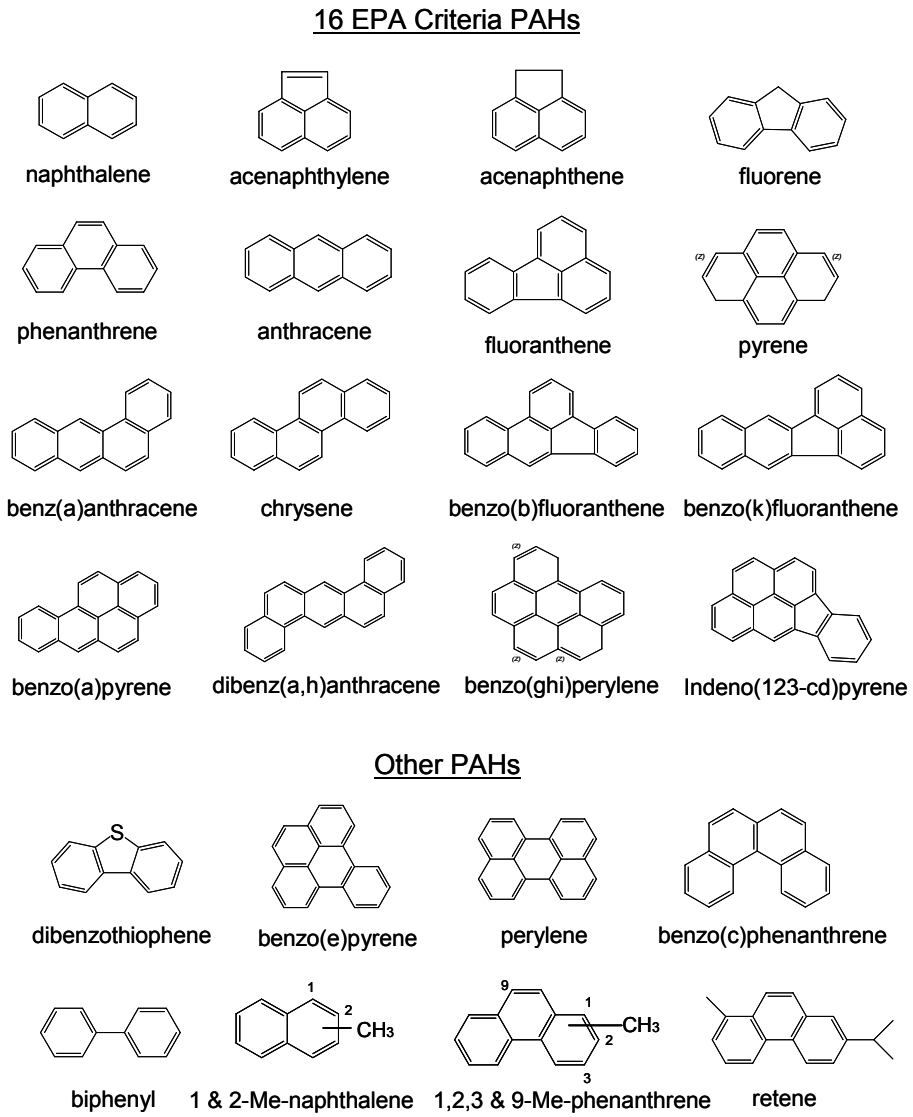

Figure 2.1. Molecular structures of PAHs measured in this method

In ambient air, PAHs with 2-3 aromatic rings exist almost exclusively in the gaseous phase, while compounds with 4 or more aromatic rings are primarily associated with the particulate fraction (Eiguren-Fernandez et al. 2004; Re-Poppi and Santiago-Silva 2005). In addition, it has been reported that particulate PAHs bound predominantly to fine particles, especially in areas with heavy traffic (Cancio et al. 2004; Duan et al. 2005). Because carcinogenic PAHs (e.g. benzo[a]pyrene and benz[a]anthrene) are mostly associated with particulate matter, many studies on PAHs in ambient air have focused on

PAHs bound to PM, particularly $PM_{2.5}$ (Ohura et al. 2004; Bourotte et al. 2005; Sanderson et al. 2004).

Typically, PAHs in ambient air are collected using high-volume samplers (U.S.EPA 1999), and the sample preparation involves extraction by either ultrasonic (Christensen et al. 2005) or Soxhlet extraction (U.S.EPA 1999; U.S.EPA 1995), both of which are laborious and time-consuming methods requiring large amounts of solvent. Other methods have been reported for extraction of PAHs from solid matrices, including microwave-assisted extraction (Pino et al. 2000), agitation (Pleil et al. 2004), supercritical fluid extraction (Chester et al. 1998), and Accelerated Solution Extraction (ASE) (Lintelmann et al. 2005; Burkhardt et al. 2005; Olivella 2005). Specifically, ASE has been reported to give higher extraction efficiency than sonication (Berset and Holzer 1999) and has been used in the analysis of PM and soil samples (Hollender et al. 2003). However, the instrument requires relatively high investment and maintenance costs, and the assembly and disassembly of sample extraction cells can be time-consuming.

We have developed a method for measuring 28 PAHs and Methyl-PAHs (Me-PAH) in ambient PM filter samples using a novel direct elution extraction method followed by isotope dilution gas chromatography/high resolution mass spectrometry (GC/HRMS) analysis. The proposed method requires a lower volume of solvent (10 mL) compared to reference methods (sonication 15 mL; ASE 30 mL), uses inexpensive consumables, and allows high sample throughput. The measured recovery is comparable or higher than commonly used sonication and ASE methods. The accuracy of the direct elution method was assessed by analyzing Reference Materials (RM 8785, Air particulate matter on filter media) from the National Institute of Standards and Technology (NIST). The developed method was then used to measure PAHs on $PM_{2.5}$ samples collected at three sites in the metropolitan Atlanta area.

Table 2.1. List of PAH analytes, abbreviations, isotopically labeled internal standards (IS) and quality control parameters

No[1]	Analyte	Abbr.	Internal Standard	LOD[2] (pg per sample)	CV[3] (%)	Concentration in RM 8785 (μg/g)			
						Direct elution	ASE	Sonication	NIST-ASE[4]
1	naphthalene	NAP	$^{13}C_6$ NAP	216	14	1418	4744	<LOD	1422
2	2-methylnaphthalene	2M-NAP	D_{10} 2M-NAP	213	6.3	920	1231	914	N/A[5]
3	1-methylnaphthalene	1M-NAP	D_{10} 1M-NAP	204	8.7	545	808	503	N/A
4	biphenyl	BIPH	$^{13}C_6$ ANYL	136	N/A	<LOD	<LOD	<LOD	N/A
5	acenaphthylene	ACNYL	$^{13}C_6$ ANYL	41	3.8	145	666	217	N/A
6	acenaphthene	ACNAP	$^{13}C_6$ ANAP	50	3.4	<LOD	<LOD	<LOD	N/A
7	fluorene	FLUO	$^{13}C_6$ FLUO	169	6.3	191	686	<LOD	236
8	dibenzothiophene	DBT	$^{13}C_6$ PHEN	41	5.3	437	3728	314	N/A
9	phenanthrene	PHEN	$^{13}C_6$ PHEN	366	6.2	1780	4282	2073	4632
10	anthracene	ANTH	$^{13}C_6$ ANTH	69	6.1	281	867	374	457
11	3-methylphenanthrene	3M-PHEN	$^{13}C_6$ PHEN	96	9.8	705	2197	769	554
12	2-methylphenanthrene	2M-PHEN	$^{13}C_6$ PHEN	68	13	671	2065	832	779
13	9-methylphenanthrene	9M-PHEN	$^{13}C_6$ PHEN	78	10	395	971	459	434
14	1-methylphenanthrene	1M-PHEN	$^{13}C_6$ PHEN	57	9.9	338	823	494	462
15	fluoranthene	FLRAN	$^{13}C_6$ FLRAN	143	3.6	3096	4975	3258	5887
16	pyrene	PYR	$^{13}C_6$ PYR	53	2.7	2680	3550	2888	3829
17	retene	RET	$^{13}C_6$ PYR	203	N/A	894	711	1839	339
18	benzo(c)phenanthrene	BCP	$^{13}C_6$ BAA	41	N/A	286	354	281	N/A
19	benzo(a)anthracene	BAA	$^{13}C_6$ BAA	41	6.3	953	1384	1072	2123
20	chrysene	CHRY	$^{13}C_6$ CHRY	41	5.1	3551	4492	3366	N/A
21	benzo(b)fluoranthene	BBF	$^{13}C_6$ BBF	41	5.4	8791	10948	8903	8155
22	benzo(k)fluoranthene	BKF	$^{13}C_6$ BKF	41	4.5	1786	2202	2001	2197
23	benzo(e)pyrene	BEP	$^{13}C_4$ BAP	41	N/A	3516	4249	3712	4013
24	benzo(a)pyrene	BAP	$^{13}C_4$ BAP	41	3.5	1213	1525	1717	2413
25	perylene	PER	$^{13}C_4$ BAP	41	N/A	368	415	408	391
26	indeno(123-cd)pyrene	IP	$^{13}C_6$ IP	41	4.7	3802	4910	4100	4159
27	dibenz(a,h)anthracene	DBA	$^{13}C_6$ DBA	41	4.6	367	487	419	<500
28	benzo(ghi)perylene	BP	$^{13}C_{12}$ BP	41	5.7	5103	6525	5008	6892

1. The numbers in this table correspond to the peak numbers in Figure 2.2
2. LOD – limits of detection
3. CV – coefficients of variation from 42 quality control samples run over 2 months
4. NIST – National Institute of Standards and Technology. Concentrations on RM 8785 from the NIST lab were referenced from NIST, 2005
5. N/A – not available

2.2 Experimental

2.2.1 Chemicals

Dichloromethane (DCM) and toluene were of pesticide grade (>99.9% purity) and obtained from Tedia Company Inc. (Fairfield, OH, USA). Ultra-high purity nitrogen and helium were obtained from Airgas South Co. (Chamblee, GA, USA). Individual neat material of benzo(e)pyrene, benzo(c)phenanthrene, perylene, retene and a standard mixture containing the 16 priority pollutant PAHs were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Methyl-PAHs and dibenzothiophene were obtained from Chiron AS (Trondheim, Norway). $^{13}C_{12}$-labeled 2,3,3',4,4'-pentachlorobiphenyl ($^{13}C_{12}$-CB105, neat material), deuterated 1- and 2-methyl naphthalene, and a standard mixture of sixteen ^{13}C-labeled PAHs were purchased from Cambridge Isotope Laboratory (Andover, MA). All native PAH analytes and their respective isotopically labeled internal standards are listed in Table 2.1. Quartz filters (Whatman, 4.7mm, Grade 5, 2.5 μm) used for method development were purchased from VWR International LLC (West Chester, PA). The $PM_{2.5}$ filters used in the method evaluation was a reference material (RM 8785) from NIST (Gaithersburg, MD, USA).

We prepared stock solutions (1 mg/mL) from neat materials by weighing approximately 5 mg of each compound into a silanized amber vial and dissolving it in toluene. The internal standard solution contained sixteen ^{13}C-labeled PAHs and 2 deuterated Me-PAHs (200 pg/μL in toluene). The recovery standard solution contained $^{13}C_{12}$-CB105 (200 pg/μL in toluene). Six calibration standards were prepared in toluene spanning the range of 4.1–1000 pg/μL for each of the 28 analytes, with the 18 internal standards present at a constant concentration of 200 pg/μL.

2.2.2 Filter Extraction Procedure

The quartz filters were punched twice using a stainless steel cutter (14 x 9.5mm) pre-rinsed with methanol. Two punches from each filter were combined for extraction and were loaded into a cartridge lined with a frit (Reservoir-2 Frits, Varian Inc., Palo Alto, CA, USA). The sample was fortified with isotopically labeled internal standards at 2 ng per compound, and then topped with another frit. Samples were extracted with DCM (5 x 2 mL) at approximately 1 mL/min. All cartridges, frits and liners were pre-rinsed with DCM prior to use. The extracts were concentrated to ~50 μL in a TurboVap LV evaporator (Caliper Life Sciences, Hopkinton, MA) under a gentle stream of nitrogen (5-10 psi) at 40ºC. The extracts were then spiked with toluene (40 μL) and recovery standard ($^{13}C_{12}$-CB105, 4 ng per sample, 20 μL). Finally, the samples were transferred to GC vials for analysis.

Accelerated solvent extraction (ASE) was carried out on a Dionex ASE 200 system (Dionex Corp., Sunnyvale, CA, USA) using Ottawa sand (20-30 mesh, pre-rinsed by DCM) as filler material. The filters were extracted through two cycles by DCM at 120 ºC and 1500 psi. The heat time was 6 minutes, static time was 5 minutes, purge time was 60 seconds, and flush volume was 50%. The extracts (~30 mL) were evaporated as described above.

Sonicate extraction was carried out by placing filter samples in a capped glass tube and sonicating the samples in an ultrasonic bath for 20 minutes (3 x 5 mL DCM). The extracts were then filtered and evaporated using the same method described above.

2.2.3 Gas Chromatography/High Resolution Mass Spectrometry

GC/HRMS analyses were performed on a MAT 95 XL HRMS instrument (Thermo Scientific, Waltham, MA, USA), coupled with a 6890 GC (Agilent Technologies, Palo Alto, CA, USA). The chromatographic separations were carried out on a RTX-440 column (30 m, 0.25-mm i.d., 0.25-μm film thickness; Restek Inc.) using helium (1 mL/min) as the carrier gas. The injector was operated in split-less mode at 270

°C. The GC oven was programmed from 80°C (hold for 1 min) to 150 °C (50 °C/min, hold for 0.5 min), then ramped to 310 °C (10 °C/min, hold for 6 min). The source temperature was 250°C in electron impact mode (50 eV). The mass spectrometer was operated under selected ion monitoring (SIM) mode. The two most abundant ions from each compound were monitored as the quantification ion and confirmation ion. For most PAH analytes, the molecular ion was the most abundant ion and was used for quantification, with the exception of retene; its M-15 ion (m/z 219) was the most intense fragment, and thus was used as the quantification ion.

The sensitivity of the instrument, operated at 10,000 resolution, was verified daily by the analysis of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD, 20 fg/μL, 1 μL injection) in SIM mode (m/z 319.8965, 321.8937, 331.9368 and 333.9338). A signal-to-noise ratio greater than 5:1 was set as sensitivity threshold for the daily sensitivity test of 2,3,7,8-TCDD (m/z 321.8937).

2.2.4 Quality Assurance and Quality Control

Quality control (QC) samples were prepared by spiking a known amount of standard mixtures onto blank filters. In this method, a set of samples was defined as 16 unknown samples, prepared and analyzed along with 2 filter blanks and 2 filter QC samples. In addition, an external recovery standard and a calibration standard were analyzed on the GC/HRMS in parallel with the sample extracts. The external recovery standard contained the 18 isotopically labeled PAH internal standards and the recovery standard ($^{13}C_{12}$-CB105) and was used as a reference for the recovery calculation. Analysis of a set of samples was considered valid only after fulfilling the following criteria: (*i*) relative retention time, defined as the retention time ratio of a native compound over its labeled internal standard, must be within ± 2% of the reference ratio set by the calibration standard run with the samples, and (*ii*) recovery of the labeled internal standard must be within the range of 25–150%. Furthermore, the following

criteria must be fulfilled for a set of samples to pass the QC requirements: (*i*) the measurement of the target analytes in the QC sample must be within 3 standard deviations of the established mean, (*ii*) 2 or more consecutive measurements may not fall above or below 2 standard deviations, and (*iii*) 10 or more consecutive measurements may not fall above or below the established mean.

The limit of detection (LOD) for this method was defined as the higher LOD calculated by two methods: (*i*) in direct relation to method blanks prepared in parallel with the unknown samples, as 3 times the standard deviation of the method blanks, and (*ii*) according to the instrumental detection limit defined as the lowest point in the calibration curve verified to give a signal with the signal to noise ratio greater than 5:1. All measurements were subtracted with the average blank levels to correct for any analytical background.

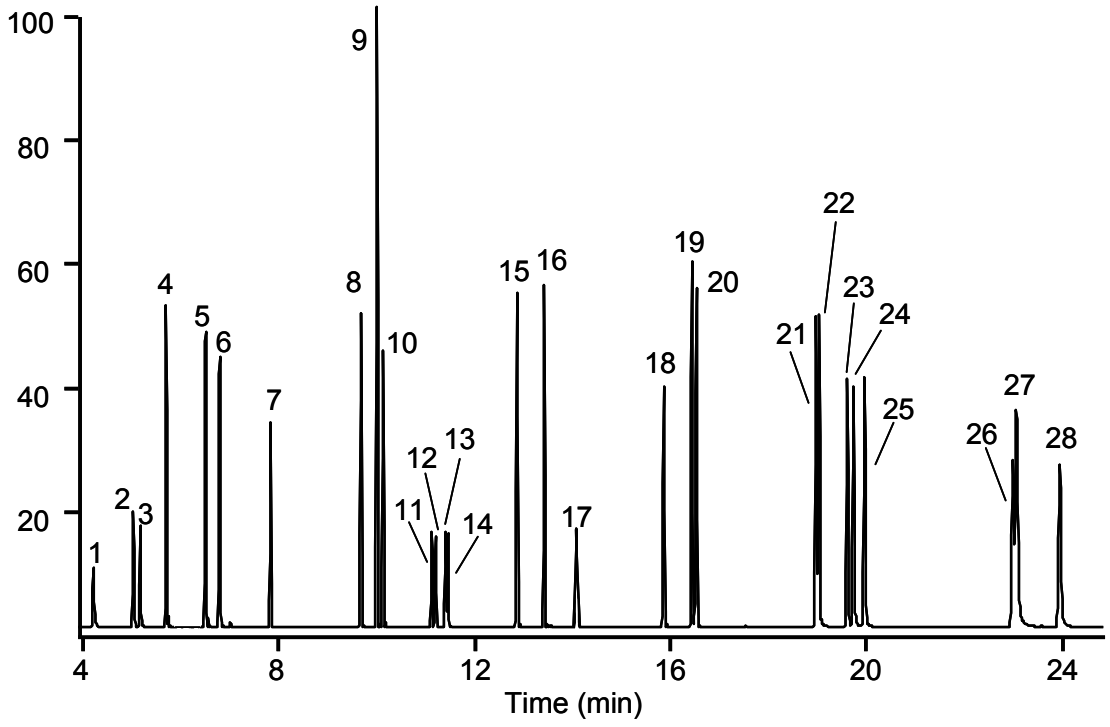

Figure 2.2. Chromatogram of the 28 PAH and Me-PAH standard mixture. Peaks are numbered according to Table 2.1

2.3 Results and Discussion

2.3.1 Method Development

We have developed a method for measuring 28 PAHs and Methyl-PAHs (Me-PAH) in ambient PM filter samples using a novel direct elution extraction method followed by isotope dilution GC/HRMS analysis. The PAHs reported in this study, as shown in Figure 2.1, include the 16 criteria PAHs that are on the U.S. EPA's hazardous air pollutant list, retene – a proposed biomass burning marker (Ramdahl 1983), dibenzothiophene – a distinct component in petroleum fuel (Bence et al. 1996), as well as methylnaphthalenes and methylphenanthrene.

We tested several GC columns, including commonly used DB-5MS and DB-XLB, and found that full separation for all 28 analytes and the 18 labeled internal standards can be achieved by using an RTX-440 column (Figure 2.2). Chromatographic separation of benzo(b)fluoranthene and benzo(k)fluoranthrene may be difficult to achieve due to their structural similarities. In our method, we reached 25% valley-separation of these analytes, and thus, allowed us to report them individually.

The sample extraction was conducted by direct elution of PM samples. DCM was chosen as the extraction solvent because of its extraction strength that gives high recovery of PAHs. In addition, given its high volatility (b.p. 39.8 °C), the DCM extract (~10 mL) can be evaporated within 10–15 minutes, thus minimizing analyte loss during evaporation. We determined the extraction condition in a series of experiments using a Rapid Trace® SPE workstations (Caliper Life Sciences, Hopkinton, MA). First, an experiment was set up to determine the amount of solvent required to achieve complete extraction of labeled internal surrogates and reach maximum concentration of target analyte concentration on the filter samples. We collected 5 fractions of DCM extracts (2 mL in each fraction) from each sample and found that the five fractions contained on average 84%, 11%, 4%, 1% and 0% of the labeled internal standards, respectively, which

indicated complete extraction after elution with five 2mL volumes of DCM. In a follow-up experiment, we varied solvent flow rate between 0.2 mL/min and 5 mL/min and found that the flow rate was not critical at or below 1 mL/min; however, when solvent flow-rate was over 1 mL/min, lower recoveries were obtained. Therefore, we determined the direct elution extraction condition as 5 x 2 mL of DCM at a flow rate of 1 mL/min to ensure maximum extraction efficiency.

This method can be performed on a 24-port vacuum manifold routinely used for solid phase extraction. The first step in the sample preparation is setting up the extraction cartridges on the manifold and pre-cleaning the tubes and liners with 5 mL DCM (~ 15 min), followed by filter sample preparation and internal standard addition (~ 15 min). Extraction of the 24 samples required approximately 20 minutes. The total time per sample is hence approximately 2 minutes, which makes the proposed method one of the most time-efficient method for extracting PAHs from PM samples. Due to the built-in frit inside each cartridge, no post-extraction filtration step was needed, which can further reduce human labor and increase sample throughput, in comparison to commonly used sonication extraction and Soxhlet extraction methods.

Two deuterated methyl PAHs and sixteen ^{13}C-labeled PAH internal standards, corresponding to the 16 EPA criteria PAHs, were used for quantification. The ^{13}C-labeled standards elute at the same time as the native analytes, allowing accurate analyte identification in complex samples where retention times may shift due to potential interference from co-extracting compounds. The isotope dilution method, i.e. the use of isotopically labeled PAHs as internal standards, can also correct for analyte loss during sample preparation and instrument performance fluctuation and, thus, ensure accurate quantification.

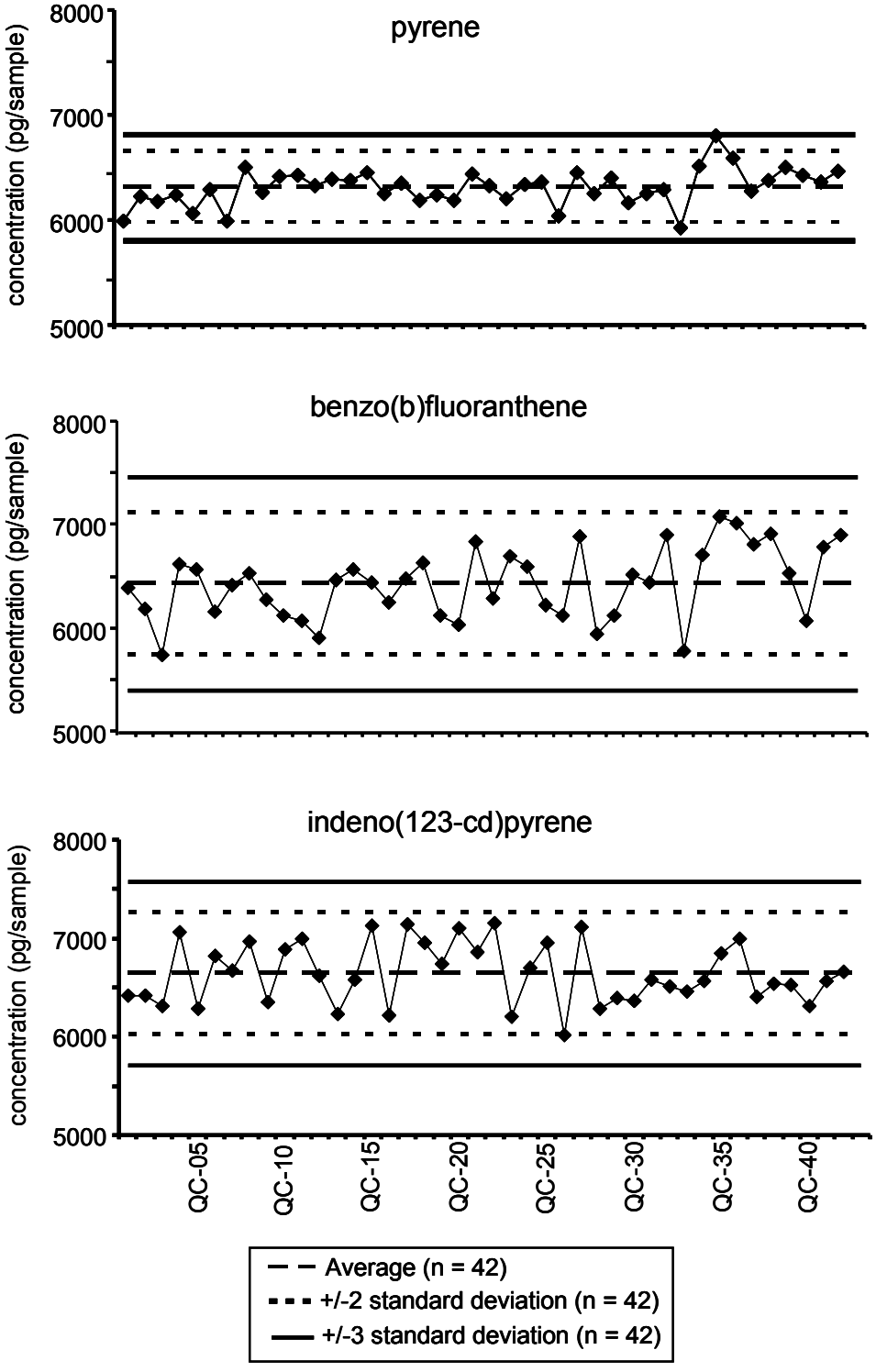

Figure 2.3. Concentration plots for pyrene, benzo(b)fluoranthene and indeno(123-cd)pyrene from 42 spiked filter samples (quality control samples) over a 2-month period

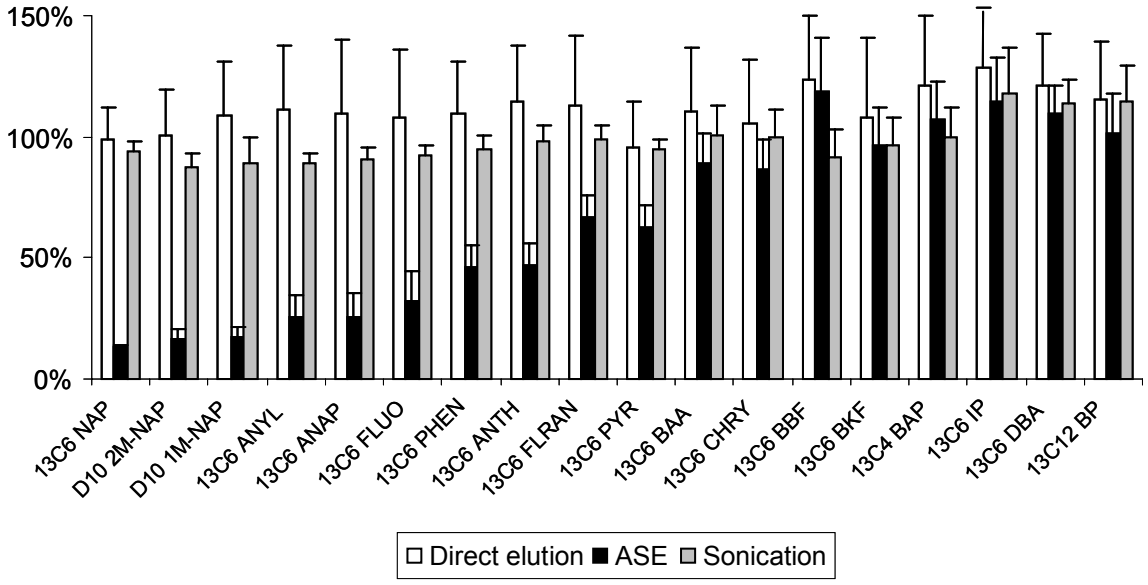

Figure 2.4. Average (with standard deviation) recoveries of the isotopically labeled internal standards from three extraction methods – direction elution, accelerated solvent extraction (ASE) and sonication extraction

2.3.2 Method Evaluation

The LODs for this method ranged from 41 to 366 pg per sample for all analytes (Table 2.1). PAHs with low molecular weight (2-3 aromatic rings) tended to have higher background levels in method blanks; therefore, their LODs were set by 3 times the standard deviation of 25 method blanks analyzed in parallel with the unknowns over a period of one month. PAHs with higher molecular weights (4 or more aromatic rings) had no or low background levels, and their LODs were determined by the lowest point of the calibration curve. Compared to other published methods measuring ambient PAHs (Ravindra et al. 2006; Burkhardt et al. 2005), the LODs achieved by this method were up to two orders of magnitude lower and, thus, enabled the detection of particulate-PAHs collected on air samplers running at relatively low flow rates, in contrast to high-volume samplers.

Along with the analysis of unknown filter samples and method blanks, two spiked filter QC samples were also analyzed as part of the quality control system. The overall

coefficients of variance (CV) for 42 QC samples prepared over a 2-month period were at ≤10% or below for most of analytes, with the exception of naphthalene (14%) and 2-methylphenanthrene (13%, cf. Table 2.1), demonstrating the reproducibility of this analytical method. QC charts for pyrene, benzo[b]fluoranthene, and indeno[123-cd]pyrene (representatives of 4, 5 and 6-ring PAHs) are given in Figure 2.3.

Recovery of the isotopically labeled internal standards spiked to all samples prior to extraction were close to 100% in the proposed method, which is comparable to sonicate extraction (Figure 2.4). Recoveries from ASE, however, ranged from 15-47% for small PAHs (2-3 rings) to 67-119% for PAHs with 4 or more aromatic rings (Figure 2.4), despite the fact that ASE extraction is known for its high extraction efficiency by employing high pressure (1000 psi), elevated temperature (120 ºC) and multiple extraction cycles (Prycek et al. 2006; Lintelmann et al. 2005; Bergvall and Westerholm 2008). Therefore, the recovery loss for the small PAHs using ASE most likely took place in the evaporation step (~30 mL extract, in comparison to 10-15 mL extracts from the direct elution method and sonication extraction), during which compounds with lower boiling points were affected more adversely than larger compounds.

To evaluate the accuracy of the proposed method, we analyzed RM8785, a reference material of $PM_{2.5}$ filter samples, which is a fine fraction of the urban dust standard reference material (SRM 1649a) obtained from NIST (NIST 2005). As a comparison, we also analyzed the RM8785 filters using ASE and sonication extraction. The results demonstrate that the direct elution performance is similar to that of the commonly used ASE and sonication extraction methods, which were also in good agreement with analytical results from NIST (Table 2.1). Figure 2.5 demonstrates agreement of the concentrations of pyrene, benzo(b)fluoranthene and indeno(123-cd)pyrene as determined by our laboratory using direct elution, ASE, and sonication extraction, with those reported by NIST (ASE extraction) and 8 other laboratories that used ASE, sonication or microwave-assisted extraction, a variety of sample clean-up

procedures, and GC/MS or LC-fluorescence detection (Table 2.2) (NIST 2005). Overall, among the 19 analytes reported by both NIST and our method, analytical results from the direct elution extraction were on average 12% lower than those from the NIST lab. Results obtained from direct elution were also consistent with those from sonication extraction, with on average 8.8% difference between these two methods. It is a potential risk that small PAHs with low molecular weight (2-3 rings) may re-volatilize during PM sample storage which could lead to inconsistent results, and therefore, they are often not included in studies reporting particle-bound PAH concentrations (Tsapakis and Stephanou 2005; Pleil et al. 2004). When excluding the small PAHs and only considering PAHs with 4 and more aromatic rings, the differences were 7.5%, 10%, and 19% between direct elution results and those from NIST, sonication extraction, and ASE, respectively.

Table 2.2. Brief description of methods used in the NIST interlaboratory comparison

Lab code in this paper	Lab code in NIST report	Extraction method	Extraction solvent	Extract clean-up method	Instrument method
NIST	1a	ASE[1]	DCM[2]	SPE[3]	GC/MS[4]
Lab1	1b	ASE	DCM	silica	GC/MS
Lab2	1c	ASE	DCM	SPE	GC/MS
Lab3	3a	ASE	DCM	filtration	HPLC FLD[5]
Lab4	3b	Sonication	2x5mL cyclohexane/DCM (4:1)	centrifuge	GC/MS
Lab5	4	ASE	acetone/DCM (1:4)	alumina	GC/MS
Lab6	7	Microwave-assisted extraction	acetone/hexane (1:1)	silica	GC/MS
Lab7	9	ASE	toluene	none	HPLC FLD, GC/MS
Lab8	14	Sonication	DCM/acetonitrile (2:1)	filtration	HPLC FLD

1. ASE: Accelerated solvent extraction, or other equivalent pressuized fluid extraction systems
2. DCM: dichloromethane
3. SPE: solid phase extraction
4. GC/MS: gas chromatography mass spectrometry
5. HPLC FLD: high performance liquid chromatography fluorescence detection

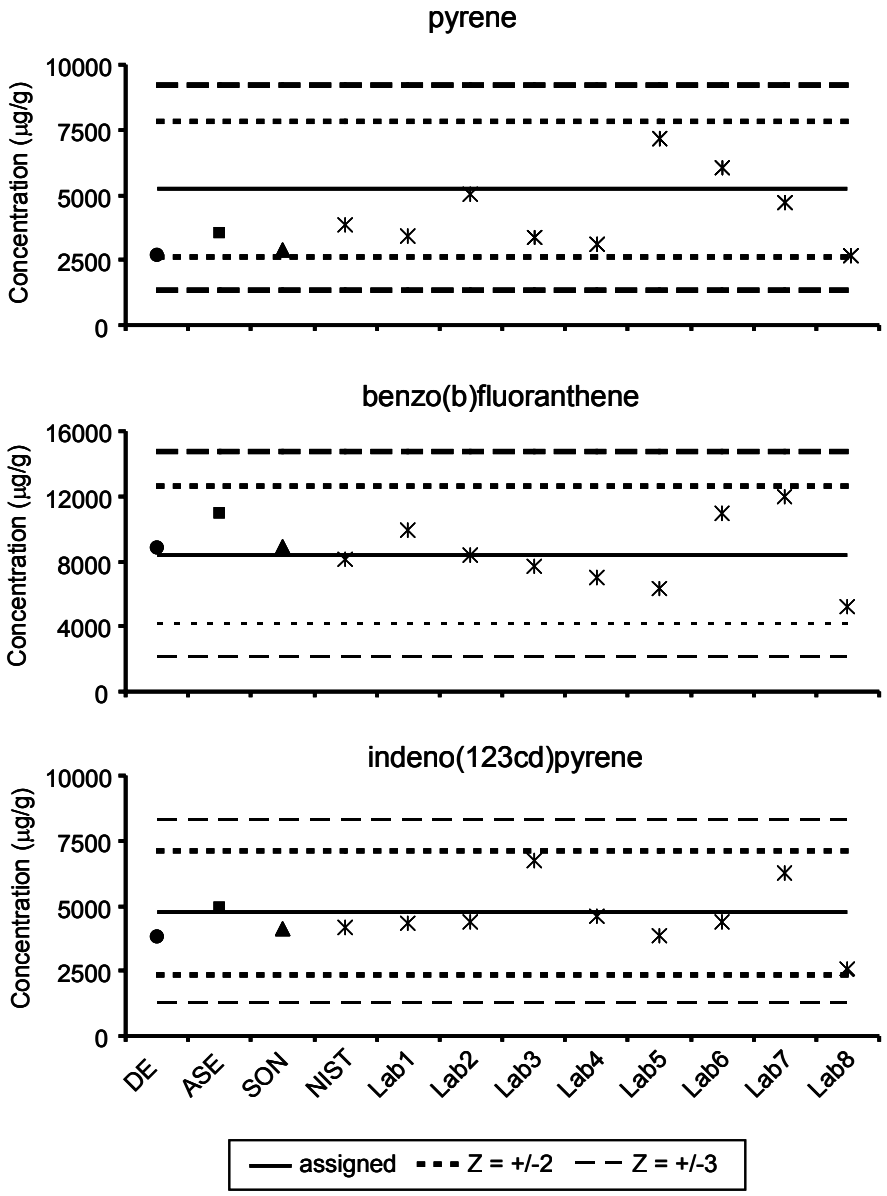

Figure 2.5. Inter-laboratory comparison on $PM_{2.5}$-bound PAH concentrations in NIST Reference Material 8785. Results from our lab are labeled as DE (direct elution), ASE (accelerated solvent extraction) and SON (sonication); results from the NIST lab are labeled as NIST

2.3.3 Concentrations of $PM_{2.5}$-bound PAHs in Atlanta

The developed direct elution method was used to analyze $PM_{2.5}$ samples taken from three sites (urban, suburban and rural) in the metropolitan Atlanta area during 2004, as described in Chapter 2. Six 24-hour samples from each site and month were analyzed,

and the results were compared to a pilot study reporting levels of 19 PAHs on $PM_{2.5}$ samples taken from the same sites but measured using a different method (Li et al. 2009). As shown in Figure 2.6, results from the current method were consistent with findings from the earlier study during which samples from December 2003 and June 2004 were extracted by ASE and analyzed by an Agilent GC/MSD system. These findings demonstrate comparability between the current direct elution method and the more established and commonly used ASE method.

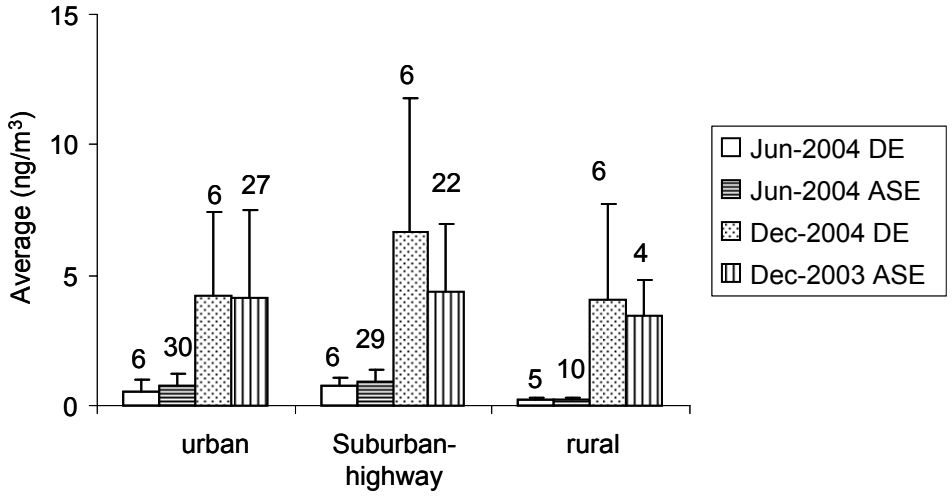

Figure 2.6. Average (with standard deviation) summed concentration of 19 PAHs from $PM_{2.5}$ samples taken in Atlanta, comparison between results from current study (DE or direction elution) and an earlier pilot study using ASE as extraction method. Also given are numbers of samples in each category

CHAPTER 3

CHARACTERIZATION OF $PM_{2.5}$-BOUND POLYCYCLIC AROMATIC HYDROCARBONS IN ATLANTA

(Li Z, Sjodin A, Porter EN, Patterson Jr. DG, Needham LL, Lee S, Russell AG and Mulholland JA. *Atmospheric Environment*, 43:1043-1050, 2009

Li Z, Porter EN, Sjodin A, Needham LL, Lee S, Russell AG and Mulholland JA, *Atmospheric Environment*, Submitted)

Abstract

Twenty-eight polycyclic aromatic hydrocarbons (PAH) and methylated PAHs (Me-PAH) were measured in daily $PM_{2.5}$ samples collected at an urban site, a suburban site, and a rural site in and near Atlanta during 2004 (5 samples/month/site). The suburban site, which is impacted by a nearby major highway, had higher $PM_{2.5}$-bound PAH concentration than did the urban site, and the rural site had the lowest PAH levels. Monthly variations are described for concentrations of total PAHs and individual PAHs. PAH concentrations were much higher in cold months than in warm months, with average monthly total PAH concentrations at the urban and suburban-highway monitoring sites ranging from 2.12 to 6.85 ng/m^3 during January-February and November-December 2004, compared to 0.38-0.98 ng/m^3 during May-September 2004. Total PAH concentrations were found to be well correlated with $PM_{2.5}$ and organic carbon (OC) within seasons but not over the entire year, and the fractions of PAHs in $PM_{2.5}$ and OC were higher in winter than in summer. Methyl phenanthrenes (MePhe) were present at higher levels than their un-substituted homologue (phenanthrene or Phe), suggesting a significant petrogenic (unburned petroleum products) input. Retene, a

proposed tracer for biomass burning, peaked in March, the month with the highest prescribed burning and unplanned fires, and in December during the high wood-burning season, which gives a strong indication that retene might be a good marker for burning of all biomass materials; in contrast, potassium peaked more sharply in December, indicating that it might be a more specific tracer for wood burning.

3.1 Introduction

Particulate matter (PM) air pollution has been linked to increased morbidity and mortality rates, as well as various adverse health effects such as respiratory and cardiovascular diseases (Cakmak et al., 2007; Kappos et al., 2004; Lebowitz, 1996; Ostro et al., 2007; Pope, III et al., 2004). Particulate matter with aerodynamic diameters less than 2.5 μm ($PM_{2.5}$ or fine particulate matter) are of special concern because, when inhaled, they can penetrate effectively into the respiratory system and deposit deep in the bronchioles and alveoli of the lungs. The association of fine particles with mutagenic and carcinogenic chemicals — such as polycyclic aromatic hydrocarbons (PAHs) — may contribute to these acute health effects or potentially result in long-term health risks. PAHs are a group of environmental pollutants formed during incomplete combustion of organic materials and are ubiquitously present in the ambient air, soil/sediments, water, and food such as grilled meat (ATSDR, 1995; Bostrom et al., 2002; Guillen et al., 1997; IARC, 1983). Several PAHs, including benzo(a)pyrene, benz(a)anthracene and chrysene, have been classified as probable human and animal carcinogens (ATSDR, 1995). PAHs have also been reported to possess reproductive, developmental, hemato-, cardio-, neuro-, and immuno-toxicities (ATSDR, 1995). The general population is primarily exposed to PAHs through inhalation of polluted air or cigarette smoke and through ingestion of PAH-containing foods (ATSDR, 1995; Bostrom et al., 2002; Ramesh et al., 2004). Given their carcinogenic and mutagenic potential and their wide distribution resulting in

exposure in the general population, PAHs have generated considerable interest; 16 PAHs have been included in U.S. EPA's list of 188 hazardous air pollutants (U.S.EPA, 1990).

PAHs in the atmosphere can come from natural sources, such as forest fires and volcanic eruptions. However, their presence, especially in densely populated areas, is significantly affected by anthropogenic emissions, such as automobile exhaust, fossil fuel combustion, biomass burning, cigarette smoking, and industrial activities. On the other hand, alkylated PAHs have often been found at comparable or even higher levels than their un-substituted parent compounds and have been primarily attributed to mature organic matter or petrogenic sources (Bostrom et al., 2002; Yunker et al., 2002). Examples of such sources that can be significant in urban areas include evaporation of fossil fuel drippings on the roadway, evaporative losses during motor vehicle refueling, and venting of restaurant frying oils. The ratio of methylated PAHs (Me-PAH) to their un-substituted homologue has been used as an indicator to un-burnt fossil fuel sources in the atmosphere, soil and aquatic environments (Simo et al., 1997; Vondracek et al., 2007; Yunker et al., 2002). Me-PAHs, specifically monomethylated phenanthrenes (MePhe) and naphthalenes (Me-Nap), have been recommended for inclusion in ambient air monitoring based on their high abundance in air samples and association to un-combusted petroleum fuel (Bostrom et al., 2002).

Atmospheric PAHs are found in both gaseous and particle phases. PAHs with 2-3 aromatic rings exist predominantly in the gaseous phase, while compounds with 4 or more aromatic rings are primarily associated with the particulate fraction (Eiguren-Fernandez et al., 2004; Li et al., 2006; Re-Poppi and Santiago-Silva, 2005). In addition, it has been reported that PAH-associated particles exist predominantly as fine particles, i.e., in the $PM_{2.5}$ size range, especially in areas with heavy traffic (Cancio et al., 2004; Duan et al., 2005; Ohura et al., 2004). Because the carcinogenic PAHs (e.g. benzo[a]pyrene and benz[a]anthrene) are mostly associated with particulate matter, many studies on PAHs in ambient air have been focused on PAHs bound to PM, particularly $PM_{2.5}$ (Borlakoglu et

al., 1993; Bourotte et al., 2005; Ohura et al., 2004; Papageorgopoulou et al., 1999; Sanderson et al., 2004; Sklorz et al., 2007).

Particulate matter pollution is of interest in metropolitan Atlanta, Georgia, one of the fastest growing regions in the United States. Atlanta's traffic congestion is the second worst in the country, following Los Angles, CA (Texas Traffic Institute, 2007). Air quality in this region is often problematic; currently, the twenty-county Atlanta metropolitan area is in non-attainment for $PM_{2.5}$ and ozone (U.S.EPA, 2007). PM source apportionment analyses by two groups of researchers indicated that 30 to 40% of Atlanta's $PM_{2.5}$ can be attributed to mobile sources and biomass burning (Kim et al., 2004; Marmur et al., 2007), and a recent epidemiologic study indicated that these components are associated with both cardiovascular and respiratory emergency room visits in Atlanta (Sarnat et al., 2008).

We conducted two studies characterizing PAHs in $PM_{2.5}$ samples collected at three sites (urban, suburban-highway and rural) in the metropolitan Atlanta area. First, a pilot study was conducted reporting levels of 19 particle-bound PAHs in daily $PM_{2.5}$ samples taken in December 2003 and June 2004 (Li et al., 2009b). We found strong seasonal variation for particulate PAHs and positive correlations of PAHs to other pollutants including $PM_{2.5}$, organic carbon (OC) and elemental carbon (EC). As an expansion of the pilot study, we conducted a year-round study by measuring 28 particulate PAHs and Me-PAHs in outdoor $PM_{2.5}$ samples collected at the three sites during the year of 2004 (5 samples/month/site). We examined the concentrations and profiles of these pollutants by month and site. We investigated the potential of retene as a marker for biomass burning and relative concentration of methyl phenanthrenes as an indicator for petrogenic sources. We also examined correlations of PAH levels with other air pollutants such as $PM_{2.5}$ mass and OC and the temperature dependency of these correlations.

3.2 Experimental

3.2.1 Sampling Sites and Method

Three sampling sites in the metropolitan Atlanta region were chosen to characterize particle-bound PAHs (Figure 3.1). The sampling sites were selected from the Assessment of Spatial Aerosol Composition in Atlanta (ASACA) network, a project started in early 1999 to study the spatial and temporal variation of $PM_{2.5}$ and its chemical components in Atlanta (Butler et al., 2003). One of the sites, Fort McPherson (FM), is located in southwest urban Atlanta on a military base, well within I-285, the Atlanta perimeter interstate highway. A second site, South Dekalb (SD), is located in a primarily residential suburban area less than 1 km south of the I-285, which carries on average over 130,000 vehicles per day and is the main detour route around Atlanta for large, heavy service diesel trucks. The third sampling location, Fort Yargo (YG), is a rural site at a state park with heavy vegetation about 80 km northeast of Atlanta.

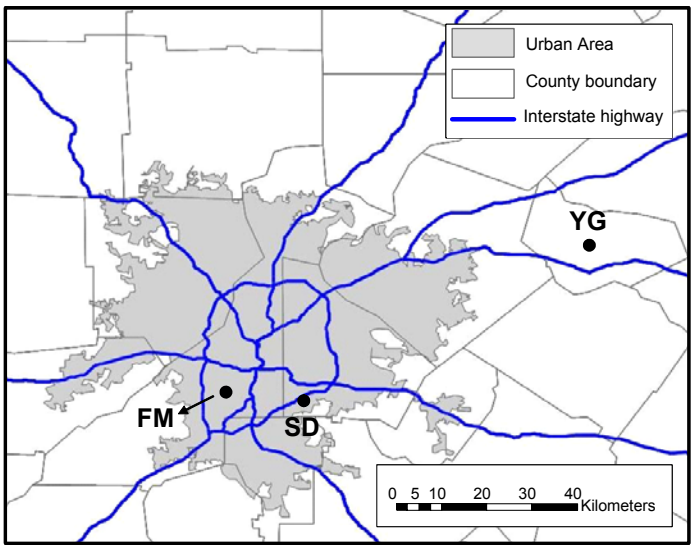

Figure 3.1. Map of the metropolitan Atlanta area with major interstate highways, county boundaries, and air monitoring stations. Data for this study were taken from three sites, Fort McPherson (FM, urban), South Dekalb (SD, suburban-highway), and Fort Yargo (YG, rural)

The $PM_{2.5}$ particle composition monitor (PCM) sampling system has been described in detail previously (Butler et al., 2003), and is only briefly described here. Twenty-four hour $PM_{2.5}$ samples were collected using three-channel PCM systems placed on top of single-story trailers, with sampling inlets located approximately 4 m above ground level, except at YG, where the inlet is approximately 2 m above ground. Particle-bound PAHs were captured on a quartz filter (Whatman, 4.7mm, Grade 5, 2.5 μm) after the ambient air sample has passed through a Teflon-coated cyclone with a 10 μm cut-point, an activated carbon parallel-plate denuder to remove organic gases, and a Well Impactor Ninety Six (WINS) impactor with a 2.5 μm cut-point. The flow rate was 16.7 L/min, resulting in a total sampled air volume of 24 m^3 for each sample. Prior to sampling, quartz filters were baked at 550 ºC for 12 hours to remove organic impurities. After sampling, the filters were put into individual Petri dishes and kept in a -20 ºC freezer until analysis. Samples analyzed in the pilot study were taken in December 2003 and June 2004. For the expanded year-round study, five samples each month in 2004 from each of the 3 sampling sites were analyzed for PAHs.

3.2.2 Sample Analysis

The detailed methods for measuring concentrations of $PM_{2.5}$, OC, EC and ionic species have been described elsewhere (Cobb, 2006). Briefly, continuous $PM_{2.5}$ mass concentrations (at 10 min sampling intervals) were collected using tapered element oscillating microbalances (TEOM), with which we calculate 24-hr averaged $PM_{2.5}$ mass. OC and EC concentrations were quantified using thermal optical transmittance (Sunset Labs Inc., Tigard, OR). Major ion species were analyzed on a Dionex Ion Chromatography system (Dionex Corp., Sunnyvale, CA). Metals were analyzed using x-ray fluorescence (XRF).

3.2.2.1 Extraction and Instrument Methods in Pilot Study

Nineteen PAHs were measured in all samples using a method that included Accelerated Solvent Extraction (ASE) and isotope-dilution GC/MS quantification. Filter samples were cut in half and weighed. One half was extracted and analyzed for PAHs, while the other half was used for OC and EC analysis. The filters were fortified with a mixture of 15 ^{13}C-labeled internal standards in toluene, at 2 ng per compound per sample, then loaded onto a pressurized solvent extraction system (ASE 200, Dionex Corp., Sunnyvale, CA) using hydromatrix (pre-rinsed by dichloromathene) as filler. The filters were extracted three times by dichloromethane, at 120 ºC and 1500 psi. The extracts (~25 mL) were concentrated to ~100 μL in a TurboVap LV evaporator (Caliper Life Sciences, Hopkinton, MA), under a gentle stream of nitrogen (5-10 psi) at 40ºC, then spiked with a recovery standard ($^{13}C_{12}$ labeled 3-methoxy-3,3',4,5'-tetrachlorobiphenyl, 3 ng per sample, in toluene) to track recovery from the sample preparation process. Finally, the samples were transferred to GC vials for analysis.

GC/MS analyses were performed on a 6890 GC coupled with a 5973 mass selection detector and a 7683 auto-injector (Agilent Technologies, Palo Alto, CA). The chromatographic separations were carried out on an RTX-440 column (30 m, 0.25-mm i.d., 0.25-μm film thickness; Restek Inc.) using helium (1 mL/min) as the carrier gas. The injector was operated in the splitless mode at a temperature of 270 °C. The GC oven was programmed from 100°C (hold for 1 min) to 200 °C (30 °C/min), then ramped to 290 °C (10 °C/min), and finally to 320 °C (20 °C/min, hold for 4 min). The mass spectrometer was operated under the selected ion monitoring (SIM) mode. Only the two most abundant ions from each compound were monitored as the quantification ion and confirmation ion. For most PAH analytes, the molecular ion was the most abundant ion and was used for quantification, with the exception of retene, the only alkylated PAH (1-methyl-7-isopropyl phenanthrene) on the analyte list. Retene's M-15 ion (m/z 219) was the most intense fragment, and thus was used as the quantification ion. The 19 PAHs measured in this study are listed in Table 3.1, along with their formulas, molecular weights, vapor

pressures, ^{13}C labeled internal standards, limits of detection (LOD), and recoveries. Retene, an alkylated phenanthrene, has 18 carbons and it elutes after pyrene, therefore, it is included in the 4-ring or C16-C18 group (Table 3.1).

Table 3.1. List of PAHs reported in the pilot study, including the abbreviations, formula, molecular weights, vapor pressure, ^{13}C-labeled internal standards, limits of detection, and percent recoveries

Compound	Abbr.	Formula	Molecular weight	Vapor pressure (mm Hg) [a]	Internal standard	LOD (pg/m^3) [b]	Recovery (%)
3-ring, C12-C14							
acenaphthylene	acny	$C_{12}H_8$	152	0.029	acny $^{13}C_6$	9.4	32%
acenaphthene	acne	$C_{12}H_{10}$	154	$4.47x10^{-3}$	acne $^{13}C_6$	9.4	36%
fluorene	fluo	$C_{13}H_{10}$	166	$3.2x10^{-4}$	fluo $^{13}C_6$	24.6	55%
phenanthrene	phen	$C_{14}H_{10}$	178	$6.8x10^{-4}$	phen $^{13}C_6$	51.8	68%
anthracene	anth	$C_{14}H_{10}$	178	$1.7x10^{-5}$	anth $^{13}C_6$	9.4	64%
4-ring, C16-C18							
fluoranthene	flran	$C_{16}H_{10}$	202	$5.0x10^{-6}$	flran $^{13}C_6$	11.3	91%
pyrene	pyr	$C_{16}H_{10}$	202	$2.5x10^{-6}$	Pyr $^{13}C_6$	9.4	92%
retene[c]	retene	$C_{18}H_{18}$	234	n/a	Pyr $^{13}C_6$	9.4	92%
benzo(c)phenanthrene	bcp	$C_{18}H_{12}$	228	n/a	baa $^{13}C_6$	9.4	110%
benzo(a)anthracene	baa	$C_{18}H_{12}$	228	$2.2x10^{-8}$	baa $^{13}C_6$	9.4	110%
chrysene	chry	$C_{18}H_{12}$	228	$6.3x10^{-7}$	chry $^{13}C_6$	9.4	103%
5-ring, C20							
benzo(b)fluoranthene	bbf	$C_{20}H_{12}$	252	$5.0x10^{-7}$	Bbf $^{13}C_6$	9.4	102%
benzo(k)fluoranthene	bkf	$C_{20}H_{12}$	252	$9.59x10^{-11}$	Bkf $^{13}C_6$	9.4	97%
benzo(e)pyrene	bep	$C_{20}H_{12}$	252	$5.7x10^{-9}$	bap $^{13}C_4$	9.4	98%
benzo(a)pyrene	bap	$C_{20}H_{12}$	252	$5.6x10^{-9}$	bap $^{13}C_4$	9.4	98%
perylene	per	$C_{20}H_{12}$	252	n/a	bap $^{13}C_4$	9.4	98%
6-ring, C22							
benzo(ghi)perylene	bgp	$C_{22}H_{12}$	276	$1.03x10^{-10}$	bgp $^{13}C_6$	9.4	112%
dibenz(a,h)anthracene	dba	$C_{22}H_{14}$	278	$1.0x10^{-10}$	dba $^{13}C_6$	9.4	108%
indeno(123-cd)pyrene	inp	$C_{22}H_{12}$	276	$\sim10^{-11}$-10^{-6}	Inp $^{13}C_6$	9.4	104%

a As compiled in ATSDR, 1995
b Limit of detection, based on 16.7 L/min flow rate and 24 hour air sampling
c Full nomenclacture: 1-methyl-7-isopropyl phenanthrene

3.2.2.2 Extraction and Analysis Methods in Year-round Study

Filter samples in the year-round study were extracted through direct elution and quantified by isotope-dilution gas chromatography/high resolution mass spectrometry (GC/HRMS). The method has been described in detail in Chapter 2 (Li et al., 2009a). Briefly, each filter sample was loaded into a cartridge lined with a frit, fortified with a mixture of 18 isotopically labeled internal standards, and then topped with another frit. Samples were extracted with dichloromethane (DCM, 5 x 2 mL) at approximately 1 mL/min. The extracts were concentrated to ~50 μL under a gentle stream of nitrogen (5-10 psi, 40ºC), spiked with toluene (40 μL) and a recovery standard ($^{13}C_{12}$-labeled PCB105, 4 ng per sample). Finally, the samples were transferred to GC vials for analysis.

GC/HRMS analyses were performed on a MAT 95 XL HRMS instrument (Thermo Scientific, Waltham, MA, USA), coupled with a 6890 GC (Agilent Technologies, Palo Alto, CA, USA). The chromatographic separations were carried out on an RTX-440 column (30 m, 0.25-mm i.d., 0.25-μm film thickness; Restek Inc.) using helium (1 mL/min) as the carrier gas. The injector was operated in split-less mode at 270 °C. The GC oven was programmed from 80°C (hold for 1 min) to 150 °C (50 °C/min, hold for 0.5 min), then ramped to 310 °C (10 °C/min, hold for 6 min). The mass spectrometer was operated under selected ion monitoring (SIM) mode. Only the two most abundant ions from each compound were monitored as the quantification ion and confirmation ion.

3.2.3 Quality Assurance and Quality Control

Field and method blanks were analyzed to monitor background contamination. Method blanks were measured along with samples, and all results reported here were blank-subtracted. Secondary filters were installed after the primary filters in the PCM sampler to study the breakthrough of particulate PAHs. Levels of particulate PAHs on the

secondary filters were very low — in most cases, they were below the limit of detection (LOD).

Deuterated or ^{13}C-labeled PAH internal standards were used for quantification. The ^{13}C-labeled standards elute at the same time as the native analytes, allowing accurate analyte identification in complex samples where retention times may shift due to potential interference from co-extracting compounds. The isotope dilution method, i.e. the use of isotopically labeled PAHs as internal standards, can also correct for analyte loss during sample preparation and instrument performance fluctuation and, thus, ensure accurate quantification.

Quality control (QC) samples were prepared by spiking a known amount of standard mixtures onto blank filters. In this method, a set of samples was defined as 16 unknown samples, prepared and analyzed along with 2 filter blanks and 2 filter QC samples. In addition, an external recovery standard and a calibration standard were analyzed on the GC/HRMS in parallel with the sample extracts. Analysis of a set of samples was considered valid only after fulfilling a series of criteria on elution time, recovery and QC concentrations as described in Chapter 2 (Li et al., 2009a).

3.2.4 Data and Statistical Analysis

All concentrations were blank-subtracted; concentrations below the LOD were replaced with the LOD divided by the square root of 2 prior to statistical analysis. Fire data were obtained through personal communication with Georgia Forestry Commission. All statistical analyses were performed using the Statistica 7.1 software (StatSoft Inc., Tulsa, OK). Results were considered statistically significant if the *p*-value was equal to or less than 0.05.

3.3 Results and Discussion

3.3.1 Results and Discussion from the Pilot Study

3.3.1.1 Ambient Levels of Particulate PAHs – Seasonal Variation

Median concentrations of the 19 particle-bound PAHs obtained in this study are presented in Table 3.2, arranged by sampling site and month. The median total PAH concentrations were 3.40, 4.13, and 3.16 ng/m^3 in December, and 0.24, 0.74, and 0.60 ng/m^3 in June, for YG, SD, and FM, respectively (Figure 3.2). At all three sites, there were large differences between the winter month (December) and the summer month (June); total PAH concentrations in December were 4.2, 5.6, and 5.3 times higher than in June for YG, SD, and FM, respectively. As shown in Table 3.3, the PAH concentrations observed in this study are similar to those reported in Chapel Hill, NC, USA (Pleil et al., 2004), Los Angeles, CA, USA (Eiguren-Fernandez et al., 2004) and Japan (Tham et al., 2008). But the concentrations are lower than those in urban environments in Croatia (Sisovic et al., 2005), Brazil (Bourotte et al., 2005), China (Li et al., 2006), and Greece (Tsapakis and Stephanou, 2005b).

The increase in particulate PAH concentration during the winter time has been reported in a number of previously published studies (Eiguren-Fernandez et al., 2004; Li et al., 2006; Papageorgopoulou et al., 1999; Sanderson et al., 2004; Sisovic et al., 2005; Tsapakis and Stephanou, 2005b). Several factors may contribute to the strong seasonal trend. During the cold season, PAH emissions from automobile exhaust are higher due to low ambient temperature and increased cold start impacts (Ludykar et al., 1999). Biomass burning and fireplace usage are also likely to contribute to increased PAH emissions in winter. Reduced atmospheric dispersion and atmospheric reaction can lead to higher PAH concentrations in winter as well. Furthermore, low atmospheric temperature can affect the distribution of PAHs between the gas and particle phases and result in a relatively larger portion of PAH partitioning to the particle phase in winter. Finally, potential re-volatilization of PAHs, particularly smaller PAHs, may also contribute to relatively lower particle phase concentrations during the summer season.

Table 3.2. Median PAH concentrations (pg/m^3) from three sampling sites during June 2004 and December 2003

PAH Analytes	YG (rural)		SD (suburban-highway)		FM (urban)	
	June	December	June	December	June	December
acenaphthylene	<LOD	<LOD	<LOD	8.6	<LOD	<LOD
acenaphthene	<LOD	<LOD	<LOD	36.4	<LOD	68.3
fluorene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
phenanthrene	<LOD	38.7	47.0	151.8	<LOD	<LOD
anthracene	<LOD	<LOD	<LOD	15.7	<LOD	7.3
fluoranthene	12.8	101.9	35.6	140.9	32.9	110.9
pyrene	10.3	119.5	39.1	167.4	31.7	140.5
retene	<LOD	290.3	<LOD	137.1	<LOD	175.6
benzo(c)phenanthrene	<LOD	22.0	<LOD	25.3	<LOD	21.0
benzo(a)anthracene	<LOD	123.9	26.3	188.6	16.5	120.8
chrysene	10.6	153.8	49.3	233.1	33.9	156.0
benzo(b)fluoranthene	19.6	544.4	78.6	607.6	59.0	468.9
benzo(k)fluoranthene	<LOD	139.7	22.3	179.4	17.4	140.7
benzo(e)pyrene	14.4	307.0	54.5	375.2	46.3	345.6
benzo(a)pyrene	<LOD	234.3	48.8	415.5	29.1	275.1
perylene	<LOD	53.1	9.1	90.9	<LOD	160.7
benzo(ghi)perylene	16.8	257.2	64.6	312.7	56.7	244.0
dibenz(a,h)anthracene	<LOD	15.7	<LOD	18.1	<LOD	12.3
indeno(123-cd)pyrene	25.2	372.2	131.0	585.9	110.1	514.8

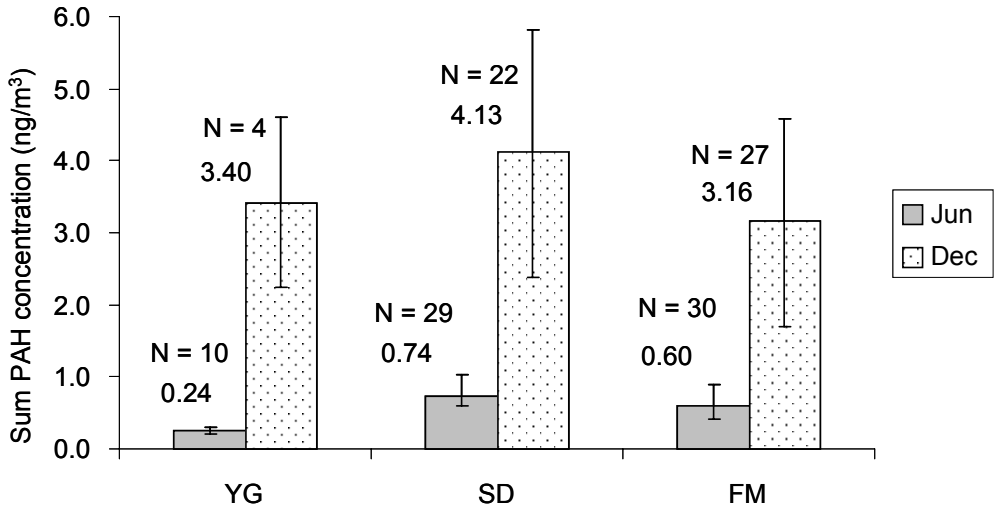

Figure 3.2. Median and quartile ranges of summed PAH concentration from three sampling sites, in June 2004 and December 2003. Also shown are the numbers of valid sampling days under each category

Table 3.3. Average $PM_{2.5}$-bound PAH concentrations (ng/m^3) in selected studies. Data from Fort McPherson (urban) site are used in this table

Location	Atlanta, urban	Atlanta, urban	Croatia, urban	Croatia, urban	Chapel Hill, USA	Brazil, urban	China, urban	Los Angeles, USA	Greece, power plant	Greece, Urban
Sampling season	Jun, 04	Dec, 03	Jul	Jan	winter, 02-03	winter, 02	all year, 01-02	all year, 01-02	all year, 01	all year, 00-02
Sampling days	30	27	30	30	26	70	51	~50	9	16
Reference	This study	This study	Sisovic, 2005	Sisovic, 2005	Pleil, 2004	Bourotte, 2005	Li, 2006	Eiguren-Fernandez, 2004	Kalaitzoglou, 2004	Tsapakis Stephanou 2005
fluoranthene	0.04	0.10	0.09	3.68	0.09	0.68	1.5	0.03	0.1	0.18
pyrene	0.04	0.16	0.07	4.65	0.09	0.52	1.6	0.05	0.17	0.31
benzo(a)anthracene	0.02	0.19	-	-	0.06	0.46	1.4	0.03	0.08	0.63
chrysene	0.05	0.23	-	-	0.12	0.51	2.7	0.05	0.11	1.65
benzo(b)fluoranthene	0.09	0.60	0.09	3.52	0.25	1.23	2.6	0.08	0.26	1.34
benzo(k)fluoranthene	0.03	0.17	0.05	2.10	0.14	0.76	2.7	0.04	0.09	1.72
benzo(e)pyrene	0.06	0.43	-	-	0.21	0.57	-	-	0.31	1.55
benzo(a)pyrene	0.04	0.37	0.05	3.18	0.09	0.52	2.3	0.08	0.12	1.07
indeno(123-cd)pyrene	0.14	0.67	0.16	4.10	0.19	2.36	2.7	0.08	0.05	3.27
dibenz(a,h)anthracene	<LOD	0.02	-	-	0.04	-	0.3	0.01	0.42	0.12
benzo(ghi)perylene	0.07	0.32	-	-	0.17	2.47	3.1	0.17	0.27	2.4

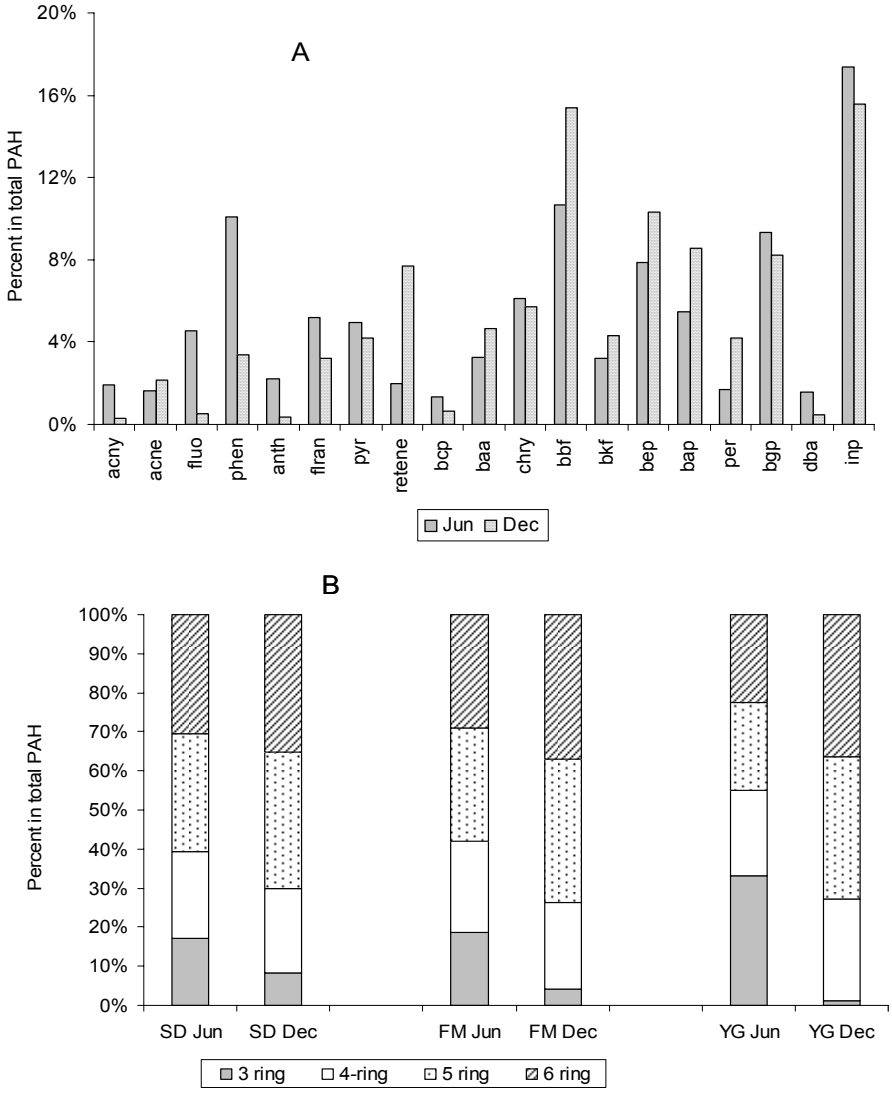

Figure 3.3. Distribution of individual PAHs (A) and grouped PAHs by ring size (B). Data are taken from all three sites

We also found large differences in concentrations among different PAHs, spanning several orders of magnitude (Table 3.2). Generally, the concentrations were related to the size of the compounds; i.e., the higher the molecular weight, the higher the particle-bound PAH concentrations. The median concentrations for PAHs with 3 aromatic rings were less than 100 pg/m^3 or at the LOD, while concentrations of PAHs with 5–6 rings were elevated, with benzo(b)fluoranthene and indeno(123-cd)pyrene being the most abundant compounds (Figure 3.3A). As a result, 55–70% of total PAH

concentrations consisted of PAHs with 5–6 rings while less than 20% (in June) or 10% (in December) consisted of PAHs with 3 rings (Figure 3.3B). This distribution pattern is similar to that found in a study conducted in Brazil (Bourotte et al., 2005). The low concentrations of small PAHs are consistent with their physio-chemical properties. With high vapor pressure and high volatility, these compounds exist primarily in the gaseous phase (Re-Poppi and Santiago-Silva, 2005; Tsapakis and Stephanou, 2005b; Tsapakis and Stephanou, 2005a). Pleil et al. (2004) assessed the measurement of particle-bound PAHs in archived filter samples and concluded that the 5-6 ring PAH measurements are quantitatively robust, as are the measurements of 4-ring compounds — if filter samples are stored under refrigeration. Because of re-volatilization, concentrations of small PAHs (i.e. those with fewer than 4-rings) in archived PM filters can be biased and are therefore excluded from further analysis in this study.

3.3.1.2 Ambient Levels of Particulate PAHs – Site Variation

SD is located about 1 km from a major highway and — not surprisingly — recorded significantly higher levels than did FM, the more central site (Wilcoxon Matched Pairs Test, $p = 0.013$). The median total PAH concentrations at SD were 31% and 23% higher than those at FM in December and in June respectively (Figure 3.2). The urban site (FM) and suburban site (SD) had significantly higher particulate PAH levels than did the rural site (YG) (Mann-Whitney U test) in June. This is consistent with their locations in densely populated and heavily trafficked areas with a high PAH contribution from traffic emission and anthropogenic input. In December, however, between YG and the urban/suburban sites, no significant differences appeared in total PAH concentrations. That said, the sample size for YG in December was small (n = 4), preventing us from drawing any conclusion from this observation. An expanded follow-up project that studies PAH levels from the same three sampling sites throughout the year 2004 is currently underway, which will give more insight into the site variation.

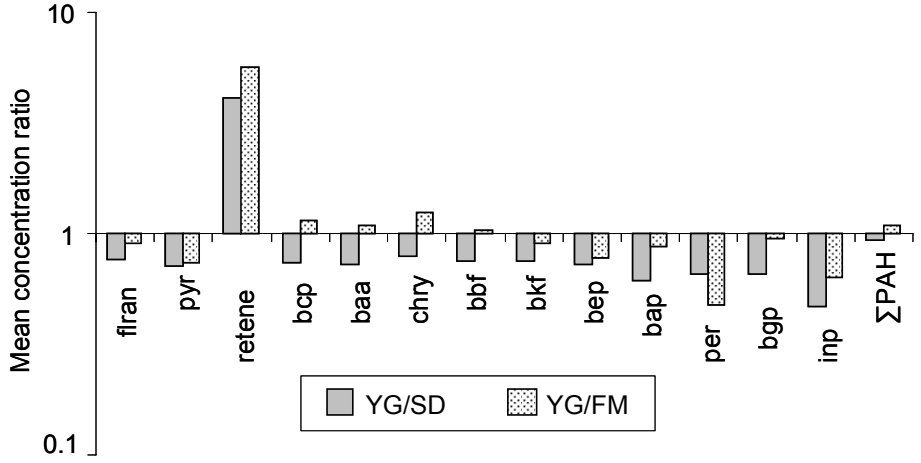

Figure 3.4. Median ratio of individual PAH concentration at Fort Yargo (YG, rural) to South Dekalb (SD, highway) and Fort McPherson (FM, urban). Samples were taken during December 2003

To examine the site variation of individual PAHs, we calculated the ratios of PAH concentrations measured at YG to those at SD and FM on the same day (paired data were available on 3 days in December 2003, i.e. 12/25/2003, 12/28/2003, and 12/31/2003). As shown in Figure 3.4, the median ratios of PAH concentrations on those 3 days were at or below 1 for most of the 4-or-more aromatic ring PAHs, with the exception of retene, an alkylated phenanthrene. Collectively, the median retene concentration at YG in December was 290 pg/m^3 (n = 4), higher than the urban site (FM, 176 pg/m^3, n = 27) and the suburban site (SD, 137 pg/m^3, n = 22). Furthermore, linear regression analysis showed that retene was either not correlated or correlated poorly with the other PAHs measured (Table 3.4), indicating a different source/formation mechanism or environmental fate pathway for this compound. The different patterns of retene compared with other PAHs has been noted in several other studies, including a temporal PAH deposition study (Lima et al., 2003) and a year-round Mexican City air study (Villalobos-Pietrini et al., 2006). The uniqueness of retene might be related to its source-specific formation mechanism. Ramdahl (1983) has demonstrated the formation of retene by thermal degradation of resin compounds in wood and proposed it as a marker in ambient

Table 3.4. Correlation matrix for the individual PAH compounds. Data from December at all three sites were used

	acny	acne	fluo	phen	anth	flran	pyr	retene	bcp	baa	chry	bbf	bkf	bep	bap	per	bgp	dba	inp
					3-Ring														
acny	**1.00**																		
acne	0.17	**1.00**																	
fluo	*0.57*[#]	0.20	**1.00**																
phen	*0.28**	-0.11	*0.40*	**1.00**															
anth	*0.54*	0.01	*0.38*	*0.67*	**1.00**														
flran	*0.43*	-0.12	*0.22*	*0.57*	*0.68*	**1.00**					*4-Ring*								
pyr	*0.43*	-0.08	*0.21*	*0.42*	*0.57*	*0.93*	**1.00**												
retene	0.00	0.19	-0.01	-0.06	0.01	0.16	*0.30*	**1.00**											
bcp	0.12	0.05	0.02	0.09	0.18	*0.47*	*0.63*	*0.64*	**1.00**										
baa	*0.23*	0.01	0.15	0.16	*0.23*	*0.53*	*0.70*	*0.54*	*0.84*	**1.00**									
chry	*0.29*	0.02	0.17	0.19	*0.28*	*0.58*	*0.75*	*0.55*	*0.84*	*0.99*	**1.00**								
bbf	*0.33*	-0.10	*0.22*	0.11	*0.26*	*0.49*	*0.68*	*0.35*	*0.67*	*0.86*	*0.88*	**1.00**						*5-6 Ring*	
bkf	*0.33*	-0.05	0.18	0.11	*0.27*	*0.52*	*0.69*	*0.35*	*0.68*	*0.87*	*0.89*	*0.98*	**1.00**						
bep	*0.32*	-0.03	0.17	0.06	*0.24*	*0.45*	*0.64*	*0.31*	*0.61*	*0.79*	*0.81*	*0.96*	*0.97*	**1.00**					
bap	*0.26*	-0.05	0.18	0.08	*0.21*	*0.45*	*0.62*	*0.32*	*0.63*	*0.84*	*0.84*	*0.96*	*0.98*	*0.98*	**1.00**				
per	0.09	*0.32*	-0.03	-0.22	0.02	0.16	*0.42*	*0.33*	*0.48*	*0.58*	*0.60*	*0.66*	*0.69*	*0.76*	*0.72*	**1.00**			
bgp	*0.34*	-0.08	*0.21*	0.10	*0.26*	*0.49*	*0.66*	*0.30*	*0.61*	*0.81*	*0.83*	*0.99*	*0.99*	*0.98*	*0.98*	*0.69*	**1.00**		
dba	*0.33*	-0.06	*0.38*	0.11	0.17	*0.37*	*0.50*	*0.35*	*0.57*	*0.70*	*0.70*	*0.86*	*0.82*	*0.75*	*0.80*	*0.46*	*0.84*	**1.00**	
inp	*0.32*	-0.03	0.17	0.09	*0.26*	*0.44*	*0.63*	*0.27*	*0.59*	*0.78*	*0.80*	*0.95*	*0.96*	*0.99*	*0.97*	*0.76*	*0.97*	*0.73*	**1.00**

* Statistically significant correlation coefficients ($p < 0.05$) are in italics

[#] Correlation coefficients over 0.5 are underlined

air for wood combustion. McDonald et al. (2000) further identified this compound as an indicator for softwood burning. Retene has also been found at levels similar to our results (in pg/m^3 range) in aerosols over the rural western U.S (Simoneit and Mazurek, 1982). Our observation of the high retene concentration at YG is consistent with that air monitor located in a heavily vegetated rural forest park with wood burning as a potential prime source for atmospheric PAHs in the area.

3.3.1.3 Relationship of PAH Concentrations with Other Air Pollutants

Correlation analysis between concentrations of PAH and other air pollutants has been used to deduce the emission source, fate and transportation pathway for these compounds in the atmosphere (Crimmins et al., 2004; Lodovici et al., 2003; Ohura et al., 2004; Papageorgopoulou et al., 1999; Wu et al., 2005). We performed linear regression analyses on the concentrations of individual PAHs as well as between the total PAH concentration and other pollutants concurrently measured on all samples. Not surprisingly, individual PAHs of similar size were well correlated with each other (Table 3.4), and overall, larger PAHs are more correlated than smaller PAHs. An exception is the case of retene, which is an alkyl-substituted three-ring PAH. The correlation coefficients (r) were -0.11 – 0.67, 0.16 – 0.99, and 0.46 – 0.99 between PAHs with 3, 4, and 5–6 aromatic rings, respectively (Table 3.4). Similar observation for the correlation among PAHs has also been reported in dust fall samples in China (Wu et al., 2005) where PAHs with comparative ring sizes were better correlated, suggesting a similarity both in source and in physico-chemical properties (i.e. similar atmospheric fate).

Correlations between total PAH concentration and $PM_{2.5}$ mass and its chemical components, i.e., OC, EC, potassium ion (K^+), sodium ion (Na^+), ammonium ion (NH_4^+), nitrite ion (NO_3^-), and sulfate ion (SO_4^{2-}), were season-specific. The total PAH concentrations were significantly correlated with total $PM_{2.5,}$ OC and EC in both December and June (Figure 3.5). However, in these two months, there was a large

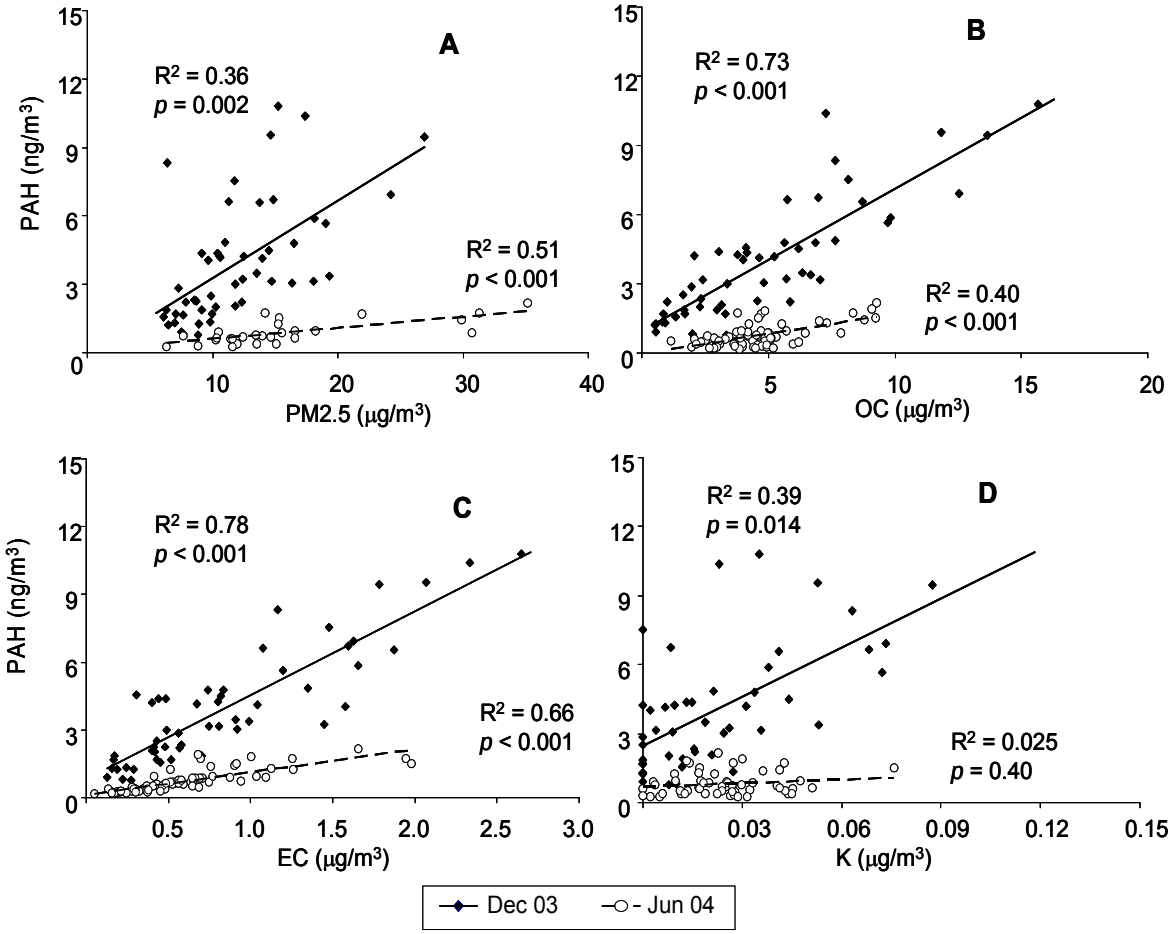

Figure 3.5. Correlations of the total PAH concentration to (A) $PM_{2.5}$, (B) OC, (C) EC, (D) potassium ion. Data from all sites are used, with the exception of the PAH vs. $PM_{2.5}$ plot (A) in June 2004. In this latter instance only data from South Dekalb (suburban, highway) were used, given that in June 2004 the $PM_{2.5}$ data were not available from the Fort McPherson (urban) and Fort Yargo (rural) sites

difference in the percent-fraction contribution of the $PM_{2.5}$ and in the OC concentrations from PAHs. The total PAH concentration represented on average 0.033% of the $PM_{2.5}$ mass and 0.137% of the OC concentrations in December, compared with 0.006% and 0.017% of the $PM_{2.5}$ and OC in June. This is a 30-fold lower percent fraction which is possibly a result of lower particle phase partition and higher atmospheric degradation caused by higher ambient temperature in June. Potassium ion, a tracer for biomass burning, was positively associated with PAH concentration in December ($r^2 = 0.39$, $p = 0.014$), but not in June (Figure 3.5), suggesting biomass burning can potentially be an

important source for particulate PAH in winter. On the other hand, PAHs were also associated with NH_4^+, NO_3^-, and SO_4^{2-} in June (r^2 = 0.53, 0.51, and 0.52, $p < 0.001$, data not shown), but not in December. Our findings differ from a study conducted on the northern Indian Ocean atmosphere, where particulate PAHs were correlated with SO_4^{2-}, K^+ and EC, but not with OC (Crimmins et al., 2004). Still, such differences are not surprising, given the various sources and atmospheric chemistry affecting the Atlanta airshed as compared with air over the northern Indian Ocean.

3.3.2 Results and Discussion from the Year-round Study

3.3.2.1 Ambient Levels of Particulate PAHs – Seasonal Variation

Average concentrations of the 28 particle-bound PAHs obtained in this study are presented in Table 3.5, arranged by sampling site and quarters of the year. Figure 3.6 illustrates the average total PAH concentrations (sum of the 28 analytes) at the three sampling sites in each month as well as in the whole year of 2004. Strong monthly trends were observed for both individual compounds and the total PAH concentrations at all three sites (Table 3.5 and Figure 3.6). Particle-bound PAH concentrations varied inversely with temperature; i.e. high in the cold months and low in the warm months (Figure 3.7A). For example, monthly average total PAH concentrations were 0.38 ng/m^3 to 0.98 ng/m^3 during May-September 2004 (monthly average of daily maximum temperature 26-31ºC), compared to 2.12-6.85 ng/m^3 during January-February and November-December 2004 (monthly average of daily maximum temperature 11-18ºC) at the urban and surburban-highway sites (Figure 3.6). The particle-bound PAH levels and seasonal trend in the current study are consistent with the pilot study and has been discussed in the previous section 3.3.1.1 (Li et al., 2009b).

Table 3.5. Average concentrations (ng/m^3) of $PM_{2.5}$-bound of PAHs at three sampling sites in Atlanta during year 2004

Analyte	Abbr	LOD[2] (ng/m^3)	FM (urban)				SD (suburban-highway)				YG (rural)			
			Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec
naphthalene	NAP	0.009	0.015	0.014	0.013	0.011	0.019	0.013	0.014	0.017	<LOD	<LOD	<LOD	0.012
2-methylnaphthalene	2M-NAP	0.009	0.022	0.014	0.012	0.020	0.027	0.016	0.017	0.033	0.010	<LOD	<LOD	0.013
1-methylnaphthalene	1M-NAP	0.009	0.011	0.010	<LOD	0.010	0.014	<LOD	<LOD	0.015	<LOD	<LOD	<LOD	<LOD
biphenyl	BIPH	0.006	0.011	0.012	0.010	0.012	0.010	0.009	0.011	0.011	0.009	0.011	0.008	0.007
acenaphthylene	ACNYL	0.002	0.009	0.005	0.003	0.009	0.010	0.005	0.004	0.010	0.006	0.003	<LOD	0.006
acenaphthene	ACNAP	0.002	0.005	0.007	0.005	0.005	0.005	0.005	0.006	0.004	0.004	0.007	0.006	0.006
fluorene	FLUO	0.007	0.051	0.080	0.080	0.084	0.044	0.076	0.071	0.060	0.046	0.088	0.050	0.063
dibenzothiophene	DBT	0.002	0.003	<LOD	<LOD	0.002	0.002	<LOD	<LOD	0.002	<LOD	<LOD	<LOD	<LOD
phenanthrene	PHEN	0.015	0.042	0.017	0.021	0.041	0.044	0.021	0.024	0.065	0.024	<LOD	<LOD	0.023
anthracene	ANTH	0.003	0.008	0.005	0.004	0.012	0.009	0.005	0.005	0.015	0.007	<LOD	<LOD	0.007
3-methylphenanthrene	3M-PHEN	0.004	0.009	0.007	0.006	0.015	0.014	0.010	0.007	0.021	0.005	<LOD	<LOD	0.006
2-methylphenanthrene	2M-PHEN	0.003	0.038	0.034	0.027	0.042	0.043	0.041	0.032	0.051	0.027	0.022	0.021	0.030
9-methylphenanthrene	9M-PHEN	0.003	0.008	0.004	<LOD	0.013	0.012	0.006	0.004	0.017	0.007	<LOD	<LOD	0.005
1-methylphenanthrene	1M-PHEN	0.002	0.009	0.003	<LOD	0.012	0.011	0.005	0.003	0.017	0.006	<LOD	<LOD	0.005
fluoranthene	FLRAN	0.006	0.083	0.028	0.021	0.088	0.071	0.035	0.023	0.101	0.050	0.017	0.007	0.040
pyrene	PYR	0.002	0.083	0.027	0.018	0.097	0.079	0.034	0.021	0.113	0.050	0.011	0.004	0.039
retene	RET	0.008	0.481	0.030	<LOD	0.155	0.394	0.017	<LOD	0.162	0.377	0.033	<LOD	0.133
benzo(c)phenanthrene	BCP	0.002	0.016	0.004	0.002	0.019	0.016	0.005	0.003	0.026	0.011	0.003	<LOD	0.011
benzo(a)anthracene	BAA	0.002	0.068	0.020	0.011	0.106	0.085	0.031	0.018	0.170	0.053	0.008	0.003	0.053
chrysene	CHRY	0.002	0.133	0.039	0.023	0.177	0.152	0.053	0.031	0.261	0.104	0.017	0.008	0.091
benzo(b)fluoranthene	BBF	0.002	0.302	0.091	0.050	0.438	0.425	0.120	0.057	0.664	0.283	0.042	0.017	0.409
benzo(k)fluoranthene	BKF	0.002	0.091	0.028	0.016	0.137	0.134	0.041	0.018	0.219	0.087	0.014	0.005	0.137
benzo(e)pyrene	BEP	0.002	0.175	0.068	0.039	0.265	0.253	0.093	0.045	0.398	0.155	0.030	0.013	0.237
benzo(a)pyrene	BAP	0.002	0.142	0.040	0.020	0.265	0.219	0.066	0.025	0.492	0.129	0.015	0.006	0.199
perylene	PER	0.002	0.025	0.008	0.005	0.045	0.038	0.013	0.005	0.080	0.022	0.003	<LOD	0.040
indeno(123-cd)pyrene	IP	0.002	0.185	0.074	0.044	0.280	0.299	0.094	0.044	0.420	0.172	0.035	0.017	0.233
dibenz(a,h)anthracene	DBA	0.002	0.022	0.008	0.004	0.032	0.033	0.010	0.003	0.051	0.023	0.004	<LOD	0.039
benzo(ghi)perylene	BP	0.002	0.297	0.155	0.091	0.465	0.445	0.206	0.092	0.668	0.214	0.048	0.032	0.294

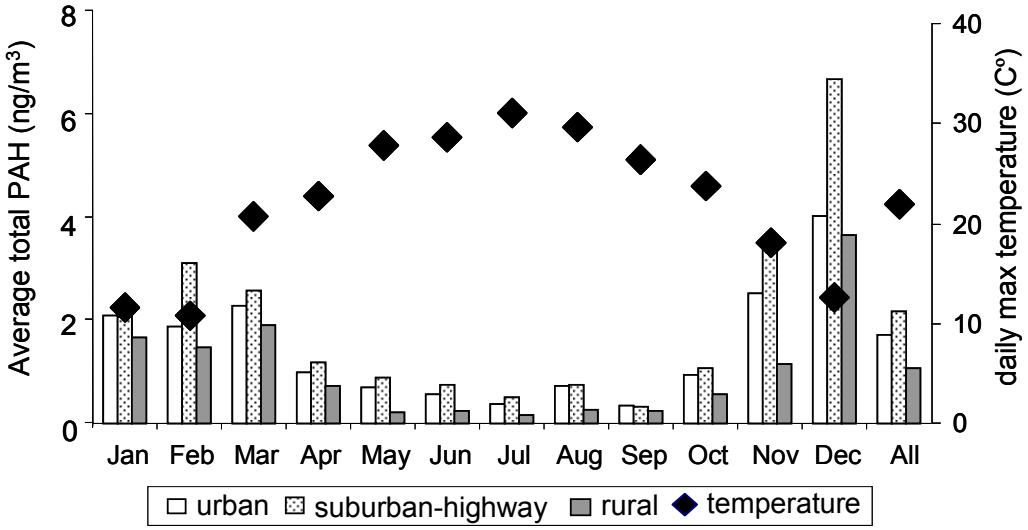

Figure 3.6. Average concentrations of total PAHs at three sampling sites in 2004. All shown are monthly average of daily maximum temperatures in Atlanta during year 2004

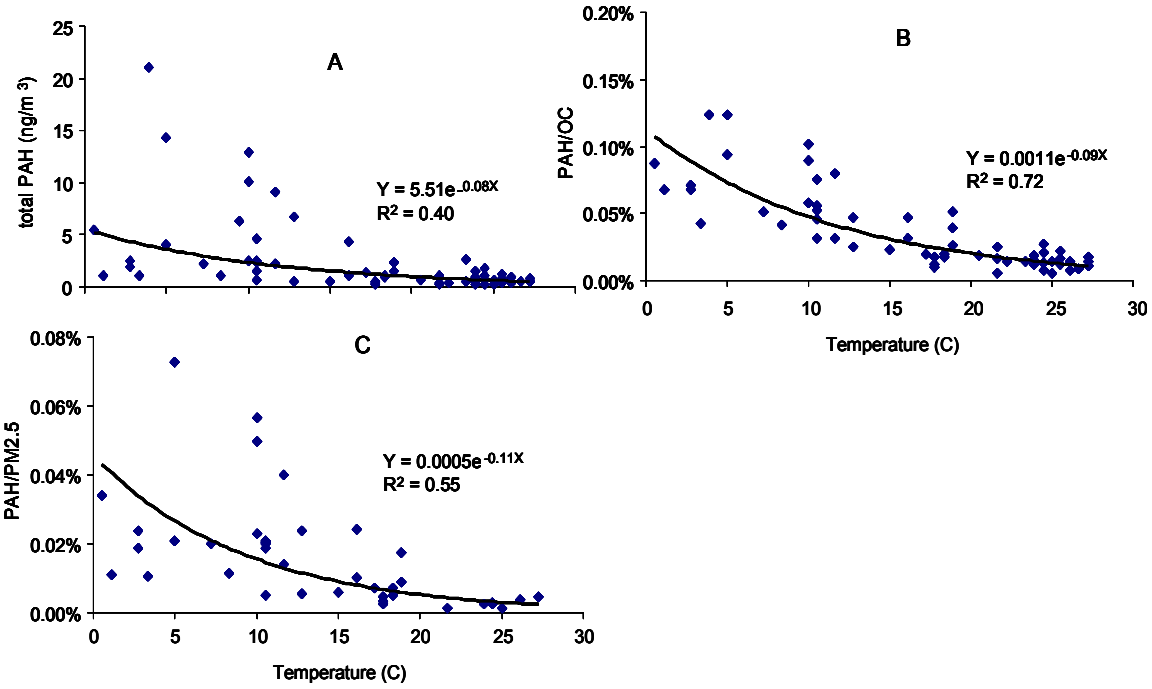

Figure 3.7. Relationship between ambient temperature and total PAH concentrations (A), PAH/OC ratio (B) and PAH/$PM_{2.5}$ ratio (C). Data from South Dekalb (suburban-highway) site were used

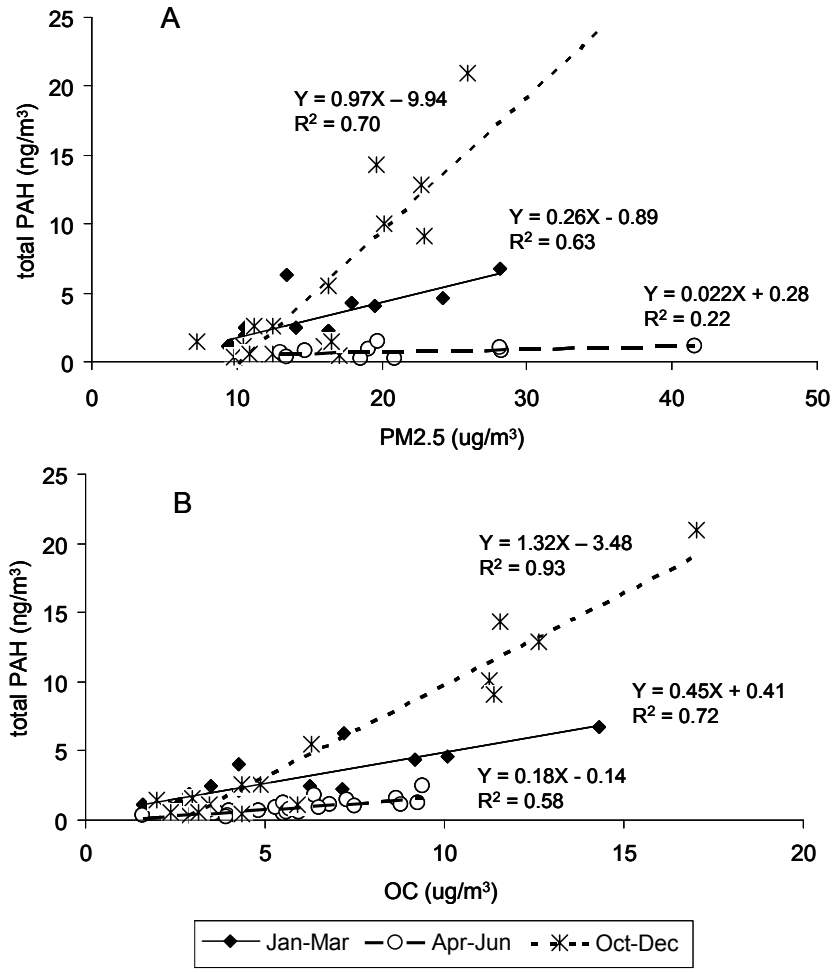

Figure 3.8. Correlations between concentration of total PAHs and $PM_{2.5}$ (A) and OC (B). Data from South Dekalb (suburban-highway) site were used

3.3.2.2 Relationship of PAH Concentrations with $PM_{2.5}$, OC and Other Air Pollutants

Overall, PAHs were poorly correlated to $PM_{2.5}$ and OC when combining the data from the whole year. However, when considering individual seasons, as shown in Figure 3.8, total PAH concentration was positively correlated to $PM_{2.5}$ and OC, which is consistent with the pilot study (Li et al., 2009b). Specifically, the slopes of the correlations, i.e. fractions of particulate PAHs in the $PM_{2.5}$ and OC, were highest during the October-December period, followed by the first quarter of the year (January-March), and were lower during the spring and summer quarters (April-September). Similar to the particle-bound PAH concentrations, the PAH fractions in $PM_{2.5}$ and OC were inversely

related to the temperature (Figures 3.7 B and C), which is likely due to the semi-volatile nature of PAHs. At the higher temperatures of summer, a larger fraction of PAHs will be in the gas phase instead of the particulate phase. Moreover, sulfate, a secondary air pollutant and a major component of $PM_{2.5}$ in this region (Butler et al., 2003), is formed more at higher temperatures, contributing to higher $PM_{2.5}$ concentrations in summer. Thus, the opposite seasonal variation of total $PM_{2.5}$ mass further drives the seasonal trend of lower PAH-to-$PM_{2.5}$ in the summer.

While PAHs in ambient air are mostly from primary sources (i.e., from direct emissions), sources of OC in the atmosphere are both primary and secondary. Although overall OC concentrations peaked in winter, the secondary components of OC tend to be higher in summer when elevated ozone oxidizes gaseous organics to form compounds in the particle phase (Marmur et al., 2007). On the other hand, ozone has been found to be negatively correlated with PAH concentrations in the atmosphere, suggesting that oxidation by ozone is a potential sink for atmospheric PAHs (Tham et al., 2008). Therefore, the PAH-to-OC ratio is inversely related to temperature.

3.3.2.3 Ambient Levels of Particulate PAHs – Site Variation

Not surprisingly, the suburban site (SD), located about 1 km from a major highway, recorded significantly higher PAH levels than did FM, the more central urban site (paired t-test, $p = 0.005$, Figure 3.6). PAH emissions from roadways are known to be a significant contributors to airborne PAHs in the central parts of large cities (Bostrom et al., 2002; Narvaez et al., 2008). On the other hand, the rural site (YG) had the lowest PAH levels of the three sampling locations (paired t-test, $p < 0.01$, Figure 3.6). The average total PAH concentration over the whole year at YG was 1.78 ng/m^3, compared to 2.92 ng/m^3 and 1.92 ng/m^3 at SD and FM, respectively. This is consistent with the locations of the samples sites – the rural site was in a heavily vegetated forest park with minimum anthropogenic PAH emissions, whereas the urban and suburban-highway sites

were located in densely populated and heavily trafficked areas with a high anthropogenic PAH input.

3.3.2.4 Methyl Phenanthrenes – Indicator for Fuel Sources

PAHs in aerosols can come from two types of sources, petrogenic (i.e. unburned petroleum products) or pyrogenic (i.e. combustion of fossil fuels and biomass) (Simo et al., 1997). Pyrogenic PAHs are generally abundant with non-substituted or parent PAHs, whereas petrogenic PAHs include more alkylated PAHs than their parent homologues (Simo et al., 1997; Yunker et al., 2002). Therefore, the relative concentration of the sum of methylated phenanthrenes (MePhe) to their un-substituted parent compound (phenanthrene or Phe), i.e. MePhe/Phe or MePhe/(MePhe+Phe), has been used as an indicator for petrogenic inputs (Yunker et al., 2002; Zakaria et al., 2002).

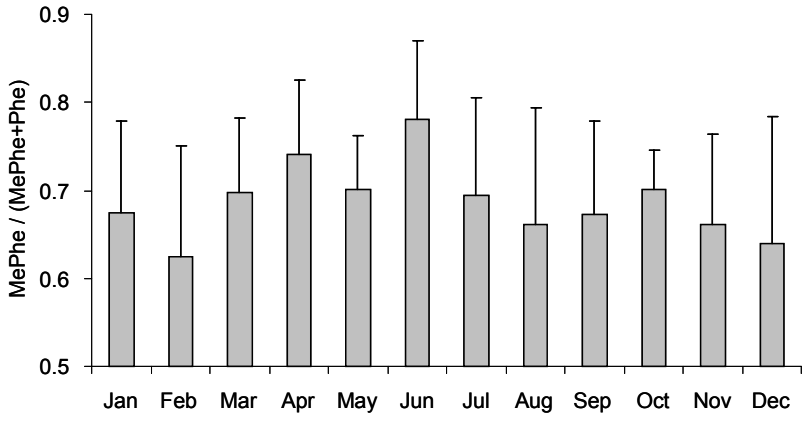

Figure 3.9. Average (with standard deviation) ratio of summed methyl phenanthrenes (MePhe) to the total of phenanthrene and methyl phenanthrenes, stratified by month. Data from the urban and suburban-highway sites were used

Among the four methyl-substituted phenanthrenes measured in this study, 2-MePhe was present at the highest levels (Table 3.5), consistent with what was found in used crankcase oil samples (Zakaria et al., 2002) and in the urban air particulate Standard Reference Material (SRM) 1649 from the National Institute of Standards and Technology (NIST), USA (NIST, 2007). Interestingly, 2-MePhe was also reported to have the highest

aryl hydrocarbon receptor (AhR) inducing potency in rat liver cell lines among the four Me-Phe compounds (Vondracek et al., 2007). The MePhe/(MePhe+Phe) ratio from the urban and suburban-highway sites averaged 0.69 and ranged from 0.62 in February to 0.78 in June (Figure 3.9), indicating that petrogenic input is an important source for $PM_{2.5}$-bound PAHs in this area. Our MePhe/(MePhe+Phe) ratios are in good agreement with findings from aerosol samples taken in high traffic urban area and a power plant in Spain (Simo et al., 1997), but are higher than three previous studies of urban air samples, including a study in Los Angeles and NIST SRM 1648 and 1649a (Fraser et al., 1998; NIST, 2007; Wise et al., 1988). The relative abundance of MePhe isomers to their parent compound were slightly higher during the warmer months than the cooler months (Figure 3.9), which is consistent with a greater contribution from unburned fossil fuel (e.g. fuel evaporation from the roadway) with higher ambient temperature. However, the difference of the ratios between seasons was not statistically significant, suggesting that such ratios should be used with caution and can only serve as a crude source indicator.

3.3.2.5 Retene – a Marker for General Biomass Burning

In the current study, retene had a distinct monthly variation (Figure 3.10A), different from the rest of the PAHs and other pollutants such as K^+ (Figure 3.10B). The highest retene concentrations at all three sites occurred in March, ranging from 0.81 to 1.10 ng/m^3 between the three sampling sites (Figure 3.10A). The levels in March are 3.0 to 4.2 times higher than levels in December, the month with the second highest retene concentration. Interestingly, both the subscribed burning and reported unplanned fires (wildfires) in the state of Georgia (Personal communication from the Georgia Forestry Commission) also peaked dramatically in March in 2004, in terms of total acreage burnt (Figure 3.10C) and total number of fires recorded (data not shown). Prescribed burning, a practice commonly used in forest resource management in the southeastern United State, is used primarily to burn forest fuels (a.k.a. “roughs”) accumulated under southern pine

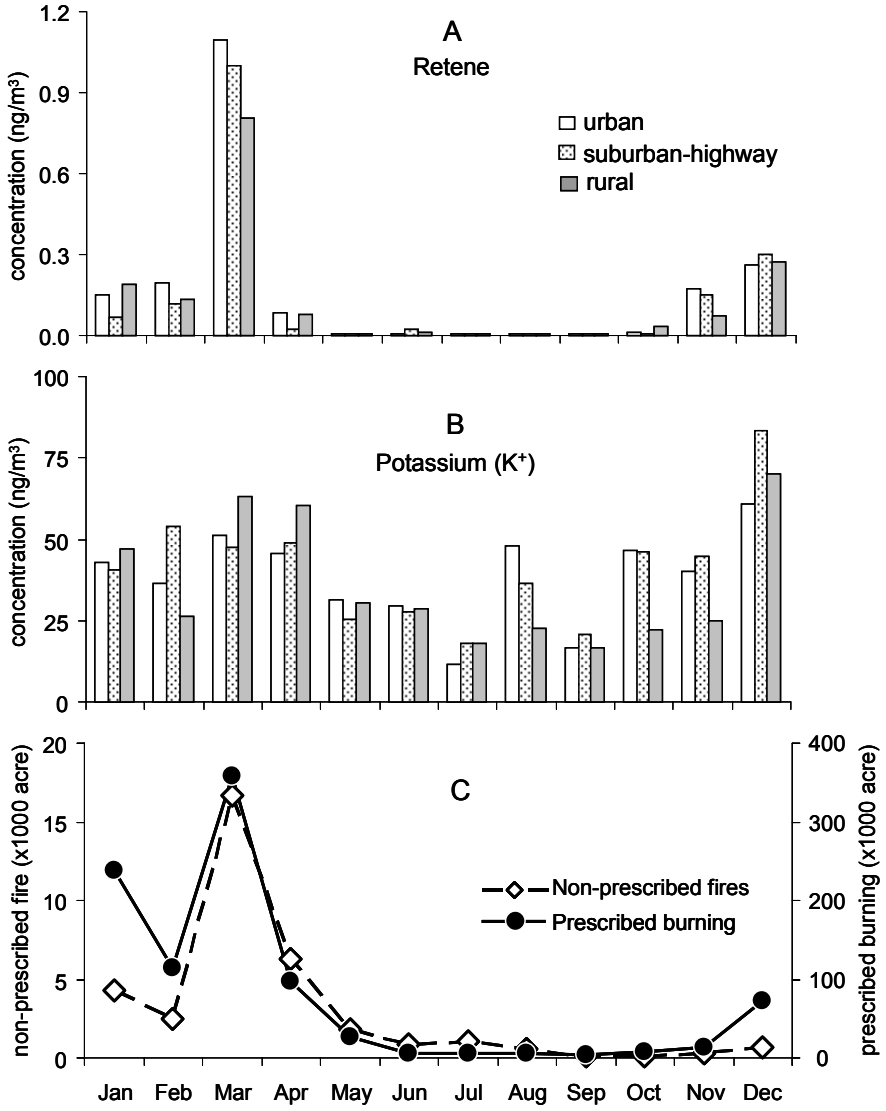

Figure 3.10. Average retene (A) and potassium ion (B) concentrations from three sites, as well as reported non-prescribed and prescribed burning (C) in Georgia during 2004

stands, e.g. brushes, branches, small plants and debris. Unlike intense forest fires in which large amounts of tree wood are burned, unplanned fires in this region are comprised of mostly burning of leaves and branches. December had the second highest retene concentration in the year 2004, possibly due to increased wood burning during this cold time of the year. In contrast, K^+, a tracer for biomass burning, was not elevated in March, but was higher in December and the other winter months and was lower during

warmer months. K^+ is considered to be present at relative high concentration in wood burning plumes, and its concentrations are readily available; therefore, it has been used as a biomass burning tracer in source apportionment studies (Sheffield et al., 1994). However, recent studies have shown that K^+ has other significant sources in urban areas, including refuse incinerators, meat cooking, and coal usages, which could lead to the application of K^+ as the biomass burning marker problematic (Wang et al., 2007). Overall, retene concentrations were correlated with K^+ (all sites included, correlation coefficient $r = 0.46$, $p < 0.001$) and this correlation improved when the high fire days in March were excluded ($r = 0.62$, $p < 0.001$). These results suggest that retene might be a better marker for all biomass burning because it captured both the high leaves-grasses-bushes-branches burning season in March and the high wood burning in winter; K^+, on the other hand, might be a more specific tracer for wood burning with its peak in December. Further, levoglucosan (1,6-anhydro-β-D-glucopyranose) has been used as a marker for biomass burning, because it is a pyrolysis product of cellulose and is one of the major organic components in ambient PM emitted from biomass combustion. However, measurement of levoglucosan can be more difficult than K^+ and retene. Therefore, retene, in comparison to K^+ and levoglucosan, might be a better and readily measurable tracer for biomass burning.

CHAPTER 4

MEASUREMENT OF URINARY MONO-HYDROXY POLYCYCLIC AROMATIC HYDROCARBONS USING AUTOMATED LIQUID-LIQUID EXTRACTION AND ISOTOPE DILUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY

(Li Z, Romanoff LC, Trinidad DA, Hussain N, Jones RS, Porter EN, Patterson Jr. DG, and Sjodin A. *Analytical Chemistry*, 78:5744-51, 2006)

Abstract

A method for the measurement of 24 hydroxylated polycyclic aromatic hydrocarbon metabolites (OH-PAHs) in urine has been developed. The method is based on enzymatic deconjugation, automated liquid-liquid extraction, and isotope dilution gas chromatography/high resolution mass spectrometry (GC/HRMS) after derivatization of the OH-PAHs to the trimethylsilylated derivatives. The metabolites included in the current method are formed from eight different parent compounds. The limits of detection were below 7 pg/mL when using a sample size of 2 mL urine, except for 1- and 2-naphthols (12 and 18 pg/mL respectively). The enzymatic deconjugation efficiency, verified by deconjugation of urine samples spiked with α-naphthyl-β-D-glucuronide sodium salt (1-NAP-GLU) and pyrene-1-sulfate potassium salt (1-PYR-SULF), was determined to be 97% for 1-NAP-GLU conjugate, and 84% for 1-PYR-SULF. The overall coefficients of variance (CV) for six batches of quality control samples (n=42), was 2.9–11%. Mean method recoveries of the ^{13}C-labeled internal standards were 66–

72%, except for $^{13}C_6$-1-naphthol (46%). The throughput of this method has been determined to be 40 samples per day per analyst. This method is currently applied to epidemiological studies, such as the National Exposure and Nutrition Examination Survey (NHANES), to measure human exposure to PAHs

4.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of environmental pollutants formed during incomplete combustion of organic materials such as wood, fossil fuels, meat, and tobacco. They are found in automobile exhaust, wood smoke, cigarette smoke, and also found at high concentrations in certain food such as barbequed food (ATSDR, 1995; Bostrom et al., 2002; Guillen et al., 1997; IARC, 1983); thus this class of compounds is ubiquitously distributed in ambient air, food, soil, as well as in many occupational environments.

PAHs have generated considerable interest, not only because of their wide distribution in the environment, but also their carcinogenic and mutagenic potential (ATSDR, 1995; IARC, 1983). PAHs, such as benzo[a]pyrene (BAP), which is present in cigarette smoke and certain occupational environments, have been positively associated with lung cancer (Armstrong et al., 2004; Denissenko et al., 1996). In addition, PAHs have also been reported to have reproductive, developmental, hemato-, cardio-, neuro-, and immuno-toxicities in humans and laboratory animals (ATSDR, 1995; IARC, 1983). Because of their toxicity and carcinogenicity potential, 16 PAHs have been included in the U.S. Environmental Protection Agency's list of 188 hazardous air pollutants (EPA, 1990). Humans are exposed to PAHs through inhalation of ambient air or cigarette smoke and ingestion of food containing PAHs, as well as dermal absorption from soil or material that contains PAHs. For the general population, ingestion and inhalation are the two major routes of exposure, with the former as the main route of PAH exposure (ATSDR, 1995; Bostrom et al., 2002; Ramesh et al., 2004). For certain occupations, such

as coal tar roofers and coke oven workers, dermal absorption can become the primary route of PAH exposure (ATSDR, 1995).

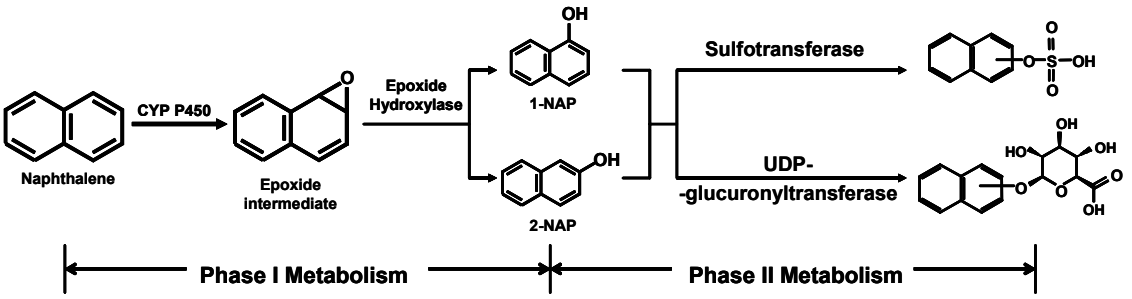

Figure 4.1. Schematic illustration of the metabolic formation of hydroxy-naphthalene glucuronic acid and sulfate conjugates demonstrating that multiple metabolic products can be formed from a single parent compound

After entering the human body, PAHs undergo rapid initial distribution to fatty tissues. The liver is often regarded as a primary site for biotransformation of PAHs regardless of the route of entry, because this organ contains many of the enzymes needed in the biotransformation of the parent compounds (Bostrom et al., 2002; Miller and Ramos, 2001; Ramesh et al., 2004). However, at lower levels of inhalation exposure, metabolism in the lungs can dominate (Bostrom et al., 2002). The biotransformation process, as illustrated in Figure 4.1, starts with Phase I metabolism in which PAHs are oxidized by the cytochrome P450 enzymes to form reactive epoxide intermediates, followed by reduction or hydrolysis to hydroxylated derivatives (OH-PAHs). In Phase II metabolism, the OH-PAHs are conjugated to glucuronic acid and/or sulfate to increase the water solubility of the metabolite and to facilitate elimination through urine, bile, or feces (Grover, 1986; Pelkonen and Nebert, 1982). These biological processes lead to the formation of multiple metabolites including epoxide, dihydrodiols, mono- and polyhydroxy PAHs. Generally, metabolites of smaller PAHs with two to three rings are excreted preferentially in the urine, whereas larger PAHs with higher molecular weight are excreted primarily in the feces (Ramesh et al., 2004). The P450 enzyme used in the Phase I and UDP-glucuronyl transferases in the Phase II metabolism are both

microsomal, while the sulfotransferase enzyme responsible for catalyzing the sulfate conjugation is cytosolic (Grover, 1986; Hakk et al., 2001). Due to the short half-lives (12~15 hours) of the urinary OH-PAH elimination (Buckley and Lioy, 1992; Viau and Vyskocil, 1995), quantitative determination of OH-PAH metabolites provides information on a subject's recent exposures to environmental PAHs.

Urinary OH-PAHs have been used as biomarkers to assess total human exposure to environmental PAHs, with 1-hydroxypyrene (1-PYR) as the most commonly used indicator in biomonitoring studies (Jacob and Seidel, 2002). Jongeneelen et al. (1985) reported one of the first analytical methods to measure 1-PYR and other OH-PAHs in urine, using enzymatic deconjugation, solid phase extraction (SPE), HPLC separation, and fluorescence detection (Jongeneelen et al., 1985; Jongeneelen et al., 1987). Gundel et al. (2000) expanded the HPLC method to include more analytes such as phenanthrene metabolites, and applied the method to various occupational exposure studies. Grimmer et al. (1997) developed a method utilizing gas chromatography/mass spectrometry (GC/MS) to measure methylated derivatives of the hydroxylated metabolites of phenanthrene, pyrene, fluoranthene, chrysene, and benzo[a]pyrene in urine. One of the most extensive OH-PAH methods which measured up to 23 OH-PAHs was developed at the Centers for Disease Control and Prevention (CDC), using enzymatic hydrolysis, solid phase extraction, derivatization of OH-PAHs to trimethylsilylated derivatives, and isotope-dilution gas chromatography/high-resolution mass spectrometry (GC/HRMS) (Romanoff et al., 2006; Smith et al., 2002). The method was used to analyze samples from the National Health and Nutrition Examination Survey (NHANES 1999–2002) (Grainger et al., 2006; Grainger et al., 2004; Huang et al., 2004), an on-going comprehensive survey CDC performs to assess exposure of the U.S. general population to environmental and industrial chemicals.

With increased interest in the biomonitoring approach for exposure assessment and with requirements for large epidemiological studies such as NHANES, it is necessary

to have a versatile, robust, and high-throughput method that measures multiple metabolites in a minimum amount of sample. In this paper, we present a new method for measuring 24 OH-PAHs (Figure 2.2) in urine using enzymatic deconjugation, liquid-liquid extraction, and GC/HRMS. We studied extensively the enzymatic hydrolysis to provide maximum deconjugation efficiency for all analytes, and we further automated the liquid-liquid extraction step not only to increase the throughput, but also to reduce variability caused by manual sample handling.

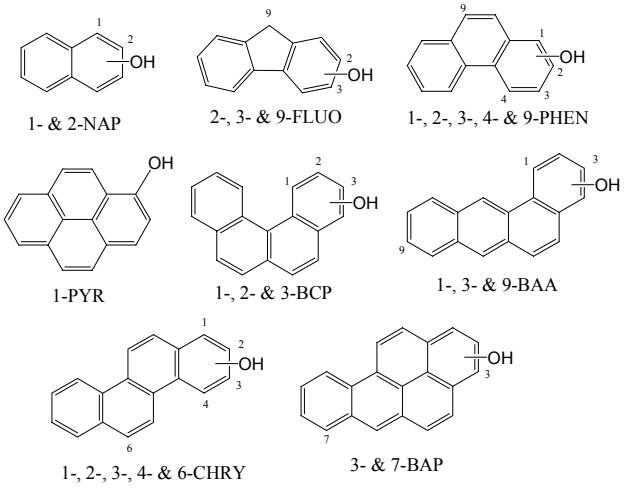

Figure 4.2. Molecular structures of the 24 OH-PAH analytes

4.2 Experimental

4.2.1 Safety Considerations

All laboratory work involving human specimens must follow universal precautions to minimize the risk of exposure. This includes the use of personal protective equipment such as gloves, lab coat, and protective eyewear. Any potential spills of human urine are decontaminated with a 10% bleach solution with a minimum contact time of 15 minutes. Any glassware or other items that come in contact with the samples are autoclaved or decontaminated with bleach prior to reuse or disposal. The experimenter must further be aware of the presence of 2,3,7,8-tetrachlorodibenzo-*p*-

dioxin (TCDD) in the instrument sensitivity verification solution and the potential health effects from exposure to TCDD (Myers and Patterson, Jr., 1987).

4.2.2 Chemicals

All reagents and solvents used in the current method were of the highest available grade or intended for pesticide residue analysis and were used only after verification by GC/HRMS. Pentane, n-hexane, acetonitrile, and toluene were of pesticide grade (>99.8%) and obtained from Tedia Company Inc. (Fairfield, OH). The glacial acetic acid (>99.8%) was purchased from J.T. Baker (Phillipsburg, NJ). Sodium acetate (>99.0%), dodecane (99%), α-naphthyl-β-D-glucuronide sodium salt, and N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) were purchased from Sigma-Aldrich (St. Louis, MO). Pyrene-1-sulfate potassium salt was purchased from Midwest Research Institute (Kansas City, MO). De-ionized water (D.I. water) was purified by the 2000^{TM} Solution Consultants Inc. Solution Water Purification System (Jasper, GA). Ultra-high purity nitrogen, helium and argon were obtained from Airgas South Co. (Chamblee, GA). $^{13}C_{12}$-labeled 3-hydroxy-3,3',4,5'-tetrachlorobiphenyl was purchased from Cambridge Isotope Laboratory (Andover, MA) and methylated in house to its methoxy derivative ($^{13}C_{12}$-3-MeO-CB79). β-Glucuronidase type H-1 enzyme with sulfatase activity, isolated from *Helix pomatia (Helix pomatia* enzyme*),* was obtained from Sigma-Aldrich (St. Louis, MO). β-Glucuronidase enzyme, isolated from *Escherichia coli (E. Coli) K12 (E. coli* enzyme*),* was purchased from Roche Diagnostic (Indianapolis, IN). All native and ^{13}C-labeled mono-hydroxylated PAH analytes, their abbreviations, together with their suppliers are listed in Table 4.1.

4.2.3 Standard Preparation

We prepared stock solutions (1 mg/mL) from neat materials by weighing approximately 5 mg of each compound into a silanized amber vial and dissolving it in

acetonitrile. The internal standard spiking solution contained thirteen ^{13}C-labeled OH-PAHs (100 pg/μL in toluene). The recovery standard solution contained $^{13}C_{12}$-3-MeO-CB79 at a concentration of 300 pg/μL in toluene. Eight calibration standards were prepared in toluene spanning the range of 0.5–1000 pg/μL for each of the 24 analytes, except for 1- and 2-OH-naphthalene (1-NAP and 2-NAP), which were present at four times higher concentration. The thirteen ^{13}C-labeled OH-PAHs were present in each calibration standard at a constant level (100 pg/μL).

4.2.4 Sample Preparation Procedure

Urine samples (2 mL) were aliquoted into glass test tubes (16 x 100 mm) and fortified with internal standards (1000 pg/compound). Sodium acetate buffer (pH 5.5; 1 M; 1 mL) containing β-glucuronidase enzyme from *Helix pomatia* (10 mg enzyme/1 mL buffer) was added to the samples. Incubation was done at 37 °C overnight (~18 hours).

D.I. water (2 mL) was then added and the samples were extracted twice with pentane using the automated Gilson 215 liquid handler (Gilson Inc., Middleton, WI). The liquid handler was equipped with an 818 automix (Gilson), and fitted with a 402 syringe pump (Gilson), which was connected to a septum-piercing probe via a coiled transfer tubing. A reservoir containing n-hexane was connected to the syringe pump and used to purge the transfer tubing and rinse the probe after drawing and dispensing reagent. The samples to be extracted were placed in the automix tray and the extraction procedure was initiated. First, pentane (5 mL) was added to all samples and the samples were mixed by rotating in the automix for 5 minutes (20 rpm). The procedure was paused and the samples were centrifuged at 2800 rpm on an Allegra^{TM} 6 centrifuge (Beckman Coulter Inc., Fullerton, CA) for 20 minutes. After returning the samples to the automix tray, the procedure was resumed and the organic phase was transferred by the probe to clean collection tubes. This extraction procedure was repeated again with additional pentane (5 mL).

Table 4.1. Measured OH-PAH metabolites, abbreviations, ^{13}C-labeled internal standards and mass spectral descriptors

Analyte Name	Abbr.	Internal Standard (I.S.)	Masses Analyte	Masses I.S.	Mass Window
1-OH-naphthalene[a]	1-NAP	$^{13}C_6$-1-NAP[f]	216.0970	222.1172	1
2-OH-naphthalene[a]	2-NAP	$^{13}C_6$-1-NAP[f]	216.0970	222.1172	1
9-OH-fluorene[a]	9-FLUO	$^{13}C_6$-9-FLUO[g]	254.1127	260.1329	2
2-OH-fluorene[a]	2-FLUO	$^{13}C_6$-2-FLUO[g]	254.1127	260.1329	2
3-OH-fluorene[a]	3-FLUO	$^{13}C_6$-3-FLUO[g]	254.1127	260.1329	2
1-OH-phenanthrene[b]	1-PHEN	$^{13}C_6$-3-PHEN[h]	266.1127	272.1329	3
2-OH-phenanthrene[c]	2-PHEN	$^{13}C_6$-2-PHEN[g]	266.1127	272.1329	3
3-OH-phenanthrene[c]	3-PHEN	$^{13}C_6$-3-PHEN[h]	266.1127	272.1329	3
4-OH-phenanthrene[b]	4-PHEN	$^{13}C_6$-3-PHEN[h]	266.1127	272.1329	3
9-OH-phenanthrene[b]	9-PHEN	$^{13}C_6$-3-PHEN[h]	266.1127	272.1329	3
1-OH-pyrene[d]	1-PYR	$^{13}C_6$-1-PYR[i]	290.1127	296.1329	4
1-OH-benzo[c]phenanthrene[d]	1-BCP	$^{13}C_6$-3-BCP[i]	316.1283	322.1485	4
2-OH-benzo[c]phenanthrene[d]	2-BCP	$^{13}C_6$-3-BCP[i]	316.1283	322.1485	4
3-OH-benzo[c]phenanthrene[d]	3-BCP	$^{13}C_6$-3-BCP[i]	316.1283	322.1485	4
1-OH-benz[a]anthracene[d]	1-BAA	$^{13}C_6$-1- BAA[h]	316.1283	322.1485	4
3-OH-benz[a]anthracene[d]	3-BAA	$^{13}C_6$-1-BAA[h]	316.1283	322.1485	4
9-OH-benz[a]anthracene[d]	9-BAA	$^{13}C_6$-1-BAA[h]			
1-OH-chrysene[d]	1-CHRY	$^{13}C_6$-3-CHRY[i]	316.1283	322.1485	4
2-OH-chrysene[d]	2-CHRY	$^{13}C_6$-3-CHRY[i]	316.1283	322.1485	4
3-OH-chrysene[d]	3-CHRY	$^{13}C_6$-3-CHRY[i]	316.1283	322.1485	4
4-OH-chrysene[d]	4-CHRY	$^{13}C_6$-3-CHRY[i]	316.1283	322.1485	4
6-OH-chrysene[e]	6-CHRY	$^{13}C_6$-6-CHRY[h]	316.1283	322.1485	4
3-OH-benzo[a]pyrene[d]	3-BAP	$^{13}C_6$-3-BAP[h]	340.1283	343.1380	5
7-OH-benzo[a]pyrene[d]	7-BAP	$^{13}C_6$-3-BAP[h]	340.1283	343.1380	5

a. Sigma-Aldrich, St. Louis, MO; b. Promochem, Teddington, United Kingdom; c. Dr. Ehrenstorfer, Augsburg, Germany; d. Midwest Reseach Institute, Kansas City, MO; e. AccuStandard Inc., New Haven, CT; f. Synthesized in-house; g. Los Alamos National Laboratory, Los Alamos, NM; h. Cambridge Isotpe Laboratories, Andover, MA; i. Chemsyn Laboratories, Lenexa, KS

The combined pentane extract was spiked with dodecane (5 μL) as a keeper and concentrated to ~ 5 μL in a TurboVap LV® evaporator (Caliper Life Sciences, Hopkinton, MA), under a gentle stream of nitrogen (5-10 psi) at 40 °C. The extract was then reconstituted into toluene (20 μL), spiked with recovery standard ($^{13}C_{12}$-3-MeO-CB79, 300 pg/μL, in toluene, 5 μL), and transferred to GC vials. Finally, prior to GC/HRMS analysis, the samples were derivatized to their trimethylsilylated derivatives by adding MSTFA (10 μL) and incubating at 60 °C for 30 minutes.

4.2.5 GC/HRMS Method

GC/HRMS analyses were performed on a MAT 95 XL HRMS instrument (Thermo Finnigan, Bremen, Germany), coupled with a 6890 GC (Agilent Technologies, Palo Alto, CA). The chromatographic separations were carried out on a DB-5MS column (30m, 0.25 mm i.d., 0.25 μm film thickness; Agilent Technology, Palo Alto, CA) using helium (1mL/min) as the carrier gas. The injector was operated in the splitless mode at a temperature of 270°C. The column oven was programmed from 95°C (2min) to 160°C (15°C/min; 0min), then to 230°C (10°C/min; 5 min), and finally to 320°C (23°C/min; 3min). The source temperature was 250°C in the electron impact mode (50 eV). The descriptor used for the GC/HRMS analyses are given in Table 4.1. The sensitivity of the instrument, operated at a resolution of 10,000; was verified daily by the analysis of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD, 20 fg/μL, 1 μL injection) in the selective ion monitoring mode (SIM) [m/z 319.8965, 321.8937, 331.9368 and 333.9338]. For the daily instrument sensitivity test, the signal-to-noise ratio (S/N) needed to be at a minimum of 3 for 2,3,7,8-TCDD (m/z 321.8937).

4.2.6 Deconjugation Conditions

We determined conditions for enzymatic deconjugation in a step-wise manner. First, an experiment was set up to determine the amount of enzyme needed to achieve

maximum yields of deconjugated products, for β-glucuronidase enzymes from *E. coli* and *Helix pomatia* respectively. We performed this by varying the amount of enzyme for the incubation (24 hours, 37 °C), and comparing the yield of deconjugated products. In a follow-up experiment, the time required to reach the maximum yield of deconjugated products was determined by varying the incubation time using a fixed amount of enzyme.

The effect of pH on deconjugation kinetics was evaluated using the *Helix pomatia* enzyme. For this experiment, buffers were prepared in the pH range of 3.5 to 7, with increments of 0.25 units between pH 4 and 7. Three replicate urine samples were prepared for each pH-value, and the deconjugation reaction was interrupted after 3 hours (i.e., before complete deconjugation for most of the analytes). Yields of deconjugated products were plotted against pH to obtain a relative measure of the reaction kinetics at different pH-values.

With the chosen conditions, deconjugation efficiencies for α-naphthyl-β-D-glucuronide sodium salt (1-NAP-GLU) and pyrene-1-sulfate potassium salt (1-PYR-SULF) were determined by analyzing urine samples spiked with a known amount of these two conjugates and un-spiked samples. The deconjugation efficiencies were calculated as the concentration differences between spiked and unspiked samples compared with the spiked amount.

The relative composition of glucuronic acid and sulfate conjugates in urine was determined by deconjugation with *Helix pomatia* enzyme that contains both glucuronidase and sulfatase activity and *E. coli* enzyme containing only glucuronidase activity (Elsohly et al., 2005). The results from the deconjugation with *Helix pomatia* enzyme represent the sum of glucuronic acid and sulfate conjugates, while deconjugation with E. coli selectively deconjugates glucuronides. The sulfate conjugate concentration was calculated as the difference between these two measurements.

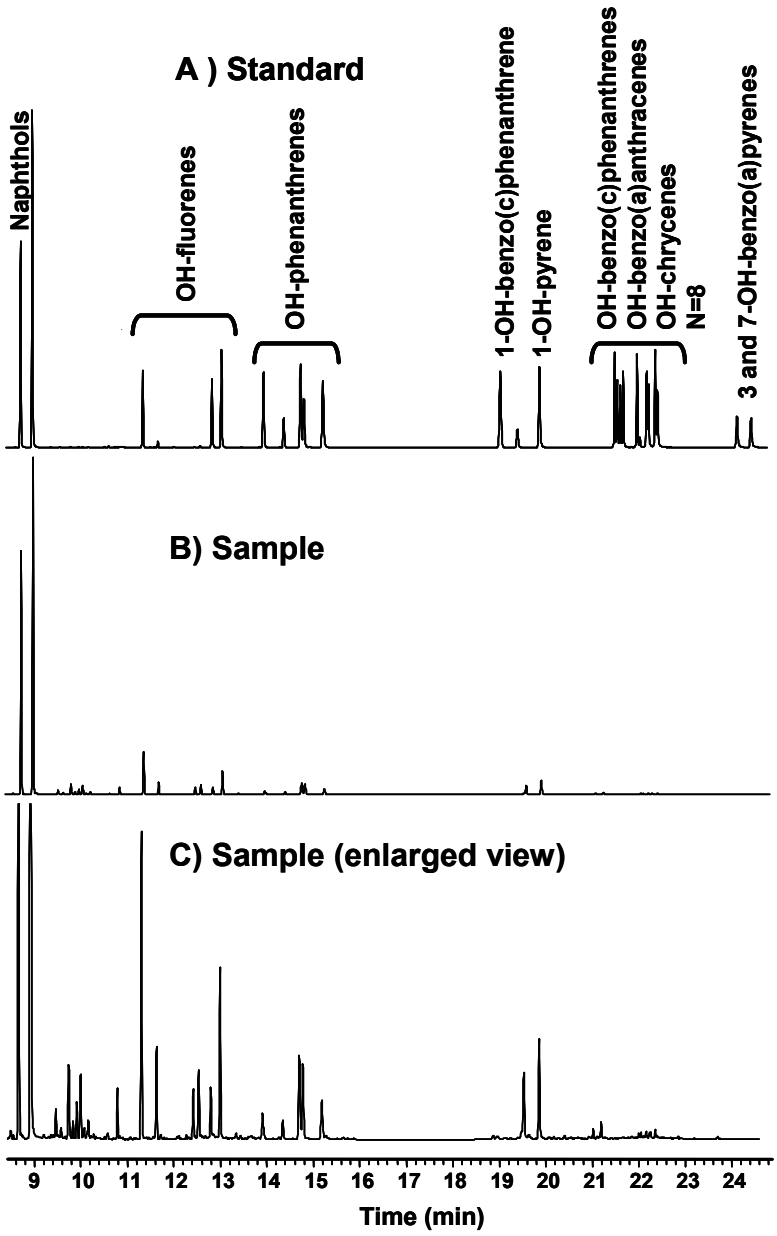

Figure 4.3. Gas chromatograms of a calibration standard (A) and a pooled urine sample (B, C).

4.2.7 Limit of Detection

The limit of detection (LOD) for this method was defined as the higher LOD calculated by two methods: (*i*) in direct relation to method blanks prepared in parallel with the unknown samples, as 3 times the standard deviation of the method blanks, and

(ii) according to the instrumental detection limit defined as the lowest point in the calibration curve (5 pg/μL) verified to give a signal with the S/N equal to or greater than 3. All valid measurements above the LOD are subtracted by the average blank to correct for any analytical background.

4.2.8 Quality Assurance and Quality Control

Anonymous urine samples were collected from multiple donors, pooled, pressure-filtered through a 0.45–μm SuporCap – 100 Capsule (Pall Corp. Ann Arbor, MI), diluted with D.I. water (1 part D.I. water to 4 parts of filtered urine), and used to prepare quality control (QC) materials. The pool was spiked with a standard mixture of the 24 native OH-PAHs in acetonitrile (500 pg/mL urine), and homogenized at room temperature overnight. The next day the spiked urine pool was aliquoted into 16 x 100 mm glass culture tubes (2 mL in each tube), and stored at -70°C until use.

In this method, a set of samples was defined as 16 urine samples, prepared and analyzed together with two water blanks and two QC samples. In addition, an external recovery standard (ERS) and a calibration standard were derivatized and analyzed on the GC/HRMS in parallel with the sample extracts. The ERS contained the ^{13}C-labeled OH-PAH internal standards and the recovery standard ($^{13}C_{12}$-3-MeO-CB79) and was used as a reference for the recovery calculation. Analysis of a set of samples was considered valid only after fulfilling the following criteria: (i) relative retention time (RRT), defined as the retention time ratio of a native compound over its ^{13}C-labeled internal standard, must be within ± 2% of the reference ratio set by the calibration standard run with the samples, and (ii) recovery of the ^{13}C-labeled internal standard must be within the range of 25–150%. Further, the following criteria must be fulfilled for this set of samples to pass the QA/QC requirements: (i) the measurement of the target analytes in the QC sample must be within 3 standard deviations (SD) of the established mean, and (ii) 10 or more consecutive measurements may not fall above or below the established mean. A SAS

computer program, developed in house, was further used to identify QC samples failing any of the criteria as defined elsewhere (Westgard et al., 1981).

Table 4.2. Quality control parameters for measuring urinary OH-PAH metabolites

Analyte Name	Recovery (%)	Conc. (pg/mL)	Coefficient of variation (%)	Limit of detection (pg/mL)
1-OH-naphthalene	46	1584	2.9	18[a]
2-OH-naphthalene	46	2912	3.3	12[a]
9-OH-fluorene	66	596	2.9	3.9[a]
2-OH-fluorene	69	559	3.0	4.5[a]
3-OH-fluorene	70	485	2.8	6.9[a]
1-OH-phenanthrene	70	391	4.4	2.6[b]
2-OH-phenanthrene	69	606	3.5	3.8[a]
3-OH-phenanthrene	70	533	3.2	2.6[b]
4-OH-phenanthrene	70	531	3.9	3.7[a]
9-OH-phenanthrene	70	191	7.1	2.6[b]
1-OH-pyrene	71	542	3.4	4.9[a]
1-OH-benzo[c]phenanthrene	69	925	8.0	2.8[b]
2-OH-benzo[c]phenanthrene	69	559	3.3	3.a[b]
3-OH-benzo[c]phenanthrene	69	522	3.0	2.6[b]
1-OH-benz[a]anthracene	70	528	2.7	2.6[b]
3-OH-benz[a]anthracene 9-OH-benz[a]anthracene	70	843	10	5.2[b]
1-OH-chrysene	69	496	11	2.6[b]
2-OH-chrysene	69	565	4.4	2.6[b]
3-OH-chrysene	69	510	3.3	2.6[b]
4-OH-chrysene	69	468	6.7	2.6[b]
6-OH-chrysene	72	509	4.5	2.6[b]
3-OH-benzo[a]pyrene	67	686	6.6	2.6[b]
7-OH-benzo[a]pyrene	67	722	8.5	2.6[b]

a. LOD determined by method blank LOD: 3 x standard deviation of blank levels
b. LOD determined by the lowest concentration from the calibration curve

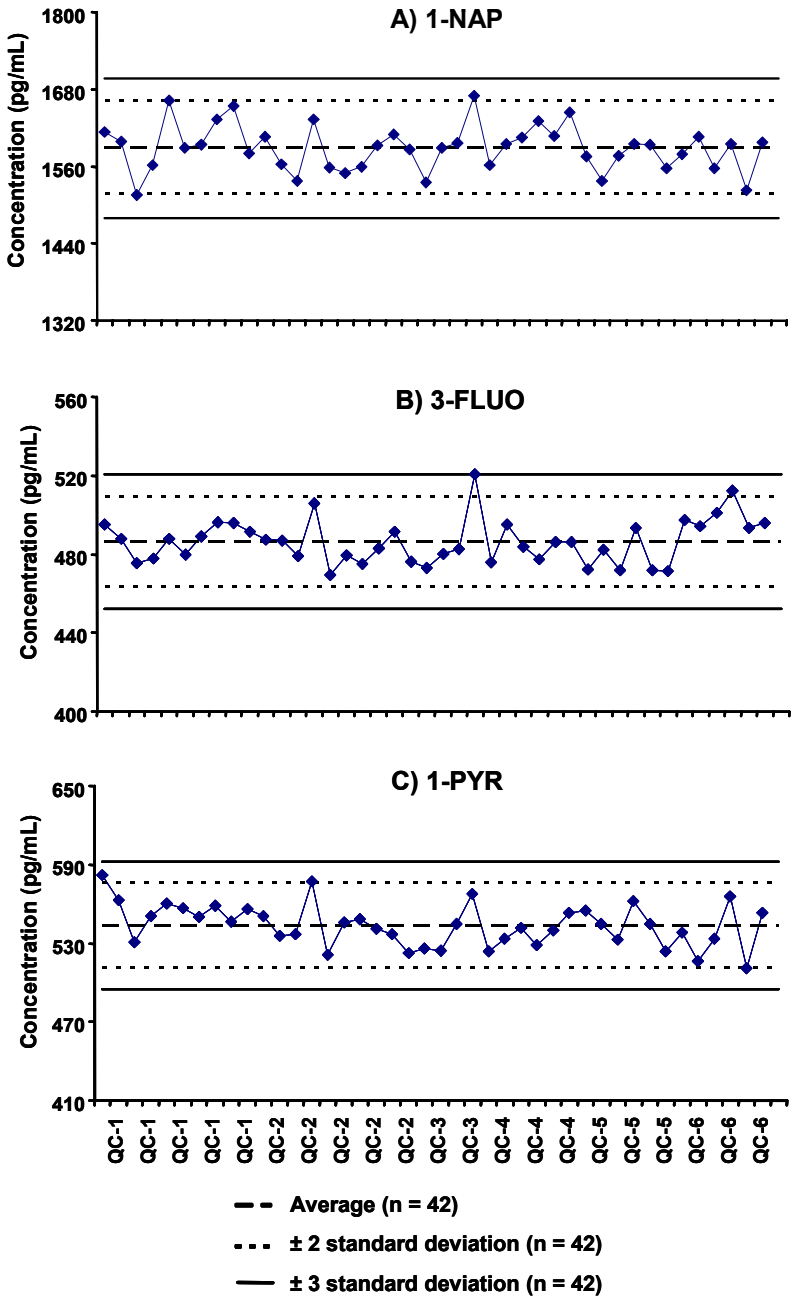

Figure 4.4. QC plots for (A) 1-naphthol (1-NAP), (B) 3-OH-fluorene (3-FLUO), and (C) 1-OH-pyrene (1-PYR) from six batches of QC samples (42 QCs in total)

4.3 Results

The sample preparation method was highly automated and required minimum manual labor. Manual tasks were limited to aliquoting the urine samples, centrifugation, evaporation, and reconstitution. The sample throughput was 40 samples per day per

analyst (including method blanks and QC samples). The current method produced cleaner extracts than previous methodology (Romanoff et al., 2006; Smith et al., 2002), with fewer interfering compounds that caused ion suppression of the lock mass channels during HRMS analysis. Example chromatograms of a standard and a urine sample are given in Figure 4.3. The method LOD was below 10 pg/mL urine for all analytes, except 1-NAP (LOD 18 pg/mL) and 2-NAP (LOD 13 pg/mL). The recoveries were approximately 70% for most compounds, with the exception of $^{13}C_6$-1-NAP (46%). The overall CV for six batches of QC samples (n=42) prepared over 3 weeks by four different analysts using two automated liquid handlers and two GC/HRMS instruments were at 10% or below (cf. Table 4.2). QA/QC charts for selected analytes (1-NAP, 3-FLUO, and 1-PYR) are given in Figure 4.4.

When using the enzyme derived from *E. coli* for selective deconjugation of glucuronides, the product yields reached maximum within 3 hours (data not shown). On the other hand, when using the *Helix pomatia* enzyme for deconjugation of both sulfates and glucuronides, the kinetics were found to be compound-specific and followed one of the three trends, as shown in Figure 4.5. One group of analytes, exemplified by 1-NAP in Figure 4.5A, followed apparent first-order reaction kinetics. The time required to reach the equilibrium phase decreased with the addition of enzyme. The second group as exemplified by 1-PYR in Figure 4.5B, reached equilibrium almost instantaneously, independent of enzyme amount. The third group, containing only one analyte 9-PHEN, reached the maximum yield within 1 hour, then the yield decreased over time (Figure 4.5C). The deconjugation efficiency was determined to be 97% for 1-NAP-GLU conjugate, and 84% for 1-PYR-SULF.

In Figure 4.6, the yields of deconjugated products for selected OH-PAHs are plotted as a function of pH, with a deconjugation time of 3 hours. In this Figure, 1-PYR reached the maximum yield within the pH range of 4.5 to 7. The yield of 3-FLUO peaked at pH 5.5, while a decrease in yield of 1-NAP was observed at a pH-value higher than 5.5

or lower than 4.5. A pH-value of 5.5 was chosen as the most suitable pH for deconjugation, considering all analytes.

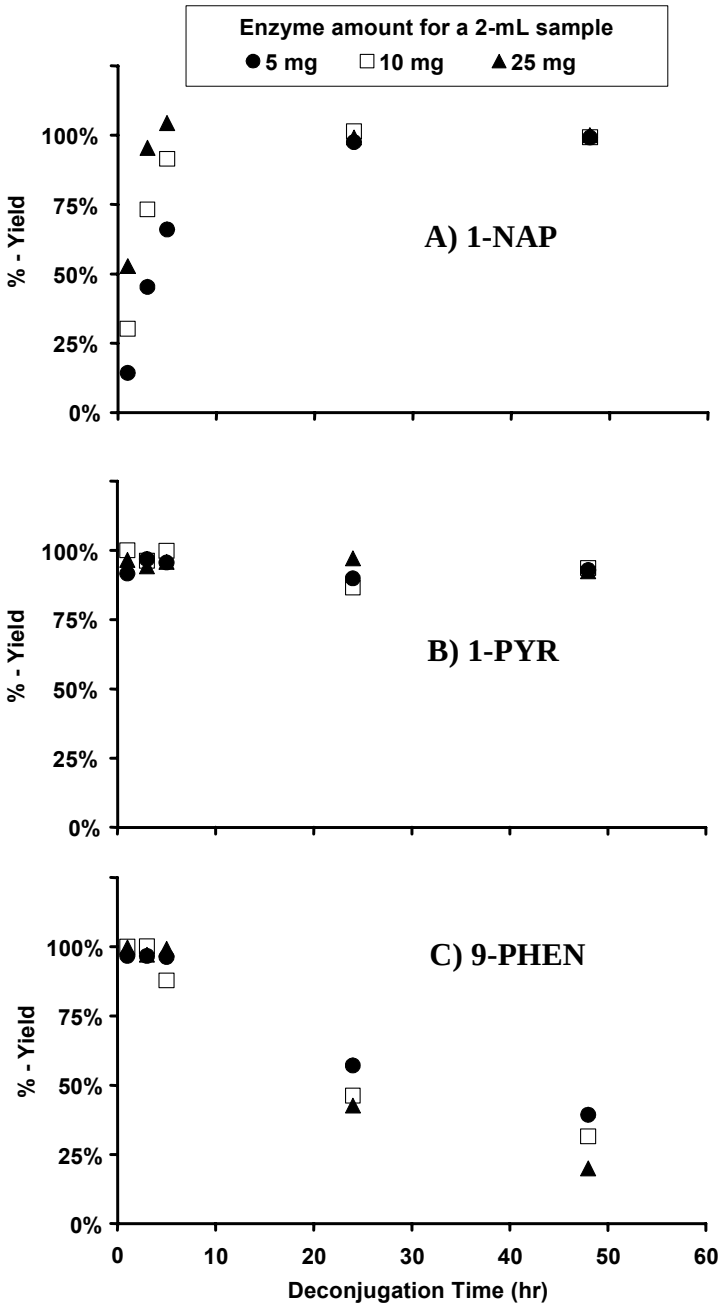

Figure 4.5. Yields of deconjugated (A) 1-naphthol (1-NAP), (B) 1-OH-pyrene (1-PYR), and (C) 9-OH-phenanthrene (9-PHEN) normalized to the maximum yield as a function of deconjugation time, for three different amounts of enzyme

The relative compositions of glucuronide and sulfate conjugates were found to be compound-specific, as shown in Figure 4.7. Overall, sulfate conjugates were present at lower levels than their glucuronide counterparts. For example, 1-PHEN was present almost exclusively as glucuronide, while up to 40% of 3-FLUO was in the form of sulfate conjugate. The percentage of sulfate conjugate in a urine pool is given in Figure 4.7B.

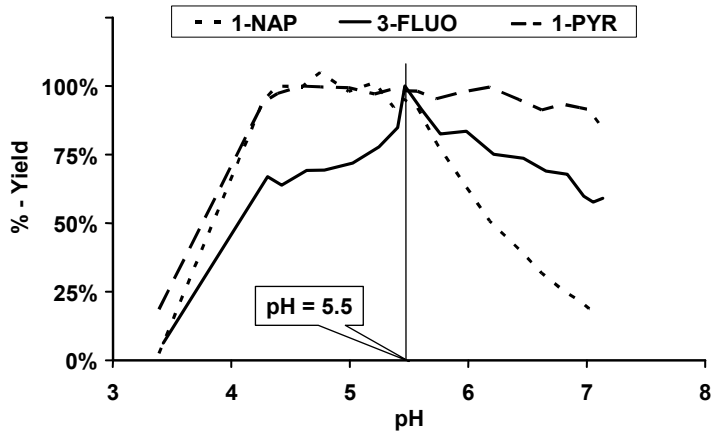

Figure 4.6. Normalized yields of 1-naphthol (1-NAP), 3-OH-fluorene (3-FLUO), and 1-OH-pyrene (1-PYR) from pooled urine samples (2-mL), deconjugated with *H. Pomatia* β-glucuronidase Type H-1 enzyme for 3 hours at 37 °C, as a function of pH

4.4 Discussion

Factors to consider when developing an analytical method for large scale biomonitoring or epidemiological studies include but are not limited to (i) identification of the chemical or composition of the chemical class investigated, (ii) an understanding of the metabolism and identification of suitable marker metabolites in those cases where the parent compound is unfeasible to monitor, (iii) choice of suitable matrix to analyze (urine, serum, adipose tissue, feces), (iv) sensitivity required for accurate identification and quantification at trace level, (v) need for automation for large scale epidemiological studies such as the NHANES survey, and (vi) limitations of various analytical approaches. In the case of PAH biomonitoring, hydroxylated metabolites (Jacob and Seidel, 2002), as well as hemoglobin adducts (Nielsen et al., 1996), have been used as

biomarkers in the past. Hemoglobin adducts have a half-life in the body equal to that of red blood cells—approximately 120 days (dell'Omo and Lauwerys, 1993). In comparison, the urinary PAH metabolites have a relatively short half-life in the range of hours (Buckley and Lioy, 1992; Viau and Vyskocil, 1995). Hence, urinary biomonitoring of PAHs represents a more recent exposure scenario. This limitation is important to take into consideration when planning epidemiological studies aimed at assessing exposure to PAHs through urinary biomonitoring or adverse health effects. Another important factor to consider is that larger PAHs, such as BAP and BAA, are excreted primarily in feces, while smaller PAHs (2–4 aromatic rings) are excreted in urine (Ramesh et al., 2004). Therefore, a low level exposure to BAP and BAA may not be detectable using urinary biomonitoring.

The first method for OH-PAH analyses developed in our laboratory utilized solid phase extraction (SPE) and included 16 OH-PAH (Smith et al., 2002). This method was then automated on the Rapid Trace® SPE workstations (Caliper Life Sciences, Hopkinton, MA), and the analyte list was increased to 23 (Romanoff et al., 2006). This method was, however, susceptible to blocked SPE cartridges as caused by particulates in the urine. When the blockage occurred, the automated process had to be stopped and extraction performed manually. Another consideration was interference by biogenic material that would lead to suppression of the lock mass channel during HRMS analysis. In the current method, we increased the number of analytes to 24 OH-PAHs compared to the previous methods (Romanoff et al., 2006; Smith et al., 2002) by adding 7-OH-benzo[a]pyrene, one of the urinary metabolites of benzo[a]pyrene (Fertuck et al., 2001; Miller and Ramos, 2001). We also developed a new extraction step based on liquid-liquid extraction and automated this method using the liquid handler. The enzymatic hydrolysis of urinary OH-PAHs conjugates was further improved in the current method, and the hydrolysis efficiency was verified with synthesized conjugates spiked into urine.

The throughput for our current method is 40 samples (including 4 method blanks and 4 QA/QC samples) per day per analyst. The sample preparation starts in the afternoon of the first day and includes dispensing the samples, adding enzyme-containing buffer, and internal standard fortification (time required ~45 minutes). After overnight hydrolysis, the samples are extracted through automated liquid-liquid extraction (4 hours or ~6 minutes per sample). The evaporation of the extract requires 10–15 minutes, followed by reconstitution in toluene, addition of the recovery standard, and MSTFA (30 minutes). The derivatization reaction with MSTFA requires 30 minutes, followed by the GC/HRMS analysis (18 hours overnight for the 40 samples). The sample size for this method is 0.25–3 mL, with 2 mL as the standard sample size to produce an adequate detection limit of 2.6–18 pg/mL, depending on the target analytes (Table 4.2).

This method has been applied to several biomonitoring studies, including the ongoing NHANES 2003-04 survey. Among over 3000 samples analyzed using the current method over a period of 10 months, only one set of samples had to be extracted manually due to an error in the automated liquid handling equipment.

4.4.1 Extraction conditions

This method employs liquid-liquid extraction to extract free OH-PAHs from the urine samples into an organic phase using a liquid handler for automation. Pentane was chosen as the extraction solvent because of low amount of co-extracted material and high recovery of OH-PAHs. In addition, given its high volatility (b.p. 36 °C), the pentane extract (~9 mL) can be evaporated within 10–15 minutes, thus minimizing analyte loss during evaporation.

During the method development, interfering peaks appeared around some of the late-eluting analytes such as chrysene and benz[a]anthracene metabolites, which caused ion suppression in this region. The source of the contamination was traced to the silicon

septa used in the sample tube caps. The interfering peaks and the ion suppression could be minimized by heating the septa to 150 ºC overnight prior to use.

4.4.2 Deconjugation Conditions and Conjugate Composition

Deconjugation is a critical step in the accurate determination of urinary OH-PAH metabolites because they are predominantly present in the conjugated form (93–100%) (Romanoff et al., 2006), and only the free OH-PAHs can be extracted from the aqueous phase. To maximize the hydrolysis yield, several factors have been evaluated, including enzyme type, enzyme amount, hydrolysis time, pH and the buffer concentration.

Enzymes from different species have different activities. For example, *Helix Pomatia* has β-glucuronidase activity as well as arylsulfatase activity (Elsohly et al., 2005; Shibasaki et al., 2001), while E. coli only has β-glucuronidase activity (Elsohly et al., 2005; Murooka et al., 1978). For this method we chose the partially purified β-glucuronidase type H-1 powder enzyme extracted from *Helix pomatia* because it has high hydrolysis activity for both conjugates, while having a lower PAH background level than the β-glucuronidase / arylsulfatase enzyme solution (from *Helix pomatia*) used in the previous methods (data not shown) (Romanoff et al., 2006; Smith et al., 2002). Moreover, every batch of enzyme has to be verified as free of interfering PAH metabolites as exemplified by our finding of a batch of enzyme containing a high level of 1-NAP (data not shown).

The rate of the hydrolysis reaction is affected by several factors, such as the amount of enzyme and pH (Dou et al., 2001; Elsohly et al., 2005). In general, the hydrolysis rate increased with increasing amount of enzyme added (Figure 4.5). Deconjugation using 10 mg of enzyme for each urine sample (2 mL) at 37 °C for 24 hours yielded the maximum amount of product, with the exception of 9-PHEN, which reached a maximum after 30 minutes and then decreased. We observed that the time required for deconjugation is structurally dependent, even among isomers. For example,

2-NAP and 3-FLUO were completely hydrolyzed within 1 hour with a small amount of enzyme (5 mg per 2-mL sample), while their isomers, 1-NAP and 9-FLUO, required 10 mg of enzyme and overnight hydrolysis. An overnight deconjugation (18–20 hours) was shown to be sufficient for deconjugating the samples (data not shown) and chosen as the standard deconjugation time.

At this time, we do not know the mechanism for the degradation of 9-PHEN during the hydrolysis step. We did, however, observe that the magnitude of the degradation was sample-dependent. We estimated the amount of the 9-PHEN loss in urine samples from six different subjects by calculating the ratio of results from overnight deconjugation to 1 hour deconjugation. The losses ranged from 30% to 73%. Thus it is not feasible to apply a single conversion factor to correct for the degradation in unknown samples. We are in the process of acquiring a custom-synthesized ^{13}C-labeled 9-PHEN standard. With this standard we can assume equal degradation of the labeled internal standard and the naturally occurring 9-PHEN to account for the loss of 9-PHEN during incubation step.

Enzymes from different species have been shown to have different optimal pH ranges when hydrolyzing glucuronides (Dou et al., 2001). To visualize the influence of pH in our method, we deconjugated several samples at different pH in the range of 3.5-7. The reaction was terminated after only 3 hours—before completion of the deconjugation for slow reacting OH-PAHs. Therefore, the quantified amount in the sample at the different pH-values is proportional to the reaction speed. Although the reaction kinetics can not be determined from this experiment, we have obtained a proportional measure of the ideal pH for deconjugation (Figure 4.6). Generally, yields of all analytes were influenced by pH, and the effect was more pronounced for some analytes. Low pH (< pH 4) was profoundly detrimental to the enzymatic hydrolysis reactions, causing yields of all analytes to drop below 20% of their maximum. All analytes reached maximum yield at pH 5.5. In light of these observations, it is important to keep the samples buffered to pH

5.5. Therefore, to provide sufficient buffering strength, we increased the molarity of the sodium acetate buffer solution (pH 5.5) from 0.1 M in previous methods (Romanoff et al., 2006; Smith et al., 2002) to 1 M in the current method. The more concentrated buffer will adjust the pH of the urine to pH 5.5 regardless of the initial pH of the urine.

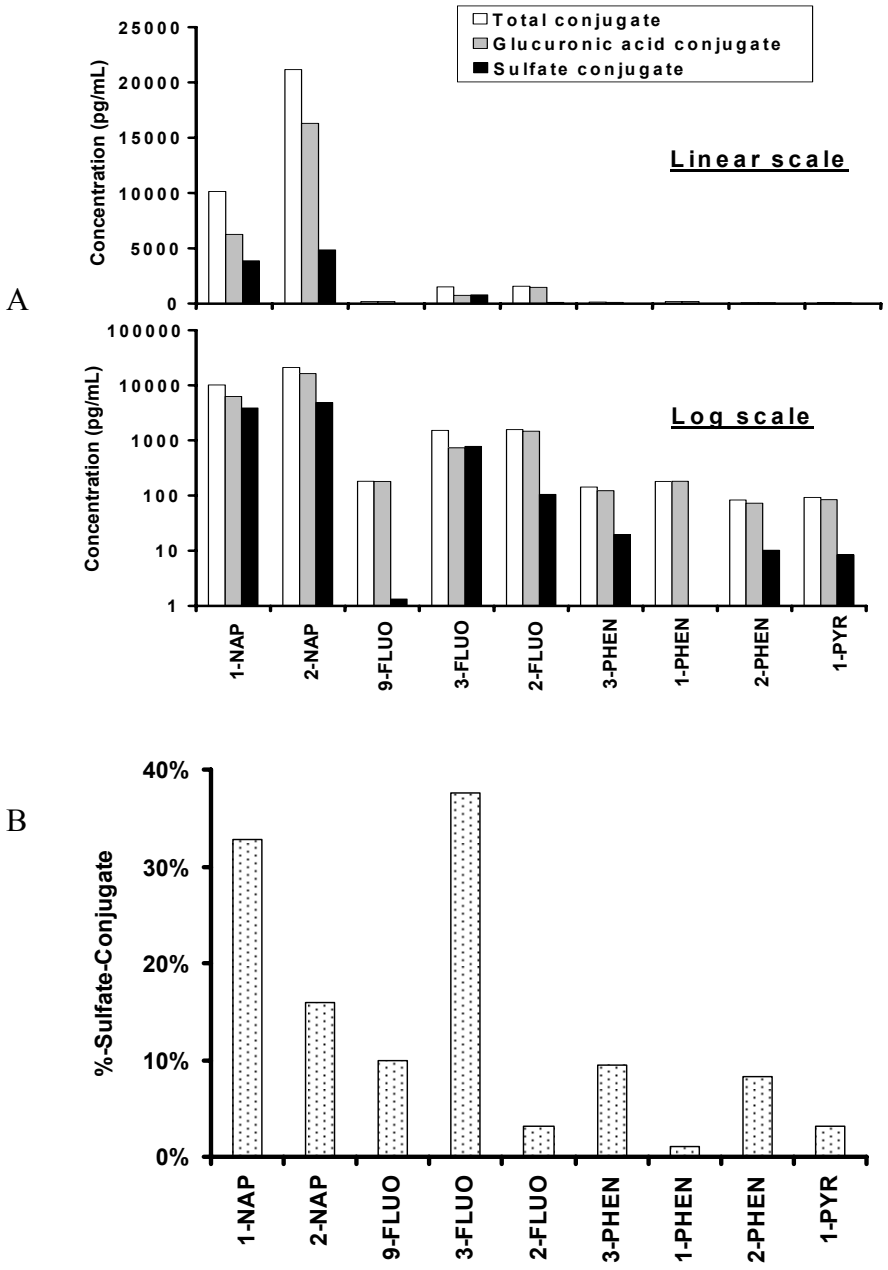

Figure 4.7. Relative distribution of glucuronic acid and sulfate conjugates for OH-PAHs: concentrations (A) and percentage of sulfate conjugates (B) in a pooled urine sample

The majority of OH-PAH metabolites are excreted in urine as conjugates and only a small amount as free OH-PAHs (Romanoff et al., 2006). We found that OH-PAHs are excreted as a mixture of glucuronide and sulfate conjugates, although glucuronides predominate (Figure 4.7). A similar observation has been reported for polychlorinated dibenzo-*p*-dioxin metabolites (Hakk et al., 2001). The reason for the differences in conjugate composition is unknown to us, but it is most likely due to enzyme kinetics. Glucuronidation is mediated by UDP-glucuronosyl transferases, which is a microsomal enzyme as are the Phase I cytochromes, while arylsulfotransferases are cytosolic (Hakk et al., 2001). Therefore, OH-PAHs formed during Phase I metabolism in the microsome may be more likely to become substrate for UDP-glucuronosyl transferases, which would explain the higher percentage of glucuronide conjugates in the urine.

CHAPTER 5

VARIABILITY OF URINARY POLYCYCLIC AROMATIC HYDROCARBON METABOLITE LEVELS IN ADULTS

(Li Z, Romanoff LC, Lewin MD, Porter EN, Trinidad DA, Needham LL, Patterson Jr. DG, and Sjodin A. *Journal of Exposure Science and Environmental Epidemiology*, Submitted)

Abstract

Urinary hydroxy polycyclic aromatic hydrocarbons (OH-PAHs) are commonly used in biomonitoring to assess exposure to polycyclic aromatic hydrocarbons (PAHs). OH-PAHs have relatively short biological half-lives (4.4-15 hours), yet little information is available on their variability over time, which can limit study design and the interpretation of analytical results. The study was designed to (*i*) study the variability of 9 urinary OH-PAH metabolite concentrations over time and (*ii*) calculate sample size requirements for future epidemiological studies. Individual urine samples were collected from 8 non-occupationally exposed adults for one week. Information on the time and volume of each urine excretion and dietary and driving history were recorded. Within subjects, the coefficients of variation (CV) for the wet weight concentration of OH-PAHs ranged from 64% to 297%; creatinine adjustment reduced the CV to 19-288%. The 24 hour void concentrations were the least variable measure with CVs ranging 13-182% for the 9 OH-PAHs. Within-day variability contributed on average 84% and between-day accounted for 16% towards the total variance of 1-hydroxypyrene (1-PYR). Intraclass correlation coefficients (ICC) of 1-PYR levels were 0.55 for spot urine samples, 0.60 for first morning voids, and 0.76 for 24-hour voids, indicating high degree of correlation

between urine measurements collected from the same subject over time. Sample size calculations were performed to estimate number of subjects needed to detect differences in geometric mean at a statistical power of 80% for spot urine, first morning and 24 hour void sampling, respectively.

5.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of pollutants formed during incomplete combustion processes and are ubiquitously distributed in the air, soil, water, food, and in many occupational settings (IARC 1983). Human exposure to PAHs can occur through inhalation of polluted air or cigarette smoke, ingestion of food containing PAHs, as well as dermal absorption from soil or other PAH containing materials such as coal tar. For the general population, ingestion and inhalation are the two dominant routes of exposure with ingestion reportedly being more important in quantity (ATSDR 1995; Bostrom et al. 2002). For certain occupations such as coke oven workers, dermal absorption can become a major exposure route (ATSDR 1995). PAHs such as benzo(a)pyrene, benz(a)anthracene and chrysene have been classified as probable human and animal carcinogens (ATSDR 1995). PAHs have also been reported to possess reproductive, developmental, hemato-, cardio-, and immuno-toxicities (ATSDR 1995).

Urinary PAH metabolites, specifically the mono-hydroxy PAH metabolites (OH-PAHs), have been used as biomarkers for assessing human exposure to PAHs, with 1-hydroxypyrene (1-PYR) as the most commonly used biomarker (Jacob and Seidel 2002). A number of studies have reported the levels of OH-PAHs in various occupational groups such as coke oven workers and road pavers (Grimmer et al. 1993; Jongeneelen et al. 1990; Levin et al. 1995), as well as in general population from various parts of the world (Hansen et al. 2005; Mucha et al. 2006). In the United State, OH-PAHs are included in the National Health and Nutrition Examination Survey (NHANES) conducted by the Centers for Disease Control and Prevention (CDC), an on-going survey reports

biannually the level of biomarkers in a statistically representative sample of the U.S. population. OH-PAHs are measured in a one-third subset of samples, corresponding to approximately 3,000 samples every two years (Li et al. 2008).

The half-life for urinary 1-PYR in humans has been reported to be 9.8 hours after inhalation exposure (Brzeznicki et al. 1997), 4.4 hours (Buckley and Lioy 1992) and 12 hours (Viau et al. 1995) after ingestion exposure, and 11.5-15 hours after dermal absorption (Viau and Vyskocil 1995). Due to the short half-lives of PAHs, the information urinary biomonitoring provides is limited to recent exposure. Further, the short half-lives will need to be considered in study design and appropriate samples will need to be collected within the window PAHs are excreted in the urine following exposure. Theoretically, a 24-hour void specimen represents the average daily metabolite excretion more reliably than a spot urine sample. Thus, 24-hour urine collection was recommended by the U.S. EPA for evaluating occupational and residential exposure to pesticides and other toxic substances (U.S.EPA 1996). However, this sampling method is burdensome on the study subjects and non-compliance can lead to compromised or biased samples. A common alternative is to collect the first morning urine as has been done in many occupational and environmental exposure investigations because the first morning urine is often correlated with the 24-hour void (Han et al. 2007; Scher et al. 2007). In epidemiological studies such as NHANES with thousands of participants, first morning voids are often difficult to obtain, hence, spot urine samples have been used as a more practical solution. However, a non-standardized sampling increases the variance especially for short-lived compounds like OH-PAHs and changes in water consumption throughout the day increase the variance even further by diluting or producing more concentrated urine. Therefore, concentration adjustments such as creatinine correction have often been used to adjust for urine excretion rate and minimize variability of the urinary biomarker concentrations caused by different hydration status of participants (Barr et al. 2005).

Epidemiological study design should include a good understanding of the variability within a normal, non-exposed population or group of interest (IPCS 1993). Furthermore, since most potential health effects are likely associated with exposure over time, understanding the temporal variability of urinary biomarker levels from subjects with no occupational exposure is essential for data interpretation and study design. At present, little is known about the variability of urinary OH-PAH levels. Siwinska et al. (1998) found that concentration of 1-PYR in first morning urine samples collected on 6 consecutive days in 30 children had a coefficient of variation (CV) within-person ranging 14-109%, whereas inter-person variation was 69-109%. Grimmer et al. (1993) reported the intra-person variation in 24-hour voids during 4 consecutive days from 4 coke workers to be 14-41% for 1-PYR and 16-94% for mono-hydroxy phenanthrene metabolites. Han et al. (2007) reported that one-third of 50 coke oven workers and 50 controls had a CV of over 50% in first morning and 24-hour voids.

The objectives of the present study were to evaluate the variability of 9 commonly detectable OH-PAHs, metabolites of naphthalene, fluorene, phenanthrene and pyrene, in adults with no occupational exposure over 7 days. Our design allowed us to determine the between- and within-subject variability, between- and within-day variability, as well as apportion the variance to subject and day effects. In addition, we calculated sample size requirements for different sampling regimes such as spot, first morning and 24-hour void sampling. The finding of this investigation is applicable to the study design of future exposure assessments or epidemiological studies.

5.2 Methods

5.2.1 Study Design

The 8 subjects in this study were healthy volunteers between 26 and 58 years of age with no known occupational PAH exposure. They were non-smokers and were not

subjected to second-hand smoking. The participants (4 males and 4 females) lived in the metropolitan Atlanta area, drove to and from work in the morning and afternoon during the weekdays (Monday – Friday) with median driving time 30 minutes (one-way, range 15 – 60 minutes) and a median distance of 21 km (one-way, range 8 – 40 km). The subjects were all working in modern office buildings at the CDC and were not occupationally exposed to PAHs. The participants did not work and had sporadic driving during the weekend (Saturday – Sunday). During the one-week study period in November 2005, participants collected all urine excretions in a graduated beaker, recorded the volume and time of each excretion, and transferred a portion (~50 mL) to a pre-labeled sterile urine cup which was then stored in an ice cooler. The urine samples were retrieved from each participant daily (or after the weekend), brought back to the laboratory, and frozen (-70ºC) until analysis. Participants also recorded detailed information on dietary intake, driving and other outdoor activities.

5.2.2 Laboratory Methods

The method used to measure the urinary OH-PAH metabolites has been described in detail previously (Li et al. 2006); hence it is only briefly outlined here. Urine samples were spiked with ^{13}C-labeled internal standards and sodium acetate buffer containing β-glucuronidase enzyme, hydrolyzed overnight at 37 ºC, and then extracted using pentene through semi-automated liquid-liquid extraction. The extracts were evaporated, derivatized, and analyzed on a 6890 gas chromatograph (Agilent Technology, Palo Alto, CA) coupled with a MAT 95XL high resolution mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA). All analyses were subjected to a series of quality control and quality assurance checks as described elsewhere (Li et al. 2006). Urinary creatinine was measured on a Roche Hitachi 912 Chemistry Analyzer (Hitachi Inc., Pleasanton, CA) using the Creatinine Plus Assay, as described in Roche's Creatinine Plus Product Application # 03631761003.

5.2.3 Statistical Analysis

All statistical analyses were performed using SAS 9.1.3 (SAS Institute, Cary, NC) and Statistica 7.1 (StatSoft Inc., Tulsa, OK). Concentrations below the limit of detection (LOD) were replaced with the LOD divided by the square root of 2 prior to data analysis. The urinary metabolite concentrations followed a log-normal distribution; therefore, all data were log-transformed before statistical analysis.

A total of 427 samples were available from 8 subjects over one week. We defined the first morning void sample as the first sample collected from each subject after 5 AM each day. The 24-hour void concentration was calculated as the volume weighted average of all urine specimens collected by an individual during a 24-hour period starting at midnight. Subject 8 (S8) had a high dietary intake of PAHs (barbecued chicken) on the fourth day of the study period and concentrations of all OH-PAHs increased up to 104-fold post exposure. Hence, data from this subject for the two days affected by the high dietary intake were excluded from further variability and sample size calculations.

To assess the between and within-subject as well as between and within-day variance, we calculated the contributions of each effect toward the total variance for four metabolites, i.e. 1-naphthol (1-NAP), 3-hydroxyphenanthrene (3-PHEN), 1-PYR, and 3-hydroxyfluorene (3-FLUO),. This was done using a random effects model fitted through the PROC NESTED, PROC MIXED, and PROC VARCOMP procedures in SAS. Since all models produced similar results we chose to only present the results from the PROC NESTED model. We calculated intraclass correlation coefficients (ICC), defined as the ratio of between-subject variance to the total variance, as an indicator of reproducibility of repeated measurements over time (McGraw and Wong 1996). A high ICC value indicates high correlation between the repeated measurements from the same subject, or in other words, high reproducibility of repeated samples. Using the findings from this study, we performed sample size calculations including from 1 to 4 repeated samples from each subject. Separate calculations were performed for spot sample, first morning

void and 24-hour void. The percent differences in geometric means (GM) used for the sample size calculation between the two groups (e.g. control vs. case) were 10%, 25%, 50%, and 100%. All of our analyses were performed on log-transformed data; hence, we converted percent differences in the GM to a difference (d) on the mean of log-transformed concentrations for the power calculation (Supplemental Material). The number of subjects (m) needed per group to achieve desired type I error ($\alpha = 0.05$) and power (P = 0.8) was calculated using the following formula (Diggle et al. 1994),

$$m = 2\,(Z\alpha + Z_\beta)^2\,SD^2\,[1 + (n\text{-}1)\,ICC]/(nd^2)$$

$$= 2\,(Z\alpha + Z_\beta)^2\,[1 + (n\text{-}1)\,ICC]/(n\Delta^2)$$

where: $\Delta = d/SD$ is the smallest meaningful difference between groups in standard deviation (SD) units; n = number of repeated samples per subject; $Z_y = y$th percentile of a standard Gaussian distribution; β = type II error = 1-P

5.3 Results

We quantified 9 OH-PAH metabolites in 427 samples collected from 8 subjects during one week. The detection rates were above 95% for all reported analytes except for 2-hydroxyphenanthrene (2-PHEN, 82%) and 3-hydroxyfluorene (3-FLUO, 87%). The GM, median and quartile concentrations from all samples, first morning voids and re-constructed 24-hour voids are given in Table 5.1. During the 7-day study period, each of the 8 subjects collected on average 53 urine samples (27-74 samples/subject, Figure 5.1). Urinary OH-PAH concentrations within subjects varied 2 to 3 orders of magnitude (Table 5.2). For example, concentration of 2-naphthol (2-NAP) in a subject identified as S7 ranged from 86 ng/L to 17069 ng/L during the study period. Within subjects, the CVs of the 9 OH-PAHs among all samples, first morning voids, and 24-hour voids were 64-297%, 26-189% and 13-182%, respectively; creatinine adjustment decreased the CV to 19-228%, 9-176% and 9-154%, respectively (Table 5.3). Levels of urinary PAH biomarkers in each subject varied throughout the day, as exemplified in Figure 5.2.

Table 5.1. Concentrations of urinary PAH metabolites for all samples, first morning voids and re-constructed 24-hour voids. Also given are reference concentrations from the U.S. adult population

Analyte	Abbr	all samples (n = 427)			1st morning voids (n=56)			24 hour voids (n = 56)			U.S. adult 2001-02[b]
		GM [a]	Median	Quartiles	GM	Median	Quartiles	GM	Median	Quartiles	
Wet weight concentration (ng/L)											
1-naphthol	1-NAP	664	693	324-1287	1355	1268	789-1941	948	866	511-1381	2190
2-naphthol	2-NAP	1041	906	507-1968	1705	1449	886-3413	1271	1016	673-2138	2620
9-hydroxyfluorene	9-FLUO	116	110	60-242	189	226	105-313	143	143	96-210	230
3-hydroxyfluorene	3-FLUO	33	35	17-56	52	50	38-68	37	35	30-47	138
2-hydroxyfluorene	2-FLUO	91	99	50-164	150	137	107-191	107	109	84-133	333
3-hydroxyphenanthrene	3-PHEN	52	51	27-99	85	79	53-125	63	60	42-80	105
1-hydroxyphenanthrene	1-PHEN	70	69	38-130	116	118	76-179	81	81	58-101	145
2-hydroxyphenanthrene	2-PHEN	29	25	14-53	43	40	26-70	33	29	23-47	57
1-hydroxypyrene	1-PYR	35	33	17-67	59	51	40-87	41	39	28-57	47
Creatinine corrected concentration (ng/g creatinine)											
1-naphthol	1-NAPc	1126	1050	638-1641	1166	1002	680-1741	1329	1168	739-1927	2070
2-naphthol	2-NAPc	1766	1657	910-3112	1468	1093	764-2437	1781	1356	925-3158	2480
9-hydroxyfluorene	9-FLUOc	196	203	125-318	163	175	117-238	201	210	158-275	217
3-hydroxyfluorene	3-FLUOc	55	53	37-77	45	45	32-58	52	52	37-72	131
2-hydroxyfluorene	2-FLUOc	155	154	113-200	129	126	108-166	150	147	116-194	314
3-hydroxyphenanthrene	3-PHENc	89	87	63-122	73	73	55-101	88	86	64-114	99
1-hydroxyphenanthrene	1-PHENc	119	122	82-173	100	96	71-143	114	116	74-165	137
2-hydroxyphenanthrene	2-PHENc	49	45	33-67	37	36	27-46	47	47	33-58	54
1-hydroxypyrene	1-PYRc	59	58	33-98	50	49	32-72	58	57	34-88	45

a. GM - geometric mean

b. Geometric mean concentrations in the general U.S. adult population (aged 20 years and older, NHANES 2001-02) were quoted from Li et al, 2008

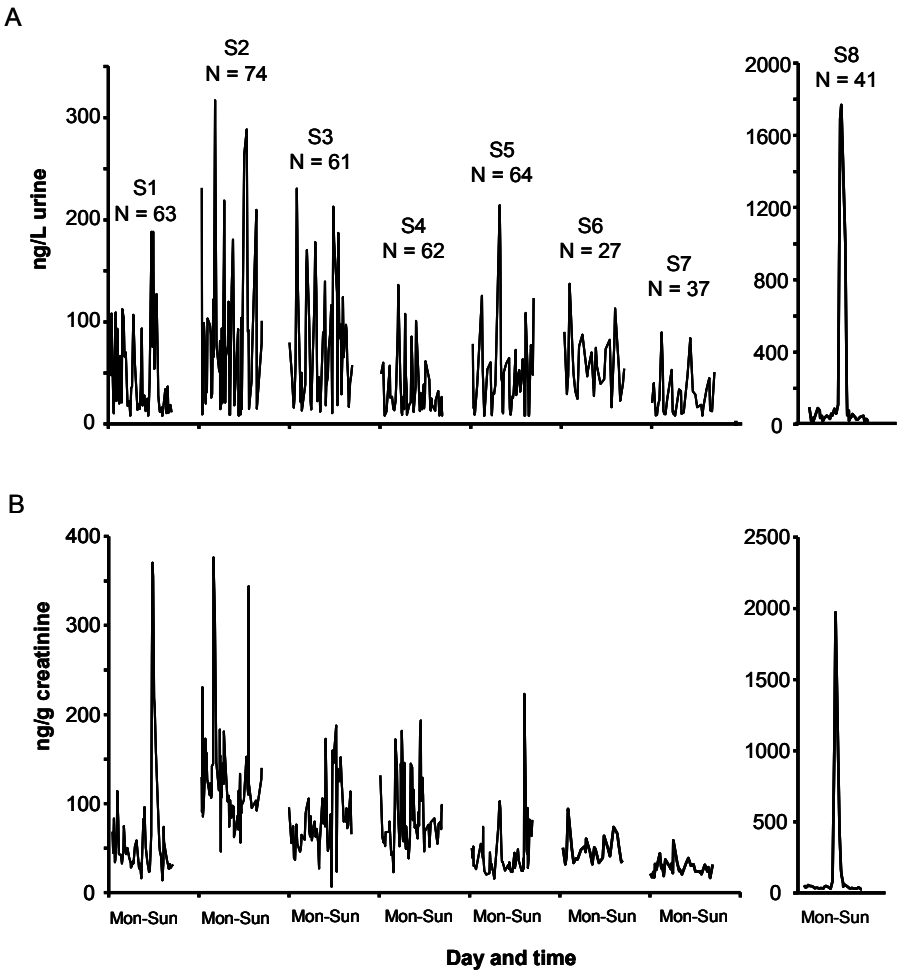

Figure 5.1. Wet weight (A) and creatinine-adjusted (B) concentrations of 1-hydroxypyrene in all individual urine samples from 8 subjects (S1-S8) during 7 days

The 1-PYR concentration over time stratified by subject is given in Figure 5.1. One of the subjects identified as S8 ate barbecued chicken at a restaurant for lunch on Thursday; as a result, levels of all the urinary metabolites increased following that exposure (Figure 5.1 and 5.3). For 1-PYR the level increased 104 fold within 12 hours after the dietary intake while the median increase in level were 41-fold among the 9 OH-PAHs monitored. Twenty-four hours after the dietary exposure, the concentration of the urinary metabolites monitored returned to pre-exposure levels. The Thursday and Friday data from subject S8 were excluded from further variability analysis and sample size calculations.

Table 5.2. Median, minimum and maximum concentrations of urinary OH-PAHs in each subject over 7 days

	1-NAP	2-NAP	9-FLUO	3-FLUO	2-FLUO	3-PHEN	1-PHEN	2-PHEN	1-PYR
Wet weight concentration (ng/L)									
S1	396 (66-2681)	593 (105-2075)	111 (30-545)	46 (10-168)	134 (19-503)	47 (10-231)	42 (10-305)	18 (10-88)	26 (8-188)
S2	663 (49-5693)	2369 (257-24263)	127 (22-911)	30 (10-160)	80 (10-419)	45 (10-327)	62 (12-369)	26 (10-190)	53 (8-317)
S3	1012 (171-3060)	746 (197-5818)	262 (34-1687)	41 (10-138)	135 (27-508)	81 (16-319)	159 (21-471)	47 (10-173)	62 (11-231)
S4	583 (100-38025)	714 (177-3628)	57 (12-491)	24 (10-168)	74 (16-368)	33 (10-210)	42 (14-318)	16 (9-100)	22 (8-136)
S5	501 (69-2013)	814 (129-4161)	84 (11-820)	35 (10-136)	112 (10-499)	84 (10-579)	98 (12-347)	44 (10-255)	32 (8-214)
S6	836 (272-2767)	1103 (422-3342)	258 (92-936)	48 (18-172)	148 (50-442)	80 (25-313)	76 (28-194)	50 (14-164)	54 (16-137)
S7	981 (163-22705)	898 (86-17069)	128 (25-829)	18 (10-86)	71 (13-231)	40 (10-139)	86 (14-332)	22 (9-86)	19 (8-90)
S8	958 (77-42606)	2759 (484-22531)	66 (12-3094)	38 (10-755)	96 (15-1904)	45 (10-2278)	66 (11-2027)	25 (10-1876)	30 (7-1761)
All	693 (49-42606)	906 (86-24263)	110 (11-3094)	35 (10-755)	99 (10-1904)	51 (10-2278)	69 (10-2027)	25 (9-1876)	33 (7-1761)
Creatinine corrected concentration (ng/g creatinine)									
S1	552 (160-3455)	935 (169-1581)	169 (51-581)	72 (10-137)	198 (27-409)	81 (16-276)	71 (23-237)	33 (10-96)	43 (14-371)
S2	1300 (425-8705)	4702 (1255-29984)	322 (94-1121)	62 (25-267)	166 (66-551)	93 (32-366)	157 (48-437)	67 (25-328)	116 (46-377)
S3	1274 (109-4035)	1021 (298-3914)	346 (22-2459)	44 (7-319)	157 (25-954)	106 (18-339)	176 (19-445)	59 (10-142)	76 (7-188)
S4	1911 (426-42439)	2617 (1076-6385)	219 (85-583)	91 (19-234)	229 (73-772)	121 (43-306)	168 (49-409)	57 (18-200)	78 (23-193)
S5	649 (257-1769)	927 (493-4152)	118 (40-1684)	44 (22-144)	123 (75-256)	93 (48-230)	111 (65-497)	50 (28-154)	35 (15-223)
S6	773 (383-1789)	981 (679-2287)	215 (155-672)	42 (26-117)	125 (102-303)	69 (38-214)	67 (54-133)	42 (28-112)	48 (32-94)
S7	1207 (765-15289)	1210 (414-16072)	185 (116-765)	29 (16-76)	94 (71-137)	52 (32-88)	105 (66-239)	31 (20-76)	27 (17-59)
S8	1034 (359-18978)	2689 (1715-14179)	63 (24-1947)	32 (17-475)	88 (58-1198)	44 (28-1762)	58 (36-1770)	27 (13-1181)	32 (16-1963)
All	1050 (109-42439)	1657 (169-29984)	203 (22-2459)	53 (7-475)	154 (25-1198)	87 (16-1762)	122 (19-1770)	45 (10-1181)	58 (7-1963)

Table 5.3. Coefficients of variability (CV) for urinary OH-PAH concentrations in all samples, first morning voids, and 24-hour voids from 8 subjects

	S1			S2			S3			S4		
	all	1stM	24hr	all	1stM	24hr	all	1stM	24hr	all	1stM	24hr
Freshweight concentration (ng/L)												
1-NAP	85%	70%	27%	111%	71%	47%	56%	40%	13%	270%	153%	140%
2-NAP	69%	51%	24%	121%	43%	61%	87%	34%	27%	80%	34%	31%
9-FLUO	81%	60%	23%	96%	54%	24%	85%	127%	61%	99%	51%	29%
3-FLUO	72%	51%	29%	85%	45%	28%	64%	47%	31%	86%	34%	37%
2-FLUO	73%	49%	26%	86%	48%	27%	67%	30%	21%	80%	27%	33%
3-PHEN	80%	66%	24%	97%	60%	26%	70%	50%	23%	87%	34%	32%
1-PHEN	87%	70%	41%	90%	47%	18%	66%	52%	27%	91%	48%	29%
2-PHEN	78%	50%	24%	94%	63%	26%	71%	59%	26%	85%	30%	24%
1-PYR	94%	99%	44%	95%	61%	38%	73%	56%	31%	88%	35%	21%
Creatinine-adjusted concentration (ng/g creatinine)												
1-NAPc	66%	32%	34%	100%	72%	59%	47%	37%	21%	182%	169%	127%
2-NAPc	29%	26%	15%	84%	56%	50%	54%	19%	21%	38%	33%	13%
9-FLUOc	59%	41%	23%	53%	50%	32%	82%	142%	67%	46%	31%	23%
3-FLUOc	30%	28%	15%	57%	35%	21%	73%	47%	29%	50%	46%	36%
2-FLUOc	30%	26%	15%	48%	13%	19%	64%	21%	11%	55%	31%	35%
3-PHENc	56%	36%	25%	58%	30%	19%	41%	53%	22%	45%	24%	27%
1-PHENc	46%	58%	37%	40%	19%	23%	36%	57%	27%	38%	26%	21%
2-PHENc	48%	26%	24%	65%	32%	30%	38%	62%	27%	54%	18%	16%
1-PYRc	104%	53%	60%	43%	19%	16%	43%	57%	30%	43%	23%	14%

	S5			S6			S7			S8		
	all	1stM	24hr	all	1stM	24hr	all	1stM	24hr	all	1stM	24hr
Freshweight concentration (ng/L)												
1-NAP	72%	47%	22%	61%	51%	41%	197%	131%	111%	205%	180%	124%
2-NAP	85%	57%	39%	52%	29%	25%	169%	140%	111%	99%	35%	64%
9-FLUO	108%	52%	22%	63%	26%	16%	87%	46%	55%	281%	75%	180%
3-FLUO	74%	46%	21%	57%	32%	30%	72%	45%	41%	178%	153%	109%
2-FLUO	83%	54%	25%	49%	30%	21%	66%	40%	31%	187%	63%	117%
3-PHEN	94%	60%	25%	64%	36%	24%	75%	51%	33%	262%	167%	171%
1-PHEN	73%	44%	20%	45%	34%	17%	71%	48%	48%	222%	173%	144%
2-PHEN	88%	55%	23%	55%	33%	26%	77%	44%	39%	297%	150%	182%
1-PYR	96%	67%	34%	48%	30%	21%	77%	44%	37%	273%	189%	177%
Creatinine-adjusted concentration (ng/g creatinine)												
1-NAPc	41%	38%	30%	40%	34%	34%	135%	109%	98%	156%	175%	117%
2-NAPc	68%	37%	30%	35%	27%	29%	127%	114%	91%	65%	25%	38%
9-FLUOc	139%	41%	40%	49%	12%	31%	51%	28%	34%	249%	62%	152%
3-FLUOc	44%	25%	24%	37%	15%	20%	36%	28%	22%	135%	133%	93%
2-FLUOc	24%	21%	14%	29%	9%	10%	19%	23%	10%	150%	47%	85%
3-PHENc	39%	31%	24%	42%	16%	12%	28%	29%	11%	256%	153%	143%
1-PHENc	46%	17%	20%	22%	16%	9%	31%	36%	28%	206%	157%	121%
2-PHENc	44%	25%	25%	36%	12%	14%	36%	27%	17%	264%	133%	154%
1-PYRc	67%	38%	38%	31%	20%	12%	28%	26%	16%	288%	176%	151%

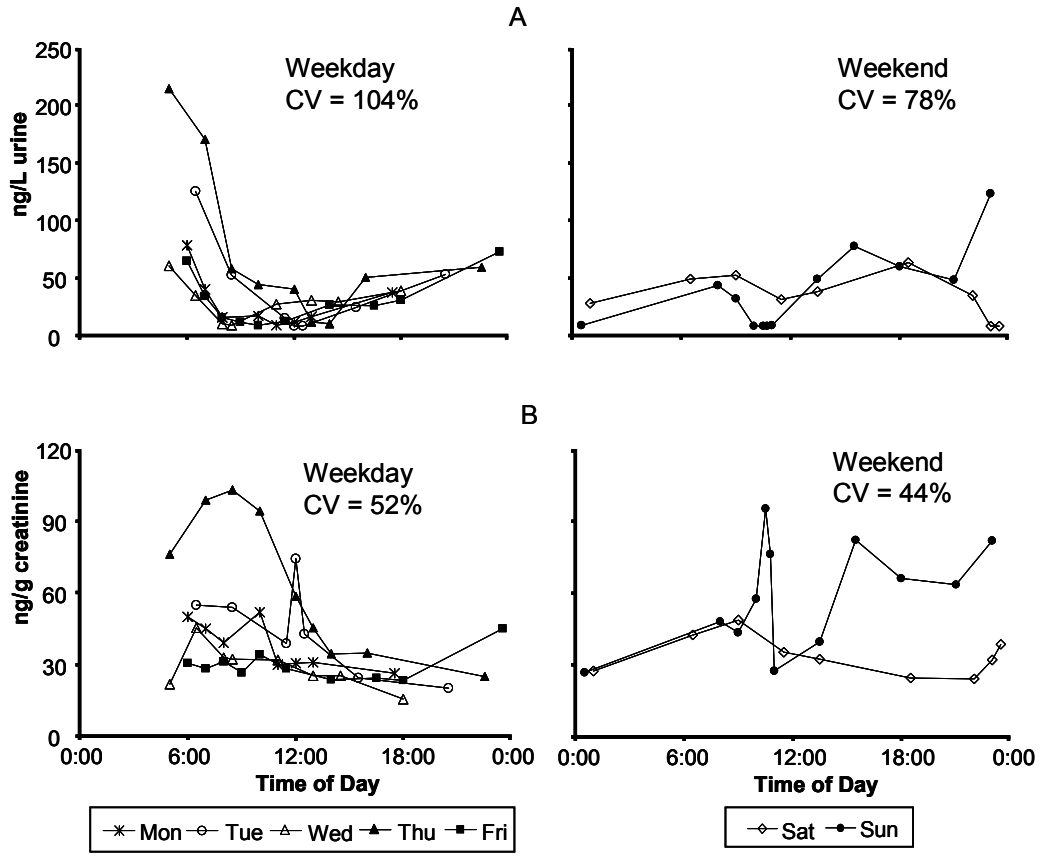

Figure 5.2. Levels of 1-hydroxypyrene in Subject 5 during one week for wet weight (A) and creatinine-adjusted concentrations (B)

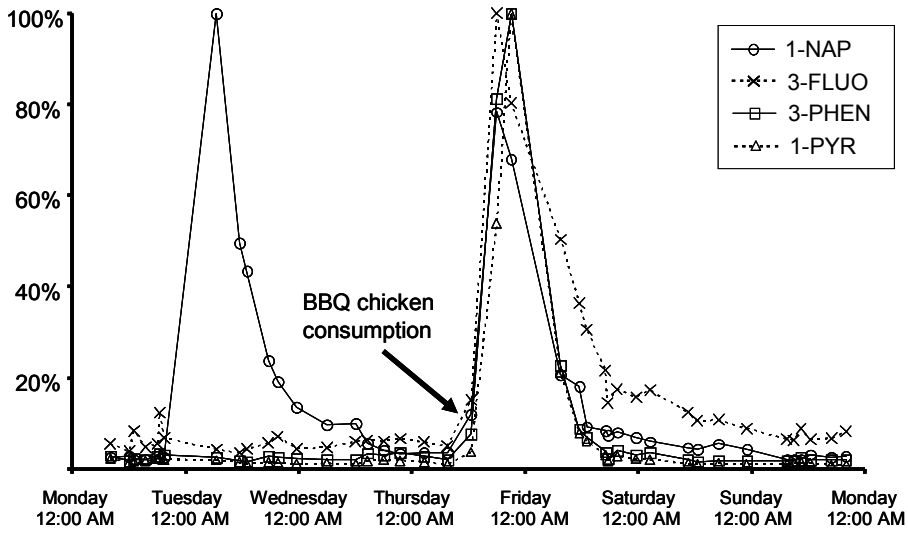

Figure 5.3. Normalized creatinine-adjusted concentrations of 4 OH-PAH metabolites from Subject 8. The subject consumed barbecue chicken for lunch on Thursday

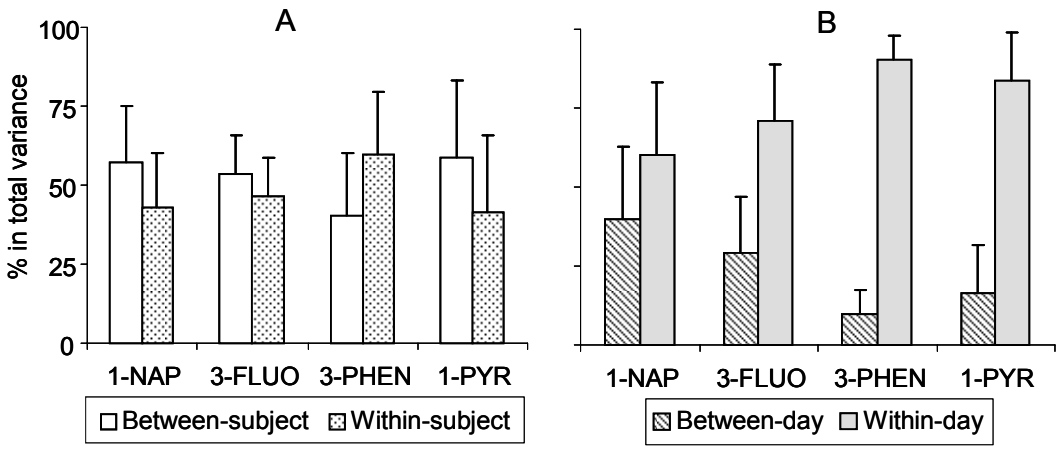

Figure 5.4. Contribution of between- and within-subject (A), between- and within-day (B) effects to total variance for 4 urinary OH-PAH metabolites

Table 5.4. Variance apportionment for creatinine-adjusted concentrations of 4 PAH metabolites in urine samples from 8 subjects over a period of 7 days

	Spot samples		1st morning voids		24 hour voids	
	Variance component	Percent of total variance	Variance component	Percent of total variance	Variance component	Percent of total variance
1-NAPc						
Subject	0.20	*30%**	0.27	*33%*	0.33	*44%*
Day	0.19	29%	0.55	67%	0.42	56%
Hour	0.27	41%	-	-	-	-
3-FLUOc						
Subject	0.14	*44%*	0.10	*50%*	0.14	*67%*
Day	0.04	13%	0.10	50%	0.07	33%
Hour	0.14	43%	-	-	-	-
3-PHENc						
Subject	0.11	*40%*	0.21	*65%*	0.25	*77%*
Day	0.01	4%	0.12	35%	0.07	23%
Hour	0.16	56%	-	-	-	-
1-PYRc						
Subject	0.26	*55%*	0.13	*60%*	0.13	*76%*
Day	0.03	7%	0.07	40%	0.04	24%
Hour	0.18	38%	-	-	-	-

* Numbers in italic are intraclass correlation coefficients (ICC), defined as the ratio of between-subject variance to total variance

Table 5.5. Estimated number of samples needed to detect a difference of 10, 25, 50, and 100% in the 1-hydroxypyrene geometric mean concentration with a statistical power of 80% (p<0.05), for single and repeated sampling for spot, first morning and 24-hour void sampling

Target % difference on GM	Number of repeated samples											
	Spot samples				First morning voids				24-Hour voids			
	1	2	3	4	1	2	3	4	1	2	3	4
Wet weight concentration (ng/L urine)												
10%	1069	624	475	401	618	420	353	320	296	230	208	197
25%	195	114	87	73	113	77	64	58	54	42	38	36
50%	59	34	26	22	34	23	20	18	16	13	11	11
100%	20	12	9	8	12	8	7	6	6	4	4	4
Creatinine-adjusted concentration (ng/g creatinine)												
10%	606	470	424	401	415	332	305	291	399	351	335	327
25%	111	86	77	73	76	61	56	53	73	64	61	60
50%	33	26	23	22	23	18	17	16	22	19	19	18
100%	11	9	8	8	8	6	6	5	8	7	6	6

Between-subject variance contributed 49-59% towards the total variance of 1-NAP, 3-FLUO, 3-PHEN, and 1-PYR (creatinine-adjusted, unless specified otherwise), and within-subject variance explained on average 41-60% for the seven days of sampling (Figure 5.4A). For the 8 subjects, within-day variance for 1-NAP, 3-FLUO, 3-PHEN, and 1-PYR, contributed to 60%, 71%, 90% and 84% (average of 8 subjects) of the total variance, respectively (Figure 5.4B). The contributions of subject, day and hour effect to the total variance of all samples, first morning voids and 24-hour voids are given in Table 5.4.

The number of subjects needed per group to detect a difference of 10%, 25%, 50% and 100% in urinary 1-PYR concentration is given in Table 5.5. Sample size calculations were performed to reflect three sampling methods: (*i*) spot sample collected anytime during the day; (*ii*) first morning void; and (*iii*) 24-hour void. Separate estimates of sample size are given for one through four repeated samples from each subject.

5.4 Discussion

We measured 9 PAH metabolites formed from four parent compounds in 8 adults during one week of continuous sampling. We quantified and apportioned variance among the subject and day factors, evaluated the effect of creatinine adjustment, and compared different sampling approaches on the reliability of these urinary biomarkers for exposure assessment purposes. Based on our findings, we also calculated sample size requirements necessary for spot urine, first morning and 24-hour void sampling, as a reference for future biomonitoring study design.

5.4.1 Concentrations and Profiles

Overall, concentrations found in this adult population with no occupational PAH exposure were comparable to the reference levels established for the U.S. general adult population (Table 5.1). This is not surprising considering that the participants were non-smoking professionals working in modern office buildings; thus their PAH exposure was relatively low. However, the number of subjects (N = 8) in this study is small and results in Table 5.1 were from repeated sampling from the 8 subjects. On the other hand, the NHANES survey that provided the reference levels for the U.S. general adult population included over 2000 subjects (Li et. al., 2008). Therefore, it is difficult to make a direct comparison between this study and the NHANES survey. Wet weight concentrations in first morning voids were significantly higher than in all samples and re-constructed 24-hour voids. The differences diminished after creatinine adjustment, indicating that the higher wet weight concentrations in the first morning voids were caused by the more concentrated urine formed overnight and that creatinine adjustment is an effective normalization method.

Within each subject, concentrations of the urinary OH-PAH metabolites varied throughout the day (Figures 5.1 and 5.2). An apparent diurnal pattern (i.e. higher concentration in the early and late hours of the day, and lower during the middle of the

day) on wet weight concentrations was observed in one subject during the weekdays but not during weekends (Figure 5.2). Creatinine normalization effectively removed or reduced this daily pattern, suggesting that urine dilution related to variation in water consumption throughout the day was the likely explanation for the higher concentrations (wet weight) at the beginning and the end of each day.

We designed and conducted this study with the intention to study variability of urinary OH-PAH levels in a non-occupationally exposed population and to conduct power calculations. Hence, we did not restrict diet and other activities during the study period. One of the subjects consumed barbecued chicken for lunch on Thursday and in the first urine sample taken 30 minutes after lunch, the creatinine-adjusted concentrations of the 9 metabolites were 1.9-6.6 folds higher than pre-exposure. Six hours later, metabolites of naphthalene and fluorene peaked with levels on average 22 times higher than pre-exposure, whereas 9 hours post exposure, metabolites of the larger PAHs, i.e. phenanthrene and pyrene, reached their highest level (41-104 fold higher than pre-exposure, Figure 5.3). Differences in urinary excretion rates for OH-PAHs have been reported previously. In an occupational study conducted in France, the time needed for urinary 1-PYR to reach maximum after work shift was approximately 8 hours, whereas the lag time was 15 hours longer for urinary 3-hydroxybenzo(a)pyrene (Gendre et al. 2004). The excretion profile of urinary OH-PAHs in our study is consistent with previously reported urinary half-lives estimates for 1-PYR ranging from 4.4 to 15 hours (Brzeznicki et al. 1997; Buckley and Lioy 1992; Viau et al. 1995; Viau and Vyskocil 1995).

It is well known that PAHs are generally present as complex mixtures in the environment, and after entering the human body, they are transformed to an array of metabolites (ATSDR, 1995). Human exposure to PAHs has been quantified based on urinary metabolites levels, and 1-PYR is the most common — often the only — biomarker measured. Indeed, concentrations of urinary OH-PAHs have been found to be

well-correlated in a large epidemiological study (Li et al. 2008), supporting the use of one biomarker as a surrogate representing PAH exposure. In our study, however, we found that certain metabolites had distinct concentration profiles over time, compared to other metabolites from the same individual. For example, 1-NAP had an elevated peak in S8 starting on Tuesday afternoon, whereas all other analytes including 1-PYR stayed at background levels during the same time period (Figure 5.3). The elevated 1-NAP concentrations could be traced back to the subject exercising at a health fitness center freshly sprayed with cleaner, and 1-NAP has been reported as a metabolite of carbaryl pesticide (Maroni et al. 2000). Similar large increases of only one or two metabolites occurred in other subjects but could not be explained by the information collected. The unique concentration profile specific to only one or two OH-PAHs demonstrated the individuality of each metabolite, the importance of a multi-analyte assay for exposure assessment, and the potential of using individual biomarkers as indicators of specific source-related exposure scenarios.

5.4.2 Variability Analysis

Few studies have reported within-subject variability of PAH metabolites in urine (Han et al. 2007; Siwinska et al. 1998). We found that levels of the PAH biomarkers spanned three orders of magnitude during a course of one week (Table 5.2). The CVs of wet weight OH-PAH concentrations within person ranged 64-270% in all samples and 26-153% in first morning voids (Table 5.3), after excluding S8 due to the high dietary exposure. Creatinine adjustment reduced the variability by 35% and 29%, respectively, indicating that creatinine adjustment corrects for differences in hydration status and reduces variability. Therefore, creatinine-corrected concentrations were used for all further variance and sample size calculations. The re-constructed 24 hour voids were shown to be the most reproducible sampling approach, reducing the CVs by 58% and 37%, compared to the individual samples and the first morning voids, respectively. No

significant difference in variability was observed after creatinine adjustment of the 24-hour void concentrations, which is consistent with the premise that humans excrete creatinine at a relatively consistent rate; approximately 2% of body creatine is converted to creatinine every 24 hours (Barr et al. 2005). Therefore, creatinine adjustment is not necessary for 24-hour voids but is essential for spot samples and first morning voids.

For individual subjects, within-day variance exceeded the between-day variance, contributing 72-89% to total variance (Figure 5.4B). This can be interpreted as the time of day is much more important than the day of the week when the sample is collected, a conclusion that stresses the importance of collecting samples at a well defined time of day in an epidemiological study. From these results, we recommend that if in a study we were to collect two samples from each subject to better reflect exposure, we would take samples on two different days at around the same time, rather than taking two samples from the same day.

In general, between-subject variance outweighed the between-day variance for 3-FLUO, 3-PHEN, and 1-PYR, whereas for urinary 1-NAP levels, the day effect contributed equally or more towards the total variance than the subject effect (Table 5.4). For spot samples, 38-56% of the total variances among the 4 metabolites could not be explained by either the subject or the day factor and could be attributed to the time of day effect — a finding that re-emphasizes the importance of collecting spot urine samples at a consistent time of the day if using this sampling approach.

ICC value, calculated as the ratio of between-subject variance to the total variance, ranged from 0.30-0.55 for spot samples, 0.33-0.65 for first morning voids, and 0.44-0.77 for 24-hour voids. The lower ICC values found in spot samples indicated higher within-subject variability and lower reproducibility from repeated urine collections from the same individual. On the other hand, 24-hour void was proven to be the most reproducible sampling technique, with markedly higher ICC values up to 0.77. As a comparison, the ICC values were 0.20-0.57 in first morning voids during an 8-

consecutive day study on urinary metabolites of phthalates, another group of non-persistent chemicals with similar half-lives (Fromme et al. 2007). In another study on urinary phthalate metabolites in spot urine samples taken over a 3-month period, the ICCs ranged from 0.28 to 0.52 (Hauser et al. 2004). The observed ICCs of urinary OH-PAH levels in our study were very similar to those reported on metabolites from other chemical classes with short half-lives, and there was a considerable degree of temporal reproducibility for these biomarkers over the study period of a week.

5.4.3 Sample Size Recommendation

In any cross-sectional or longitudinal epidemiological studies, investigators need to know in advance the approximate number of subjects required to achieve a desired statistical power. We found only one publication that reported sample size recommendations for urinary OH-PAH biomonitoring. Siwinska et al. (1998) calculated the minimum number of subjects for assessing exposure of a child population to environmental PAHs to be 164, based on variability of urinary 1-PYR concentrations in 6 consecutive first morning voids from 30 children. The method used in that estimation, however, was based on the assumption that the data were independent, which could be problematic because urinary biomarker concentrations are correlated. The ICC for 1-PYR in our study were 0.55, 0.60, and 0.76, for spot samples, first morning voids and 24-hour voids, respectively, indicating considerable agreement between repeated measurements. Using the variability and correlation findings from our study, we calculated sample size requirements for urinary 1-PYR, with one to four repeated samples, simulating the three different sampling methods, cf. Table 5.5.

As expected, number of subjects needed per group is the highest for spot samples, followed by first morning voids and 24-hour voids, due to the higher variability in the spot samples. Repeated sampling from the same subjects can reduce the sample size requirements, and the lower correlation and less reproducibility in spot samples makes

this approach benefit more from repeated sampling. For example, taking an additional sample per person reduces the sample size by 23%, 20% and 13% for spot samples, first morning and 24-hour voids, respectively. Sample size requirement can be further reduced by collecting additional repeated samples from each person; however, the benefit lessens with each additional sample, especially if 3 or more samples were taken per person. As expected, the sample size requirements when using wet weight concentration are significantly higher than for creatinine-adjusted concentrations in spot urine samples and first morning voids, which can be explained by higher variability in wet weight concentrations for these two sampling methods. For 24-hour voids, however, the sample size requirement is less when using wet weight concentrations, which is consistent with earlier conclusion that creatinine adjustment is not needed for 24-hour voids.

To detect a difference of 10% in geometric mean with a statistical power of 80%, a total of 606 spot urine samples (creatinine adjusted) will be needed per group to reach the desired statistical difference while 296 24-hour void samples (unadjusted) would be sufficient to reach the same statistical power. Hence, it is of utmost importance that these considerations are taken into account when planning an epidemiological study since we will need to balance the additional labor of collecting 24-hour void samples against the costs of recruiting and analyzing more than twice as many samples to reach the same statistical power.

Supplemental Material

Conversion of differences on geometric means (GM) between two groups to differences on the means of log-transformed data

Let: GM_1 = geometric mean of original concentration in Group 1

M_1 = mean of the log-transformed concentrations in Group 1

then: $M1 = \log (GM_1)$

Let: GM_2 = geometric mean of original concentration in Group 2

x = percent difference on GM between Group 2 and Group 1, i.e. $GM_2 = GM_1(1 + x)$

M_2 = mean of the log-transformed concentrations in Group 2

$= \log (GM_2)$

$= \log [GM_1 (1 + x)]$

$= \log (GM_1) + \log (1 + x)$

$= M_1 + \log (1 + x)$

Therefore:

d = difference on means of log-transformed concentrations between Group 1 and Group 2

$= M_2 - M_1$

$= \log (1 + x)$

For a fixed x, there is a fixed d. For example:

$x = 10\%$, $d = \ln (1 + 10\%) = 0.10$

$x = 25\%$, $d = \ln (1 + 25\%) = 0.22$

$x = 50\%$, $d = \ln (1 + 50\%) = 0.41$

$x = 100\%$, $d = \ln (1 + 100\%) = 0.69$

CHAPTER 6

ASSESSMENT OF NON-OCCUPATIONAL EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS THROUGH PERSONAL AIR SAMPLING AND URINARY BIOMONITORING

Abstract

Exposure to polycyclic aromatic hydrocarbons (PAH) can be assessed either through measuring PAHs in environmental samples (e.g. air samples) or through biomonitoring of urinary biomarker (hydroxy PAHs, or OH-PAHs) levels. This study was designed to assess non-occupational exposure to atmospheric PAHs through both personal air sampling and urinary biomonitoring. For 8-days, 8 subjects with occupational exposure to combustion sources and exposure to tobacco smoke, collected personal air samples while at home, at work or during commuting. All individual urine samples were collected from the subjects as well. We recorded detailed information on the duration and description of the personal air samples, time and volume of each urine excretion, as well as dietary intake, driving, and other activities of the participants during study period. Twenty-eight PAHs were measured in the personal air samples and 9 OH-PAHs were measured in the urine samples. PAH concentrations varied widely in personal air samples taken at home, work or while commuting. Naphthalene was found at higher concentrations in indoor air samples at home, whereas large PAHs with 4 or more rings were found at higher concentrations or with higher detection frequencies in air samples taken while commuting to/from work through driving or jogging. Naphthalene concentrations were highly elevated in one of the households, which could be a result of fresh paint and/or heavy pesticide usage a few months prior to the study. Urinary OH-

PAH biomarker levels varied throughout the study period and elevated biomarker concentrations were observed in all subjects following reported high inhalation (i.e. driving) or dietary exposures. Total inhaled naphthalene amount in 24-hour periods was well correlated with total excreted 1-naphthol, as did fluorene and its urinary metabolites. However, airborne phenanthrene and pyrene were not correlated with their urinary metabolites. These results suggest that for non-smoking reference populations without high occupational exposure, exposure to PAHs with 2-3 aromatic rings such as naphthalene and fluorene primarily through inhalation, while dietary ingestion is a major route for larger PAHs with 4 or more rings such as pyrene.

6.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of carcinogenic and mutagenic substances formed during incomplete combustion processes and are ubiquitously distributed in air, soil, water, food, and many occupational settings (IARC, 1983). Sources of PAHs include vehicle exhausts, forest fires, volcanic eruptions, and charbroiled or grilled meat. PAHs in the atmosphere, especially in densely populated areas, are primarily from anthropogenic emissions, such as automobile exhaust, fossil fuel combustion, biomass burning, cigarette smoke, and industrial activities. Human exposure to PAHs can occur through inhalation of polluted air or cigarette smoke, ingestion of food containing PAHs, and dermal absorption from soil or other PAH-containing materials such as coal tar. For the general population, inhalation and ingestion are the two dominant routes of exposure, with ingestion reportedly being more important quantitatively (ATSDR, 1995; Bostrom et al., 2002). PAHs such as benzo(a)pyrene, benz(a)anthracene, and chrysene have been classified as probable human and animal carcinogens (ATSDR, 1995). PAHs have also been reported to possess reproductive, developmental, hemato-, cardio-, neuro-, and immuno-toxicities (ATSDR, 1995).

Human exposure to environmental pollutants such as PAHs can be assessed through environmental monitoring, in which external levels in the macro (e.g. ambient air sample) or micro-environment (e.g. personal air samples) are measured and daily intake is estimated to assess exposure (Weis et al., 2005). Traditionally, epidemiologic approaches to the study of health effects of air pollution include utilizing ambient monitoring data, health outcomes, and individual-level variables. Some studies have utilized geographic information system-based models to assess exposure (Gunier et al., 2006). Numerous studies have reported on PAH levels in various matrices, such as in air (Gambaro et al., 2004; Li et al., 2009c; Ravindra et al., 2006), water or sediments (Akre et al., 2004; Oros and Ross, 2004; Zakaria et al., 2002), soil (Atanassova and Brummer, 2004; Doick et al., 2005; Golobocanin et al., 2004), house dust (Chuang et al., 1999; Fromme et al., 2004; Offenberg et al., 2004), and food (Falco et al., 2003; Stolyhwo and Sikorski, 2005). However, from an exposure assessment point of view, the selection of particular sampling sites, the relative location of the study population to the sampling sites, and individual habits and lifestyles are factors that can have a large influence on the effectiveness of exposure assessment using environmental data. Alternatively, assessing exposure at the personal level has been used to provide more accurate quantitative measures of estimate at points of human contact. Personal air sampling is a common practice in occupational studies (Bieniek, 1998; Gundel et al., 2000; VanRooij et al., 1993) as well as in studies of specific populations with suspected high exposure (Choi et al., 2008; Zmirou et al., 2000). In addition, exposure monitoring schemes can involve a combination of personal air sampling and indoor/outdoor sampling to get a better understanding of exposure at individual levels (Choi et al., 2008; Wilson et al., 1999). However, personal behavior such as physical activity and individual physiological differences can affect the uptake, absorption, distribution and metabolism of PAHs and other environmental pollutants, which present limitations of using external levels in exposure assessment.

Another method for determining exposure is biomonitoring, through which exposure is assessed by measuring the biomarkers in humans (Pirkle et al., 1995). Biomarkers of exposure refer to cellular, biochemical, analytical or molecular measures that are obtained from biological media such as tissues, cells, or fluids, and are indicative of exposure to a pollutant. Mono-hydroxylated PAHs (OH-PAHs), a group of metabolites of PAHs, have been used as urinary biomarkers of human exposure to PAHs, and 1-hydroxypyrnee (1-PYR) is the most commonly used biomarker (Hansen et al., 2008). A number of studies have reported the levels of OH-PAHs in occupational groups such as coke oven workers and road pavers (Grimmer et al., 1993; Jongeneelen et al., 1990; Levin et al., 1995), as well as in the general population from various parts of the world (Hansen et al., 2005; Li et al., 2008; Mucha et al., 2006). The half-life for urinary 1-PYR after inhalation exposure has been reported to be 9.8 hours from five volunteers at an aluminum plant (Brzeznicki et al., 1997) and 6-35 hours from 56 coke oven workers (Jongeneelen et al., 1990). For oral ingestion exposure, the half-life was reported to be 4.4 hours (Buckley and Lioy, 1992) or 12 hours (Viau et al., 1995a). Due to the short half-lives, quantitative determination of OH-PAH metabolites in urine is limited to provide information on a subject's recent exposure to environmental PAHs. Biomonitoring provides unique information for health researchers to determine what toxicants and what internal doses may be linked with adverse health effects, and markedly decreases uncertainty in assessing human risk. However, several confounding factors can affect the levels of metabolites. PAHs have rather fast clearance rate; therefore, the sampling time can have a large influence on the measurement results because urinary PAH metabolite levels reflect only recent exposure. Internal biomarker level reflects overall exposure of the subjects; however, it does not give route-specific information. In addition, individual differences in metabolism for different PAH compounds could also affect the levels of metabolites.

The objective of the present study was to investigate the feasibility of assessing exposure to ambient PAHs in an urban setting among a group of volunteers with no known occupational exposure and no or very low exposure to tobacco smoke by employing both personal air sampling and urinary biomonitoring. Eight volunteers took personal air samples while commuting to/from work, at work and at home over 8 days. All individual urine specimens excreted during the 8-day study period were also collected. The subjects followed a recommended diet plan, and maintained a detailed log with information on air samples, urine samples, dietary intake, driving and other outdoor activities. We measured 28 PAHs and methylated PAHs (Me-PAHs) in a total of 199 air samples and 9 major detectable OH-PAHs in 439 urine specimens. We studied levels of airborne PAHs in samples taken while commuting, at work and at home and computed the total amount of inhaled PAHs in each subject over 24-hour periods. We investigated excretion levels and profiles of urinary OH-PAHs and related the urine biomarker concentrations to information on driving, diet, and other activities. With the continuous air and urine sampling, we were able to study the correlation between inhaled PAHs and excreted OH-PAHs, which provided valuable information on exposure to ambient PAHs in this reference group.

6.2 Experimental

6.2.1 Study design

The 8 subjects in this study were healthy volunteers between 31 and 62 years of age with no known occupational PAH exposure. They were non-smokers who were not subjected to second-hand smoke. The participants (3 males and 5 females) lived in the metropolitan Atlanta area, and the sampling took place in November - December, 2007. Seven of them drove to and from work in the morning and afternoon during weekdays (Monday–Friday), with median driving time of 45 minutes (one-way) and a median

distance of 25 km (one-way). One subject (S12) jogged to and from work as a means of commuting with a distance of 6.5 km and on average 45 minutes one-way. All subjects worked in a modern office building at the Centers for Disease Control & Prevention (CDC) and were not occupationally exposed to PAHs. The participants did not work during the weekend (Saturday–Sunday), during which they only drove sporadically.

Detailed study design, sampling instructions and dietary recommendations are given in Appendix A. In brief, participants collected personal air and urine samples over 8 days, including four weekdays and four days over weekends. During the study period, participants followed specific diet instructions, eating only low PAH-containing food on four days (2 weekdays and 2 weekend days) while consuming flame broiled burgers (high in PAH content) as lunch on the other four days. The 8 sampling days were separated into 4 segments of 2 days each, i.e. weekend with low PAH diet, weekday with low PAH diet, weekend with high PAH lunch and weekday with high PAH lunch. Each volunteer carried a personal air sampling device and collected air samples continuously. The air sampler was comprised of a personal air sampling pump (224-PCXR4 SKC Inc., Eighty Four, PA, USA) with a flow rate set at 2.5 L/min, connected to a air sampler tube (OSHA Versatile sampler tubes, SKC Inc.). The sampler tube contained a quartz filter to collect particles, followed by two XAD sorbent layers and two PUF layers to trap gas phase organics. During the weekdays, a total of four personal air samples per day were collected by each subject, including two samples taken during morning/afternoon commute, one sample taken at home overnight, and one sample taken during the daytime at work. During the weekends, subjects were asked to minimize driving and outdoor activities while collecting air samples continuously (1-2 samples/day).

Meanwhile, participants collected all urine specimens throughout the study period. During each restroom visit, participants collected all urine excretions in a graduated beaker, recorded the volume and time of each excretion, and transferred a portion (~100 mL) to a pre-labeled sterile urine cup, which was then stored in an ice

cooler. The air and urine samples were retrieved from each participant daily (or after the weekend), brought back to the laboratory, and frozen at -70ºC until analysis. Participants kept detailed information on duration and start/end time of the air samples, time and volume of the urine samples, dietary intakes, driving, and other outdoor activities.

6.2.2 Analytical methods

Air samples were extracted through direct elution by dichloromethane (DCM) and quantified by isotope-dilution gas chromatography/high resolution mass spectrometry (GC/HRMS) (Li et al., 2009a). In brief, air sampler tubes were first fortified with a mixture of 18 isotopically labeled internal standards at 2 ng per compound, and then eluted with dichloromethane (DCM, 5 x 2 mL) at approximately 1 mL/min. The extracts were concentrated to ~50 μL under a gentle stream of nitrogen (5-10 psi, 40ºC), spiked with toluene (40 μL) and a recovery standard ($^{13}C_{12}$-labeled PCB105, 4 ng per sample) to measure recovery through the sample preparation process, and then transferred to GC vials for analysis. GC/HRMS analyses were performed on a MAT 95 XL HRMS instrument (Thermo Scientific, Waltham, MA, USA), coupled with a 6890 GC (Agilent Technologies, Palo Alto, CA, USA). The chromatographic separations were carried out on an RTX-440 column (30 m, 0.25-mm i.d., 0.25-μm film thickness; Restek Inc.) using helium (1 mL/min) as the carrier gas. The mass spectrometer was operated under selected ion monitoring (SIM) mode. Only the two most abundant ions from each compound were monitored as the quantification ion and confirmation ion.

Nine major detectable OH-PAH metabolites were measured in urine samples using a semi-automated liquid-liquid extraction and isotope dilution GC/HRMS method (Li et al., 2006). In brief, urine samples (2 mL) were spiked with ^{13}C-labeled internal standards and sodium acetate buffer (pH 5.5, 1M) containing β-glucuronidase enzyme (10 mg/sample). Samples were de-conjugated through overnight hydrolysis at 37 ºC, and then extracted by pentene through semi-automated liquid-liquid extraction (2 x 5mL) on a

Gilson 215 Liquid Handler (Gilson Inc., Middlerton, WI). The extracts were evaporated, re-constituted with 20 μL toluene, and derivatized to their trimethylsilylated derivatives. Finally, samples were analyzed on a GC/HRMS system at 10,000 resolution using a DB-5MS column (30m x 0.25 μm x 0.25-mm i.d.). Urinary creatinine was measured on a Roche Hitachi 912 Chemistry Analyzer (Hitachi Inc., Pleasanton, CA) by use of the Creatinine Plus Assay, as described in Roche's Creatinine Plus Product Application # 03631761003.

6.2.3 Quality assurance and quality control

All air sample tubes were pre-purified by the manufacturer to ensure low background interferences. In addition, all tubes were pre-rinsed by 5 mL of DCM, dried in a vacuum oven, capped, sealed in zip-loc bags, and refrigerated before being handed out to subjects. The flow rate for air sampling pumps was set at 2.5 L/min; the flow rates for pumps were measured by a flow meter (DC-Lite flow meter, SKC Inc.) before and after each sampling period. Field and method blanks were analyzed to monitor background contamination. Method blanks were measured along with samples, and all results reported were blank-subtracted. Secondary sample tubes were installed after the primary sample tubes to study the breakthrough of PAHs. Levels of PAHs on the secondary tubes were in most cases below the limit of detection (LOD).

Quality control (QC) samples were prepared by spiking a known amount of standard mixtures onto blank sample tubes (for air measurements) or into an anonymously collected urine pool (for urine measurement). In both air and urine methods, a set of samples was defined as 16 unknown samples, prepared and analyzed along with 2 blanks and 2 spiked QC samples. In addition, an external recovery standard and a calibration standard were analyzed on the GC/HRMS in parallel with the sample extracts. Analysis of a set of samples was considered valid only after fulfilling a series of

criteria on elution time, recovery and QC concentrations as described elsewhere (Li et al., 2009a; Li et al., 2006).

6.2.3 Data and statistical analysis

All concentrations were blank-subtracted; concentrations below the LOD were substituted with the LOD divided by the square root of 2 prior to statistical analysis. All statistical analyses were performed using the Statistica 7.1 software (StatSoft Inc., Tulsa, OK). Correlation analysis between inhaled PAHs and excreted OH-PAHs were carried through both linear regression analysis and non-parametric Spearman correlation analysis. Both methods gave comparable results; therefore, only results from the linear regression analysis are shown here. Results are considered statistically significant if the *p*-value was less than 0.05 and marginally significant when the *p*-value was between 0.05 and 0.1.

A total of 199 air samples were collected by the 8 subjects and all individual samples were measured for PAH (gas and particle phase combined). The 24-hour total inhaled PAH was calculated by summing up amount of PAHs from all air samples collected from one subject during a 24-hour period (~10 pm on day 1 – 10 pm on day 2) and normalizing to reference daily inhaled air volume (0.5 m^3/hr or 12 m^3/day for adults with sedentary activities) (U.S.EPA, 1997). Subject S17 did not collect continuous air samples; therefore, the 24-hour total inhaled PAH data were not available for this subject.

A total of 439 urine samples were collected from the 8 subjects. The 24-hour total excreted OH-PAH amount was calculated by summing up amount of OH-PAHs in all urine specimens collected by an individual during a 24-hour period starting at mid-day (12 pm – 12 pm). Subject S11 did not follow dietary plan and had limited number of urine samples with as low as 2 samples a day; therefore, urine results from this subject were excluded in the 24-hour total excretion calculation. When conducting correlation analysis on total inhaled PAHs and total excreted OH-PAH metabolites, we used a lag of

12 hours between the start of the air sampling and the start of urine sampling to account for biological half-lives in humans. Due to extremely high 1-NAP levels in subject S17 during the first weekend that was not likely related to PAH exposure, 1-NAP data from this subject for those two days were excluded from the correlation analysis.

6.3 Results and Discussion

Exposure assessment using both personal air sampling and biomonitoring has been applied often to study high exposure in various occupations, such as coke oven workers (Bieniek, 1998; Grimmer et al., 1993; Pan et al., 1998), electrode paste plant workers (Bentsen et al., 1998b), fireproof stone producing plant workers (Gundel et al., 2000), pavers/roofers (Hatjian et al., 1995; Vaananen et al., 2003) and policemen (Szaniszlo and Ungvary, 2001). However, studies on non-occupationally exposed reference populations are scarce. In our study, we aimed at investigating exposure of 8 volunteers to ambient PAHs and studying whether biomonitoring can capture low level exposure to environmental PAHs in an urban setting. The subjects collected personal air samples continuously and collected all urine specimens during the 8-day study period. Twenty-eight PAHs and Me-PAHs were measured in personal air samples, and 9 OH-PAH metabolites formed from 4 parent compounds were measured in urine samples. Levels of PAHs in the air samples and PAH metabolites in the urine samples in this study were, not surprising, up to orders of magnitudes lower than those found in occupational settings. We studied the concentrations and profiles of the airborne PAHs and urinary PAH metabolites. We also investigated correlations between the inhaled PAHs and excreted OH-PAH levels, and explored assessment of low-level exposure scenarios using a combination of personal air sampling and urinary biomonitoring.

6.3.1 PAHs in personal air samples

Table 6.1 Median and quartile PAH concentrations with detection frequencies in personal air samples taken at home, at work, inside an automobile or jogging while commuting to/from work. Also given are the numbers of samples, median sampling duration and volume for each category

	Abbr	LOD[1] (ng)	Detection frequency				Median and quartile concentrations (ng/m^3)			
			Drive	Jog	Work	Home	Drive	Jog	Work	Home
Number of samples			44	8	29	88				
Median sampling duration (hr)			0.83	1.0	7.6	10.6				
Median sampling volume (m3)			0.12	0.15	1.14	1.60				
PAH compounds										
naphthalene	NAP	14.5	95%	88%	93%	98%	823 (470-1537)	404 (260-476)	138 (95-222)	921 (372-3265)
2-methylnaphthalene	2-MNap	13.5	80%	50%	90%	97%	288 (276-452)	113 (69-176)	70 (41-137)	455 (105-627)
1-methylnaphthalene	1-MNap	10.7	59%	25%	83%	97%	104 (106-197)	LOD (LOD-77)	37 (18-65)	169 (51-262)
biphenyl	BIPH	3.0	77%	75%	93%	98%	47 (37-70)	25 (18-41)	17 (9-31)	43 (25-78)
acenaphthylene	ACNYL	0.20	93%	88%	79%	95%	5.1 (3.7-7.5)	3.9 (2.9-6.4)	0.7 (0.3-1.1)	1.1 (0.6-2.2)
acenaphthene	ACNAP	0.77	55%	50%	90%	98%	6.6 (6.5-9.2)	5.5 (4-7.9)	3 (2.5-4.4)	4.8 (2.5-8.5)
fluorene	FLUO	0.98	48%	38%	97%	96%	LOD (LOD-10.6)	LOD (LOD-7.9)	4.3 (3.1-6.4)	6.6 (3.7-17.6)
dibenzothiophene	DBT	0.40	18%	13%	66%	96%	LOD (LOD-LOD)	LOD (LOD-2.3)	0.7 (0.4-1)	2 (1.3-2.8)
phenanthrene	PHEN	1.63	45%	25%	100%	97%	LOD (LOD-18.2)	LOD (LOD-12)	7.5 (5.6-10.1)	10.5 (6-14.8)
anthracene	ANTH	0.09	77%	63%	83%	96%	1.6 (4.2-3)	0.7 (0.6-1)	0.3 (0.1-0.4)	0.5 (0.3-0.9)
3-methylphenanthrene	3-MPhe	0.46	18%	0%	79%	98%	LOD (LOD-LOD)	LOD (LOD-2.4)	1.1 (0.7-1.9)	2.4 (1.7-3.2)
2-methylphenanthrene	2-MPhe	0.49	20%	0%	86%	98%	LOD (LOD-LOD)	LOD (LOD-2.5)	1.4 (1-2.4)	2.9 (2-3.8)

Table 6.1 (continued)

PAH compounds	Abbr	LOD[1] (ng)	Detection frequency				Median and quartile concentrations (ng/m^3)			
			Drive	Jog	Work	Home	Drive	Jog	Work	Home
9-methylphenanthrene	9-MPhe	0.39	16%	0%	72%	97%	LOD (LOD-LOD)	LOD (LOD-2)	0.7 (0.4-1.2)	2.4 (1.8-3.2)
1-methylphenanthrene	1-MPhe	0.25	18%	0%	79%	97%	LOD (LOD-LOD)	LOD (LOD-1.3)	0.5 (0.3-0.9)	1.5 (1.1-1.9)
fluoranthene	FLRAN	0.04	80%	100%	100%	95%	1.4 (1.6-2.7)	3.5 (1.8-4.2)	0.8 (0.5-1.4)	0.4 (0.3-0.7)
pyrene	PYR	0.04	80%	100%	100%	90%	1.5 (2.4-2.8)	2.5 (0.9-3.2)	0.5 (0.2-1)	0.4 (0.2-0.6)
retene	RET	0.04	52%	38%	97%	89%	0.3 (0.8-1.2)	LOD (LOD-0.5)	0.2 (0.1-0.5)	0.2 (0.1-0.3)
benzo(c)phenanthrene	BCP	0.04	2%	13%	0%	8%	LOD (LOD-LOD)	LOD (LOD-LOD)	LOD (LOD-LOD)	LOD (LOD-LOD)
benzo(a)anthracene	BAA	0.04	9%	50%	10%	35%	LOD (LOD-LOD)	0.4 (0.2-0.8)	LOD (LOD-LOD)	LOD (LOD-0.04)
chrysene	CHRY	0.04	27%	88%	28%	77%	LOD (LOD-0.3)	0.9 (0.5-1.7)	LOD (LOD-0.06)	0.06 (0.03-0.07)
benzo(b)fluoranthene	BBF	0.04	18%	88%	28%	68%	LOD (LOD-LOD)	1.1 (0.6-2.1)	LOD (LOD-0.1)	0.05 (0.03-0.1)
benzo(k)fluoranthene	BKF	0.04	7%	50%	14%	31%	LOD (LOD-LOD)	0.4 (0.2-0.9)	LOD (LOD-LOD)	LOD (LOD-0.04)
benzo(e)pyrene	BEP	0.04	30%	88%	28%	71%	LOD (LOD-0.4)	0.9 (0.5-1.6)	LOD (LOD-0.05)	0.04 (0.03-0.08)
benzo(a)pyrene	BAP	0.04	14%	88%	14%	59%	LOD (LOD-LOD)	0.7 (0.4-1.4)	LOD (LOD-LOD)	0.04 (0.02-0.07)
perylene	PER	0.04	0%	38%	0%	10%	LOD (LOD-LOD)	LOD (LOD-0.4)	LOD (LOD-LOD)	LOD (LOD-LOD)
indeno(123-cd)pyrene	IP	0.04	27%	88%	24%	76%	LOD (LOD-0.4)	0.8 (0.5-1.4)	LOD (LOD-LOD)	0.06 (0.03-0.1)
dibenz(a,h)anthracene	DBA	0.04	2%	25%	0%	9%	LOD (LOD-LOD)	LOD (LOD-0.3)	LOD (LOD-LOD)	LOD (LOD-LOD)
benzo(ghi)perylene	BP	0.04	32%	75%	31%	78%	LOD (LOD-0.4)	1.1 (0.6-1.7)	LOD (LOD-0.06)	0.07 (0.04-0.14)

1. LOD: limit of detection, ng per sample

The median and quartile concentrations, detection frequencies and LODs of the 28 PAHs and Me-PAHs in personal air samples (gas + particle phases) are given in Table 6.1, stratified by the description of the samples, i.e. samples taken inside an automobile while computing to/from work, samples taken while jogging to/from work (as a mean of commuting, 1 subject), samples taken at work and at home. Since we combined the filter and sorbents during sample extraction and analysis, the results given in this study are a combination of both gas phase and particle phase. In general, concentrations of the PAH compounds were inversely related to their ring sizes, i.e. the lower the molecular weight, the higher the concentration in air samples. The median concentration of naphthalene (NAP) was as high as 3629 ng/m^3, followed by the two methyl naphthalenes (MNap), while concentrations of PAHs with 5-6 aromatic rings were up to 5 orders of magnitude lower, and in most cases, were below the detection limits (Table 6.1). This pattern is consistent with observations in previous studies that NAP is the most abundant PAH in ambient air and is predominantly found in the gas phase, as do the rest of small PAHs with 2-3 aromatic rings (Eiguren-Fernandez et al., 2004; Fang et al., 2004b; Ohura et al., 2004). Total concentrations (sum of gas + particle phases) of larger PAHs with 4 or more rings are generally lower, but they exist primarily in the particle phase (Fang et al., 2004b; Terzi and Samara, 2004; Tsapakis and Stephanou, 2005). Therefore, it is important to understand phase distribution when conducting research on ambient levels of semi volatile organic compounds such as PAHs.

PAH concentrations were different in air samples taken at different locations, and the variation pattern was compound specific. Overall, PAH concentrations were at the lowest levels in samples taken at work, which is not surprising because all subjects worked in a modern office building with advance air filtration devices and minimum indoor PAH sources. Large PAHs with 4 or more aromatic rings were found at higher concentrations or with higher detection frequency in samples taken while driving and jogging through traffic, compared to samples taken at home. Notably, the average

sampling volume and duration of the drive and jog samples were around 1 hour or 0.15 m^3 air sampled, which were more than 10 folds less than samples taken at home (10.4 hour or 1.56 m^3 air sampled). Yet, the jogging samples had the highest detectable rate for large PAHs despite of the smallest sampling volume and shortest sampling time, indicating that the jogging samples had the highest concentrations for the large PAHs (Table 6.1). This is consistent with conclusions from previous studies that mobile sources or vehicle exhausts can be the major contributors to PAHs, especially for large PAHs with higher molecular weight, in urban and suburban areas (ATSDR, 1995; Eiguren-Fernandez et al., 2004; Fang et al., 2004a).

NAP and MNap, on the other hand, were found at higher levels in samples taken at home than in samples taken while commuting (Table 6.1). It has been reported that these small PAHs are associated more with indoor sources such as combustion of biomass and fossil fuel at home and the use of NAP-containing moth repellent; and their concentrations in indoor air have been shown to be more elevated than in outdoor air (ATSDR, 2005; Sanderson and Farant, 2004). In our study, even though we did not collect simultaneous indoor and outdoor air samples, the fact that NAP and MNap concentrations were substantially higher in samples taken at home than in drive and jog samples is consistent with the notion that indoor sources for these small PAHs can be more important than outdoor sources. Interestingly, NAP concentrations varied largely among the air samples taken at the 8 homes, as shown in Figure 6.1 and Table B.1. Samples taken at Subject S18's home had the highest level of NAP at on average 18,700 ng/m^3, followed by those from S12's home at 4,810 ng/m^3, while the NAP concentrations ranged 118-1580 ng/m^3 in the other 6 households. S18 moved to the current house four months prior to the time when this study took place. The house was freshly painted and sprayed with pesticides. The flooring (half carpet and half hardwood) went through deep cleaning before the move and new carpet was installed in the basement, an area of approximately 167 m^2 or 1800 ft^2. NAP is not only a major product from in-complete

combustion processes and a constituent in fuel and creosote, but it also is a common industrial chemical used in the production of dyes, plasticizers, pesticides and pharmaceuticals. Therefore, the highly elevated NAP levels in S18's house could be a result of the fresh paint, pesticide usages and other activities in this household. Indoor concentrations of larger PAHs such as pyrene did not vary as much in the individual households as did naphthalene (Figure 6.1B and Table B.1). Interestingly, contrary to naphthalene levels, pyrene was found at the lowest concentration in samples taken at S18's 5-year-old house, which was the newest among the 8 households and presumably with the best insulation. This is consistent with previous conclusion that PAHs with 3 or more aromatic rings in indoor air without substantial combustion activities were mostly resulted from the infiltration of outdoor air (Li et al., 2005; Sanderson and Farant, 2004).

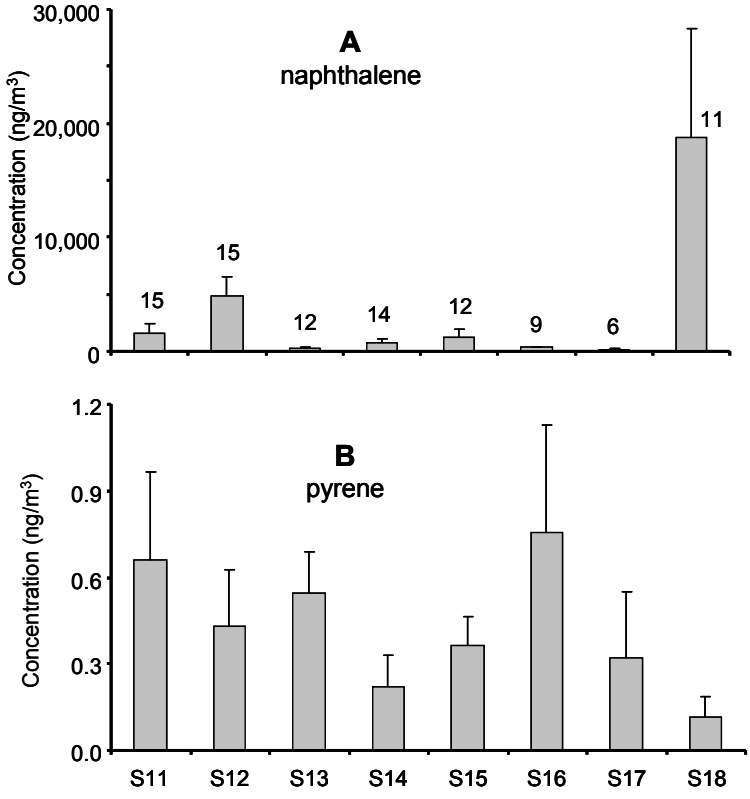

Figure 6.1 Average naphthalene (A) and pyrene (B) concentrations (with standard deviation) at individual households. Also givens are numbers of air samples taken at each home.

Table 6.2 Average PAH concentrations (sum of gas and particle phase, ng/m^3) in indoor environments from selected studies

	home[1]	low-income home[2]	daycare centers[3]	home[4]	wood-burning home[5]	reference home[5]	home[6]	home, summer[7]	home, autumn[7]
Study location	USA	USA	USA	Canada	Sweden	Sweden	Taiwan	China	China
Number of homes	8	24	9	12	13	10	n/a	8	8
Average PAH concentrations									
naphthalene	3400	2190	205	271	n/a	n/a	32208	5691	7741
acenaphthylene	1.5	43.9	2.2	n/a	n/a	n/a	198.0	1066	1388
acenaphthene	7.5	96.0	6.7	4.7	n/a	n/a	179.0	n/a	n/a
fluorene	10.5	49.7	4.1	2.1	n/a	n/a	298.0	133	94
phenanthrene	11.1	66.3	21.0	14.8	14.0	8.7	119.0	860	539
anthracene	0.67	6.26	0.83	0.06	1.30	0.41	20.80	234	230
fluoranthene	0.51	8.58	0.62	0.14	2.40	1.30	n/a	477	894
pyrene	0.43	6.73	0.37	0.31	2.00	0.96	n/a	383	667
benzo(a)anthracene	0.04	0.59	0.11	0.02	0.59	<LOD	n/a	172	208
chrysene	0.06	0.84	0.11	n/a	0.75	0.19	n/a	129	168
benzo(b)fluoranthene	0.08	1.27 [8]	0.14	0.28	0.55	0.21	n/a	n/a	n/a
benzo(k)fluoranthene	0.04		0.09	0.08	0.23	0.11	n/a	4	8
benzo(e)pyrene	0.08	1.05	0.11	0.17	0.42	0.21	n/a	27	52
benzo(a)pyrene	0.06	7.00	0.10	0.18	0.63	0.16	n/a	10	16
indeno(123-cd)pyrene	0.08	0.88	0.07	0.19	0.61	0.10	n/a	n/a	n/a
dibenz(a,h)anthracene	0.03	0.54	<0.04	0.09	0.49	0.10	n/a	n/a	n/a
benzo(ghi)perylene	0.11	1.46	0.08	0.36	0.26	0.13	n/a	n/a	n/a

1. This study; 2. Chuang et al., 1999; 3. Wilson et al., 1999; 4. Sanderson et al., 2004; 5. Gustafson et al., 2008; 6. Hung et al., 1992; 7. Liu et al., 2001;
8. Combined concentrations of benzo(b)fluoranthene and benzo(k)fluoranthene

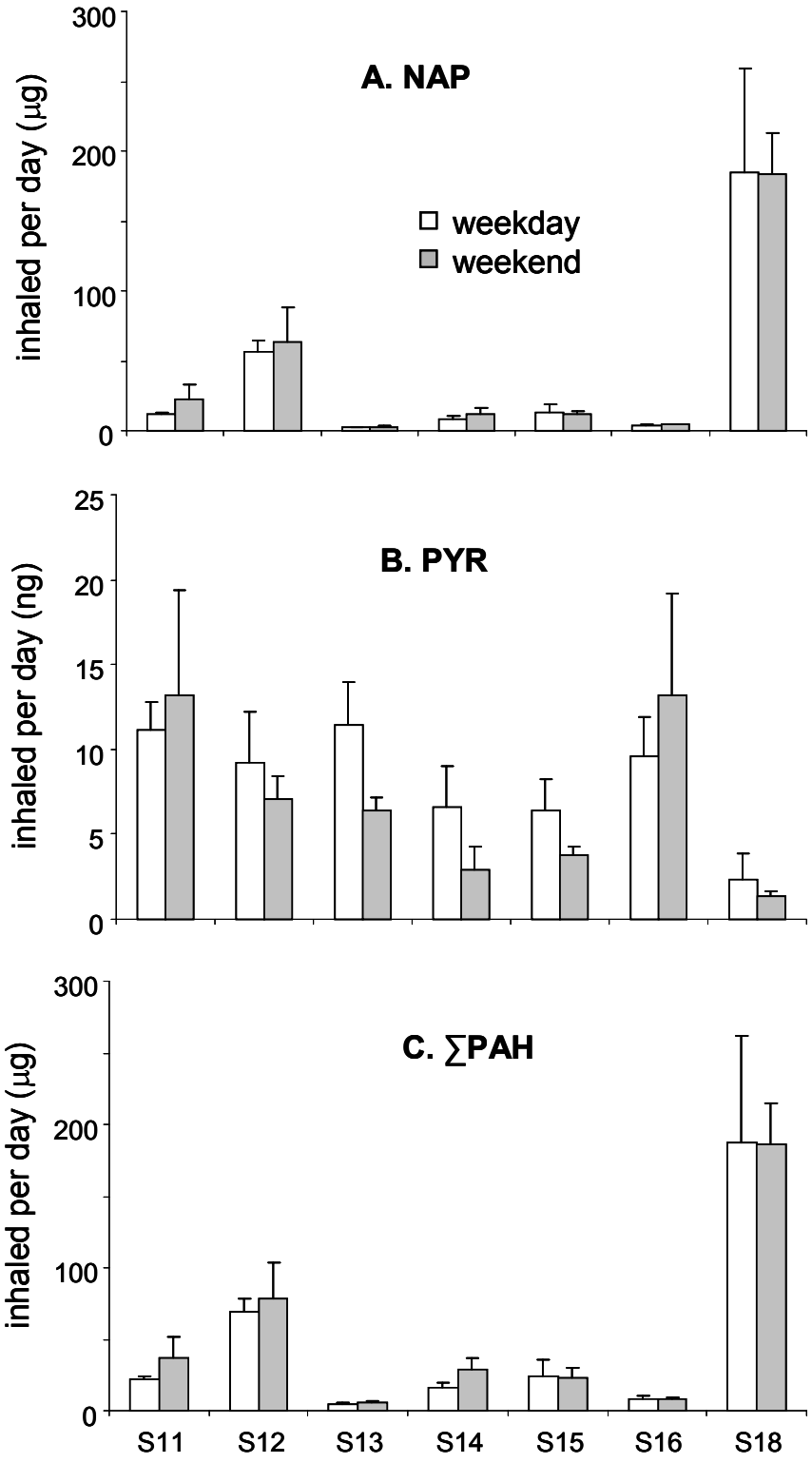

Figure 6.2 Calculated daily inhaled PAHs in 7 volunteers for naphthalene (A), pyrene (B) and summed PAHs (C), stratified by weekend and weekdays.

Average concentrations of the 16 EPA criteria PAHs and benzo(e)pyrene in air samples taken in the 8 households are given in Table 6.2. The indoor NAP concentrations in this study, as shown in Table 6.2, are similar to those reported in North Carolina, USA (Chuang et al., 1999; Day et al., 1992) and Canada (Sanderson and Farant, 2004), but are lower than the homes in Taiwan (Hung et al., 1992) and China (Liu et al., 2001). Concentrations of other PAHs, especially the ones with 4 or more aromatic rings, were much lower in this study than other published studies, including wood-burning and reference homes in Sweden (Gustafson et al., 2008), low-income homes and daycare centers in North Carolina, USA (Chuang et al., 1999; Wilson et al., 1999), homes in Canada (Sanderson and Farant, 2004), Taiwan (Hung et al., 1992), and China (Liu et al., 2001). It has been reported that in homes without substantial indoor sources, PAHs other than NAP were mostly attributable to the infiltration of outdoor air (Li et al., 2005; Sanderson and Farant, 2004). The lower concentration on larger PAHs in this study indicated overall lower PAH concentrations in the ambient air of this region and potentially better insulation in the homes involved in this study.

Overall, PAHs were not well correlated with each other when studying all air samples (n = 170, Table B.2), with the exception of the isomeric Me-PAHs. However, when only considering samples taken at work, the PAHs were highly correlated despite their low concentrations (Table B.4), which is consistent with the fact that all subjects worked in the same building with a homogenous indoor environment. In general, the correlations between PAH are higher when at a specific home, or at work, or driving, than the correlations for all samples (Tables B.2 -14). Specifically, PAHs with 4 or more aromatic rings were only detectable in jogging samples and were very well correlated with each other, supporting the suggestions that these large PAHs are mainly from mobile exhaust sources (Eiguren-Fernandez et al., 2004; Fang et al., 2004a). This suggests that the distributions are functions of the environment and might be useful as fingerprints for environmental exposure.

Calculated total inhaled NAP, pyrene (PYR) and summed PAHs (∑PAH) over a 24-hour period (assuming 12 m^3 air inhaled/24-hour) for 7 subjects are given in Figure 6.2. S17 was excluded due to in-complete air sampling. Large inter-subject variations were observed for all compounds. The variation on total inhaled NAP can be attributed to the differences on indoor NAP levels, as discussed above. Variation pattern for ∑PAH is similar to that of NAP, which is caused by the high NAP contribution to the ∑PAH (on average 62% among all air samples). PYR, on the other hand, presented a different variation pattern. The daily inhaled PYR levels were higher during weekdays than weekends in 5 out of the 7 subjects. This is consistent with the suggestion that exposure of larger PAHs such as PYR are predominantly through outdoor sources, and PAHs in urban air of heavily trafficked and populated areas are primarily from mobile sources (Fang et al., 2004a). Therefore, inhalation exposure of large PAHs would have less inter-subject variation and could potentially be different between weekdays and weekends due to different driving/outdoor activity patterns.

In traditional environmental epidemiological studies, air quality data from stationary monitoring sites are used to estimate exposure of study participants to air pollutants. This approach can pose a great amount of uncertainties because, as shown above, pollutant concentrations vary largely in the air that comes in direct contact with individual subjects. In addition, air toxicants such as NAP exist at higher concentrations in indoor environments and people generally spend more time indoor than outdoor. Therefore, using data from stationary sampling locations might not give a good indication of the actual exposure among study subjects and personalized exposure assessment might be a necessary measure to provide a better understanding of the true exposure.

6.3.2 OH-PAHs in urine samples

We quantified 9 OH-PAH metabolites in 439 samples collected from 8 subjects during 8 days. The OH-PAHs reported here are metabolites of 4 parent PAH compounds,

i.e. naphthalene, fluorene, phenanthrene and pyrene. The median, minimum and maximum concentrations from each subject are given in Table 6.3. The detection rates were over 95% for all reported metabolites with the exception of 2-hydroxyphenanthrene (2-PHEN, 86%). During the 8-day study period, each of the 8 subjects collected an average of 55 urine samples (36–84 samples/subject, Table 6.3). Overall, the median 1-PYR concentration found in this adult population with no occupational PAH exposure were at similar levels as the reference range established for the U.S. adult population (Li et al., 2008) and a group of male university students in Korea (Kim et al., 2001), residents in Frankfort, Germany (Heudorf and Angerer, 2001) and a small group of subjects in Atlanta, USA (Li et al., 2009b), but were lower than those reported in Canada (Viau et al., 1995b), Italy (Roggi et al., 1997) and Germany (Goen et al., 1995) (Table 6.4).

Concentrations of 3-hydroxyfluorene (3-FLUO) from 3 subjects (S12, S14 and S15) during the whole study period are given in Figure 6.3, and concentrations of all 9 OH-PAHs in all 8 subjects are given in Appendix C. Levels of urinary PAH metabolites in each subject varied throughout the day, as exemplified in Figure 6.3. Elevated biomarker concentrations can be observed after self-reported exposure. For example, 3-FLUO concentration in S14 peaked after morning rush traffic during the weekdays (Figure 6.3A); and in S17, 3-FLUO increased from 55 ng/g creatinine to 173 ng/g creatinine after driving for 225 km (~2 hours) (Figure 6.4). Similarly, increased urinary biomarker concentrations after ingesting high PAH-containing food (flame broiled burgers) have been noticed in several subjects, as shown in Figure 6.3B and Figure 6.4. Notably, it appears that different subjects had different exposure patterns, although their occupations were all of office nature and they worked at the same location at the time of the study. S14 in general had lower exposure and less variable biomarker levels during weekends than weekdays, suggesting that this subject's 1-hour commute through congested highway might result in higher exposure during weekdays (Figure 6.3A). Diet appeared to be the major source of exposure in S15, potentially due to the shorter

Table 6.3 Median, minimum and maximum urinary OH-PAH concentrations (creatinine adjusted, ng/g creatinine) in each subject

Metabolites	Abbr.	S11 (n=36)	S12 (n=84)	S13 (n=50)	S14 (n=53)	S15 (n=61)	S16 (n=42)	S17 (n=69)	S18 (n=44)	All (n=439)
1-naphthol	1-NAP	1096 (441-5030)	2152 (708-5146)	529 (149-6936)	480 (228-1498)	1343 (444-9413)	1121 (101-23016)	1881 (454-625467)	10151 (5189-21221)	1470 (101-625467)
2-naphthol	2-NAP	3010 (1942-4671)	1994 (1078-6513)	2905 (1455-5844)	2217 (1272-2930)	1475 (825-7496)	1122 (101-9341)	962 (101-3901)	5108 (2943-17923)	2110 (101-17923)
9-hydroxyfluorene	9-FLUO	192 (106-1146)	193 (51-2810)	317 (131-5496)	103 (43-703)	269 (110-6158)	213 (66-729)	326 (109-7744)	289 (65-3704)	247 (43-7744)
3-hydroxyfluorene	3-FLUO	60 (40-225)	71 (32-378)	51 (29-124)	25 (17-86)	115 (65-1014)	79 (23-1711)	78 (41-829)	56 (29-374)	67 (17-1711)
2-hydroxyfluorene	2-FLUO	105 (67-227)	164 (110-640)	196 (117-730)	67 (43-194)	302 (206-3579)	162 (66-2942)	195 (134-1329)	157 (114-1337)	174 (43-3579)
3-hydroxyphenanthrene	3-PHEN	71 (50-324)	59 (27-289)	63 (42-312)	46 (26-119)	116 (57-2498)	85 (37-349)	81 (40-2774)	119 (60-971)	76 (26-2774)
1-hydroxyphenanthrene	1-PHEN	69 (44-182)	89 (41-312)	278 (152-1370)	51 (32-88)	92 (49-1077)	159 (56-516)	146 (86-2656)	200 (119-729)	126 (32-2656)
2-hydroxyphenanthrene	2-PHEN	31 (22-89)	46 (16-196)	69 (37-315)	31 (15-86)	51 (24-1015)	59 (23-365)	67 (30-1854)	59 (36-389)	52 (15-1854)
1-hydroxypyrene	1-PYR	25 (18-182)	51 (23-239)	79 (64-262)	32 (17-67)	77 (34-5839)	82 (41-544)	83 (46-3925)	99 (55-1035)	67 (17-5839)

Table 6.4 Comparison of median 1-hydroxypyrene (1-PYR) concentrations (creatinine-adjusted, ng/g creatinine) in selected published non-occupational studies.

Population; country	Age (years)	N	Median concentration (range)	Reference
Adults; Atlanta USA	31-62	8 (439)[1]	67 (17-5839)	This study
Adults; Atlanta USA	25-58	8 (427)[2]	58 (7-1963)	Li et al., 2009
US population; USA	≥20	1625	41 (15-233)[3]	Li et al., 2008
University students; Korea	23	129	58 (19-540)	Kim et al., 2001
Adults; Germany	≥20	495	88 (LOD-1172)	Heudorf and Angerer, 2001
Adults; Canada	n/a	140	154 (39-617)	Viau et al., 1995
Non-smoking adults; Italy	22-81	327	149 (52-654)[4]	Roggi et al., 1997
Adults; Germany	n/a	69	450 (<40-600)	Goen et al., 1995

1. A total of 439 samples were taken from 8 subjects
2. A total of 427 samples were taken from 8 subjects
3. Median concentration with 10th and 95th percentiles
4. Median concentration with 5th and 95th percentiles

commute (~25 min) which led to less inhalation exposure (Figure 6.3B). Interestingly, 3-FLUO levels in S12 were consistently higher during weekends than in weekdays regardless the diet (Figure 6.3C), indicating that this subject might be engaged in activities during weekends which resulted in higher PAH exposure.

Concentrations of 3-hydroxyfluorene (3-FLUO) from 3 subjects (S12, S14 and S15) during the whole study period are given in Figure 6.3, and concentrations of all 9 OH-PAHs in all 8 subjects are given in Appendix C. Levels of urinary PAH metabolites in each subject varied throughout the day, as exemplified in Figure 6.3. Elevated biomarker concentrations can be observed after self-reported exposure. For example, 3-FLUO concentration in S14 peaked after morning rush traffic during the weekdays (Figure 6.3A); and in S17, 3-FLUO increased from 55 ng/g creatinine to 173 ng/g creatinine after driving for 225 km (~2 hours) (Figure 6.4). Similarly, increased urinary biomarker concentrations after ingesting high PAH-containing food (flame broiled burgers) have been noticed in several subjects, as shown in Figure 6.3B and Figure 6.4.

Notably, it appears that different subjects had different exposure patterns, although their occupations were all of office nature and they worked at the same location at the time of the study. S14 in general had lower exposure and less variable biomarker levels during weekends than weekdays, suggesting that this subject's 1-hour commute through congested highway might lead to higher exposure during weekdays (Figure 6.3A). The commuting effect was not as clear on S15, potentially due to shorter commute (~25 min);

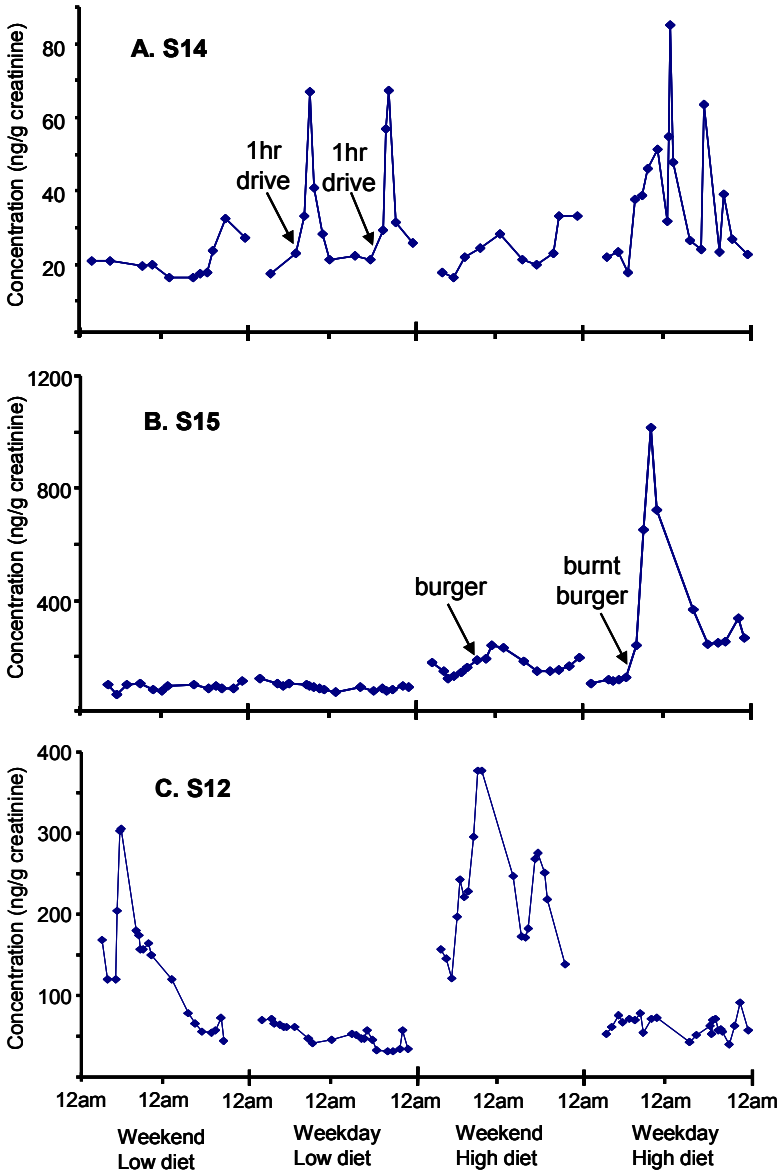

Figure 6.3 Concentration of 3-hydroxyfluorene over the 8-day study period (4 periods, 2 days in each period) in subjects S14 (A), S15 (B) and S12 (C).

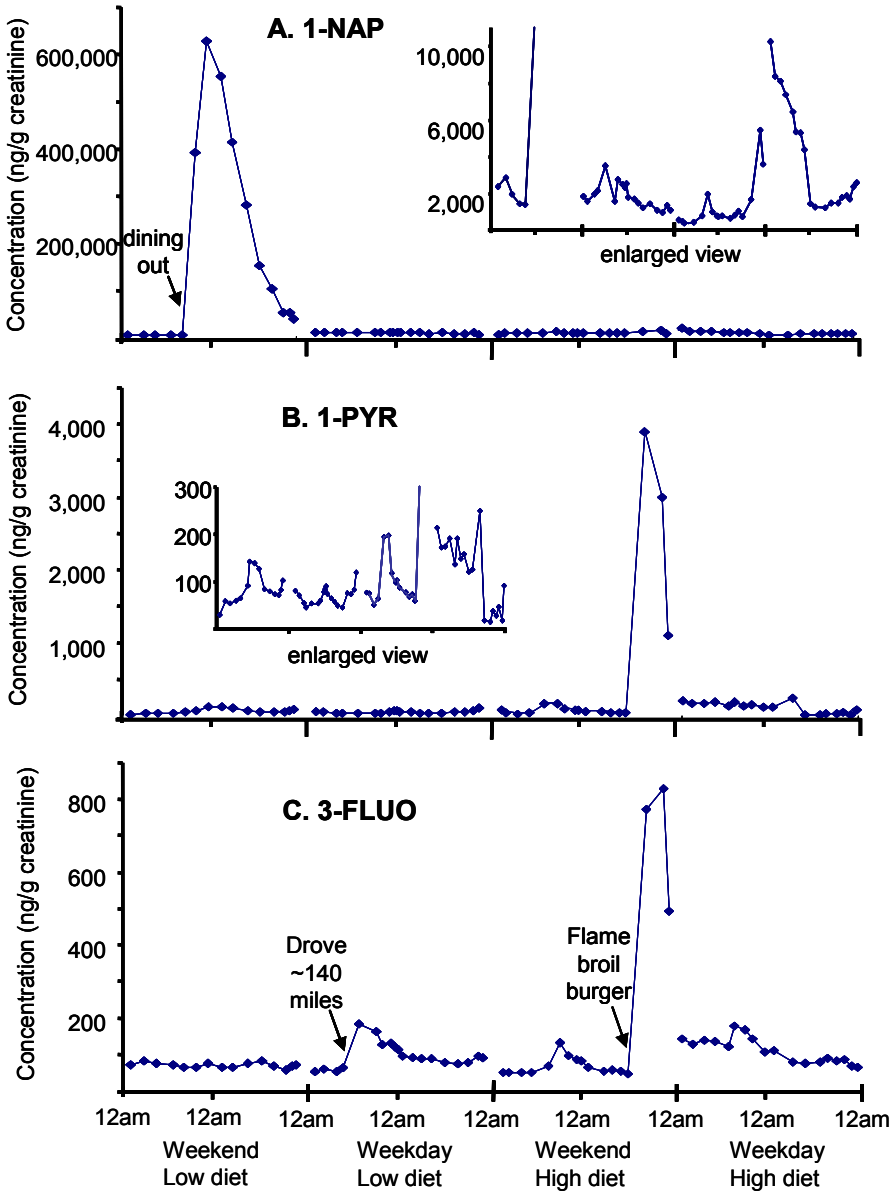

Figure 6.4 Concentration of 1-naphthol (A), 1-hydroxypyrene (B) and 3-hydroxyfluorene (C) over the 8-day study period in subject S17.

therefore, the diet was the major influence on the exposure and biomarker levels in this subject (Figure 6.3B). Interestingly, 3-FLUO levels in S12 were consistently higher during weekends than in weekdays regardless the diet, indicating that this subject might be engaged in activities during weekends which resulted in higher PAH exposure (Figure 6.3C).

It is well recognized that PAHs are generally present as complex mixtures in the environment, and are metabolized into an array of metabolites including hydroxylated metabolites (ATSDR, 1995). 1-PYR is the most common—often the only—biomarker measured to assess exposure to PAHs. Indeed, concentrations of urinary OH-PAHs have been found to be well correlated to each other in a large epidemiological study (Li et al., 2008), supporting the use of one biomarker as a surrogate representing PAH exposure. In this study, we measured 9 major detectable OH-PAHs, metabolites of NAP, fluorene, phenanthrene and PYR. Generally, the metabolites followed similar profiles, especially when high exposure occurred (Figure 6.4 B and C, Appendix C). However, we found that certain metabolites had distinct concentration profiles over time, compared to other metabolites from the same individual. For example, 1-naphthol (1-NAP) had a sharp increase in S17 after dining out at a friend's house, to 625,467 ng/g creatine, over 300 times higher than the median 1-NAP (1881 ng/g creatinine) for S17. At the same time, all other analytes, including 2-naphthol (2-NAP) and 1-PYR, stayed at background levels (Figure 6.4, Appendix C). 1-NAP has been reported as a metabolite of carbaryl pesticide (Maroni et al., 2000); therefore, the highly elevated 1-NAP could be a result of exposure to an agent(s) other than PAHs. As a result, 1-NAP data from S17 during the affected days were excluded in correlation analysis as introduced below. Concentrations of the fluorene metabolites, as exemplified in Figure 6.3, responded with both reported extended driving and high dietary PAH intake. 1-PYR, meanwhile, had the largest increase after high diet exposure in multiple subjects, as shown in Figure 6.4B, which is consistent with an earlier study reporting the increased OH-PAH levels after ingestion of barbecued chicken (Li et al., 2009b).

Among the 8 subjects, only S17 had a potential high inhalation exposure event (140 mile drive) and a high ingestion exposure (flame broiled burger), c.f. Figure 6.4 and Figure C.7 (Appendix C). In order to study the metabolite distribution under each exposure route, we calculated the percentage of each metabolite to the ∑OH-PAHs, and

plotted the percentages of the FLUO, PHEN and PYR metabolites around the time points when the long driving/burger took place, c.f. Figure 6.5. Concentrations of the two naphthalene metabolites did not have large variation during both event (Figure C.7), which is consistent with their sources being primarily indoor air; their percentages in ∑OH-PAH were as high as 53% during the time periods, overwhelming those of the rest of the metabolites; therefore, they are not included in Figure 6.5. Percentage and concentration of 9-FLUO appeared to be elevated approximately 5.75 hours after the long drive trip; the trend continued and reached maximum at mid-night, 12 hours post-driving. Levels of 2- & 3-FLUO also increases after the driving, although the trends were not as profound as for 9-FLUO (Figure 6.5A). This observation, though interesting, should be used with caution since we did not have another high inhalation exposure event and therefore, could not confirm the results. The percentages and concentrations of the PHEN and PYR metabolites, on the other hand, were not affected by the 2-hour long driving. After consuming a flame broiled burger, concentrations of all OH-PAH metabolites increased drastically (Figure 6.4 and Figure C.7). Specifically, 1-PYR had the largest increase in relation to the rest of the OH-PAHs; its percentage in total OH-PAHs increased from 3% before the burger consumption to 15% and 16% at 5 and 10 hours post the ingestion exposure (Figure 6.5 B). Therefore, 1-PYR had both highest increase in concentration and in percentage in relations to the rest of OH-PAHs, suggesting that 1-PYR is a more specific sensitive indicator to dietary input in reference populations with low PAH exposure.

It is well known that a number of enzymes are involved during the metabolism of xenobiotics such as PAHs and it has been suggested that under different exposure scenarios, different enzymes could be activated to potentially form isomeric metabolites preferentially (Baum et al., 2001; Rushmore and Kong, 2002; Wrighton and Stevens, 1992). To explore this hypothesis, we calculated the percentages of individual isomeric metabolites among the metabolites from the same parent compound, e.g. 9-FLUO/sum of

2-, 3-, and 9-FLUO, and 3-PHEN/sum of 1-, 2-, and 3-PHEN. We identified three subjects who had high biomarker spikes after consuming flame broiled burgers (Figures B.3, B.5 and B.7, Appendix C) and studied the variation of isomeric metabolite percentages around the high exposure event among the three subjects. As shown in Figure 6.6, after consuming the flamed burgers, percentage of 9-FLUO in comparison to the rest of the FLUO metabolites increased in all three subjects, indicating that out of the three FLUO metabolites measured, 9-FLUO might be a preferred metabolite formed after an ingestion exposure.

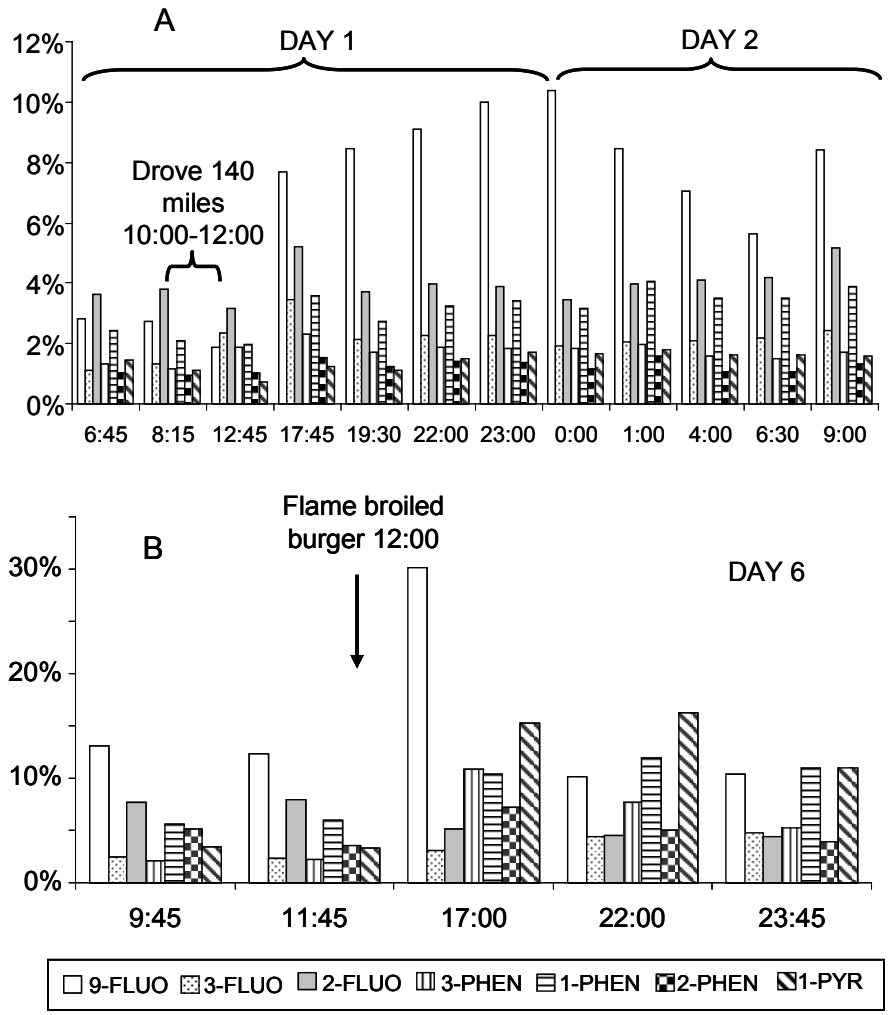

Figure 6.5 Percentage of individual metabolites to ∑OH-PAHs in subject S17 around a long driving trip on one day (A) and the consumption of a flame broiled burger on another day (B).

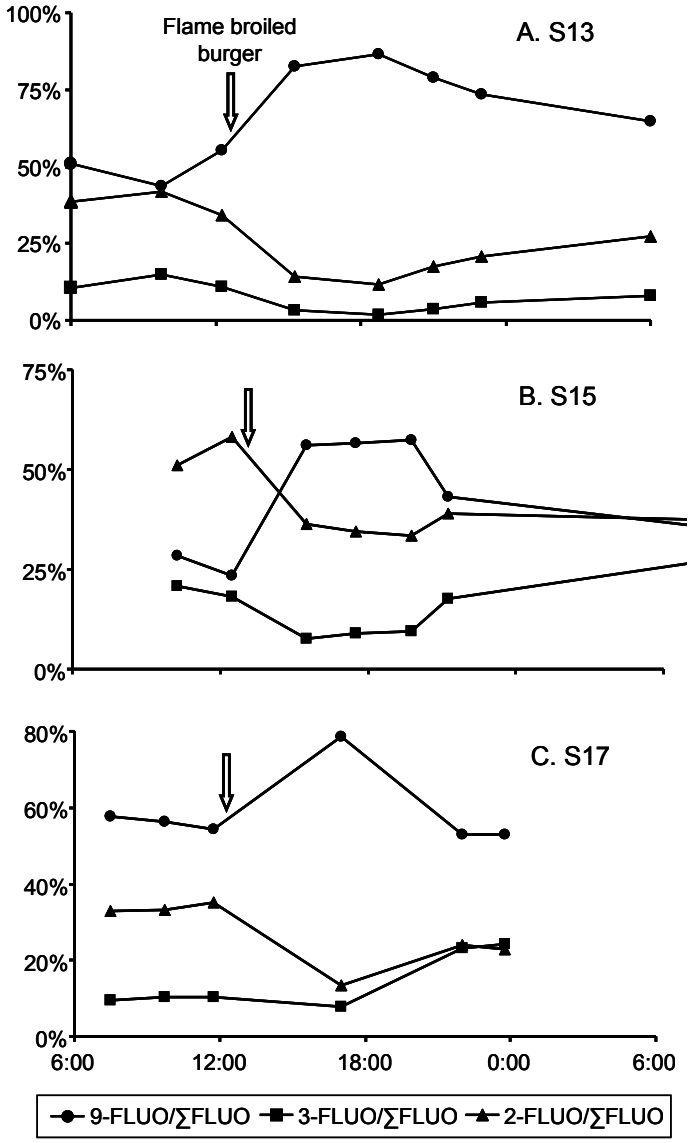

Figure 6.6 Percentages of individual fluorene metabolite to total fluorene metabolites in three subjects after consuming flame broiled burger

To sum up, we found that in this group of reference population with low over exposure, 1-PYR and 9-FLUO might be more related to ingestion exposure, comparing to the rest of the OH-PAH metabolites. We also noticed the unique concentration profiles of the individual OH-PAHs, which demonstrated the importance of a multi-analyte assay for exposure assessment, and the potential of using individual biomarkers as more specific indicators of source-related exposure scenarios.

6.3.3 Relationship between total inhaled PAHs and total excreted OH-PAHs

We found that the total inhaled NAP within 24-hour periods ranged from 2.3 μg/24-hr to 228 μg/24-hr during days with low PAH diet, while the total excreted OH-NAP in the sample period (with 12-hour lag) were 2.5 – 21.7 μg/24-hour, or on average 60.1% of the inhaled level (Figure 6.7). The lower excreted metabolite levels compared to inhalable NAP amount can be a result of respiration uptake rate, actual volume of the air breathed, and alternative metabolites of inhaled NAP. In contrast, total 24-hour inhaled pyrene ranged from 1.3 – 14.5 ng/24-hour on low PAH-diet days, while excreted urinary 1-PYR in a 24-hour period were 35-677 ng (Figure 6.7) – an average of 24 folds (5.9-74 folds) higher than inhaled PYR, demonstrating that inhalation is not the main route of exposure for pyrene, and ingestion is likely the prime source for urinary 1-PYR in this reference group.

Table 6.5 Correlation coefficients between inhaled PAHs and excreted OH-PAH metabolites on low PAH diet and high PAH diet days.

	Low PAH diet days					High PAH diet days				
	NAP	FLUO	PHEN	PYR	∑PAH	NAP	FLUO	PHEN	PYR	∑PAH
1-NAP	***0.89*** [1]	-	-	-	-	***0.76***	-	-	-	-
2-NAP	0.42	-	-	-	-	0.20	-	-	-	-
∑OH-NAP	***0.87***	-	-	-	-	***0.63***	-	-	-	-
9-FLUO	-	0.22	-	-	-	-	0.27	-	-	-
3-FLUO	-	***0.67***	-	-	-	-	0.52	-	-	-
2-FLUO	-	***0.68***	-	-	-	-	***0.54***	-	-	-
∑OH-FLUO	-	*0.55* [2]	-	-	-	-	0.41	-	-	-
3-PHEN	-	-	0.09	-	-	-	-	0.31	-	-
1-PHEN	-	-	-0.17	-	-	-	-	0.18	-	-
2-PHEN	-	-	-0.03	-	-	-	-	0.23	-	-
∑OH-PHEN	-	-	-0.09	-	-	-	-	0.13	-	-
1-PYR	-	-	-	0.38	-	-	-	-	0.11	-
∑OH-PAH	-	-	-	-	***0.84***	-	-	-	-	***0.78***

1. Numbers in bold italic are statistically significant
2. Numbers in italic are marginally significant

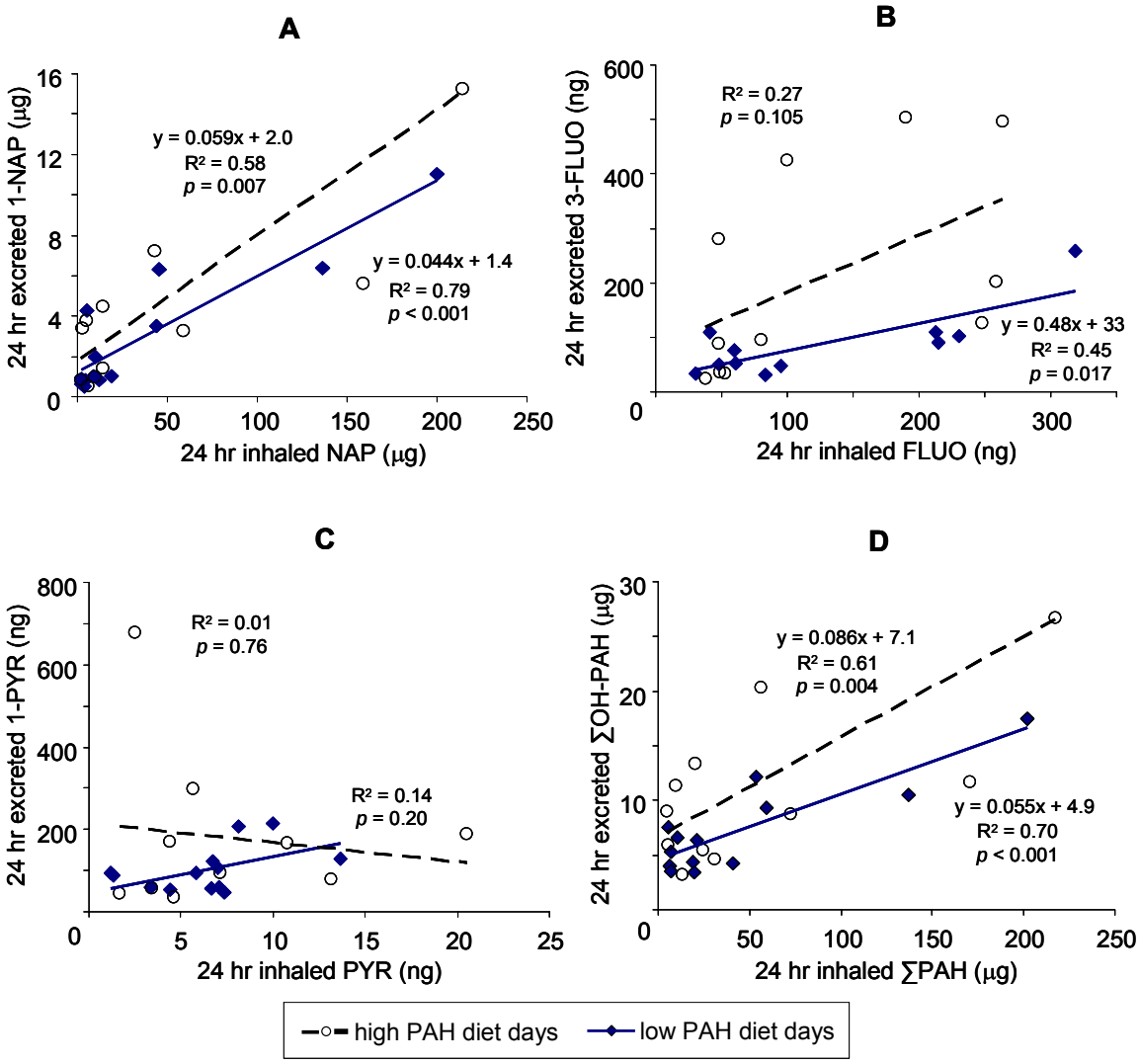

Figure 6.7 Correlations between 24-hour inhaled PAHs and 24-hour excreted OH-PAH metabolites for (A) naphthalene and 1-naphthol, (B) fluorene and 3-hydroxyfluorene, (C) pyrene and 1-hydroxypyrene, and (D) ∑PAHs and ∑OH-PAHs.

The total inhaled NAP was well correlated to 1-NAP even during the days with high PAH diet (Figure 6.7A), suggesting that 1-NAP is a prime metabolite formed from inhaled NAP and inhalation is the major route of exposure. Total excreted 2-NAP, another metabolite of NAP, was not correlated with inhaled NAP (Table 6.5). This is surprising, since 1-NAP and 2-NAP are the two major metabolites for NAP, they are the most abundant urinary PAH metabolites and are often present at similar levels, so they

both have been proposed as biomarkers for NAP or airborne PAHs exposures (ATSDR, 2005; Preuss et al., 2003; Yang et al., 1999). Moreover, due to the potential pesticide origin for urinary 1-NAP as confirmed in this study, 2-NAP has been suggested as a more specific marker for inhalation exposure to PAHs in ambient air (Kang et al., 2002; Preuss et al., 2003). The un-expected finding on non-correlated airborne NAP and urinary 2-NAP in our study could be a result of small sample size (6 subjects with matching air/urine results and 2 data points/subjects in each diet category).

Among the rest of the PAH – OH-PAH pairs (Table 6.5 and Figure 6.7), airborne fluorene were significantly correlated with 2- and 3- hydroxyfluorene during low-diet days, but not with 9-hydroxyfluorene; correlation only existed between fluorene in air and 2-FLUO on days with high PAH diet, indicating that 2-FLUO might be the preferred metabolite formed from inhaled fluorene. None of the phenanthrene and pyrene metabolites were correlated with their parent compound levels in air, suggesting that inhalation might not be their major route of exposure in this group of subjects. Total summed OH-PAH in urine were well correlated with total PAHs in air, which was likely driven by the NAP and 1-NAP contributions (NAP accounted for on average 62% towards the total PAHs in air and 1-NAP accounted for 34% of the summed OH-PAHs). Overall, correlations between inhaled PAH and excreted metabolites decreased during high PAH-diet days compared to days with low PAH diets. Biomonitoring results on urinary metabolites reflect exposure from all routes including ingestion and inhalation; therefore, when dietary input is large, correlations between inhaled PAH and urinary metabolites worsens.

Most studies reported relationships between airborne PAHs and urinary OH-PAH metabolites were focused on workers exposed to high levels of PAHs. Vaananen et al. (2003) found that among a group of pavers, urinary 1-NAP, sum of 1- and 2-NAP, and 1-PYR were significantly correlated with NAP, PYR, 2-3 ring PAHs, 4-6 ring PAHs and the total PAHs measured in personal air samples; urinary hydroxyphenanthrenes (OH-

PHEN), however, was not correlated with airborne phenanthrene levels (Vaananen et al., 2003). In another study on 170 German workers, both urinary 1-PYR and sum of OH-PHEN were correlated with PYR and phenanthrene in personal air samples (Rihs et al., 2005). Urinary 1-PYR in a group of Italian policeman exposed to traffic emissions was found to be well correlated to PYR, benzo(a)pyrene and total PAHs in the breathing zone; the correlation was further improved on samples taken during winter in the high-traffic area (Perico et al., 2001). Urinary 1-PYR in 18 electrode paste plant workers was also found to be correlated with PYR measured in the personal air samples (Bentsen et al., 1998b). However, in another study conducted by the same researchers, no significant correlation was found between urinary 1-PYR and PYR in inhalable aerosol samplers (Bentsen et al., 1998a). Studies on non-occupationally exposed populations using such methods were limited. Our results are consistent with a study on a group of Korean middle school students in which urinary 2-NAP was found significantly correlated with total suspended particulate (TSP) levels in the ambient air, but 1-PYR was not correlated with any TSP data (Kang et al., 2002).

Our results suggest that a major exposure route for NAP both for general population with low exposure and for many occupational groups is generally through inhalation. Results on other hydroxylated PAH metabolites such as 1-PYR and OH-PHEN were mixed. In general, 1-PYR was correlated to airborne PYR in occupations with suspected high inhalation exposure (Perico et al., 2001; Rihs et al., 2005), whereas such correlation did not exist when alternative exposure route such as dermal absorption (Bentsen et al., 1998a) dominated. In this study on a reference group with overall low environmental exposure, the main source for phenanthrene and PYR exposure is most likely through dietary intake.

CHAPTER 7

CONCLUSIONS AND FUTURE DIRECTIONS

Polycyclic aromatic hydrocarbons (PAHs) are distributed ubiquitously in the ambient air, soil and water environments, which lead to wide exposure in the general populations (ATSDR, 1995; Li et al. 2008). PAHs in the atmosphere can come from natural sources, such as forest fires and volcanic eruptions. However, their presence, especially in densely populated areas, is significantly affected by anthropogenic emissions, such as automobile exhaust, fossil fuel combustion, biomass burning, cigarette smoking, and industrial activities. PAHs have been reported to be carcinogenic and mutagenic, and possess toxicities towards a variety of target organs/systems, such as liver and immune system (ATSDR, 1995). Airborne PAHs, when bound to particulate matters especially the fine particles ($PM_{2.5}$), may increase potential health effects because particles carrying the toxic compounds can penetrate and deposit deep in the bronchioles and alveoli of the lungs.

Particulate matter pollution is of interest in metropolitan Atlanta, Georgia, one of the fastest growing regions in the United States. Currently, the twenty-county Atlanta metropolitan area is in non-attainment for $PM_{2.5}$ and ozone (U.S.EPA, 2007). PM source apportionment analyses by two groups of researchers indicated that 30 to 40% of Atlanta's $PM_{2.5}$ can be attributed to mobile sources and biomass burning (Kim et al., 2004; Marmur et al., 2007), and a recent epidemiologic study indicated that these components are associated with both cardiovascular and respiratory emergency room visits in Atlanta (Sarnat et al., 2008).

For general population, major routes of PAH exposure are inhalation of polluted air or cigarette smoke and ingestion of food containing PAHs. Assessment of human

exposure to environmental chemicals such as PAHs can be accomplished by two general approaches. That is, either through environmental monitoring in which concentrations of PAHs in environmental samples (air, food, water, soil, etc.) are measured to assess human intakes, or through biomonitoring in which internal levels of PAHs in human body (e.g. urinary hydroxylated PAHs, or OH-PAHs) are determined as indicators for assessing overall exposure to PAHs.

This dissertation focused on charactering $PM_{2.5}$-bound PAHs in Atlanta and assessing non-occupational exposure to PAHs through both biomonitoring and personal air monitoring. The first part of this thesis addressed PAHs levels in $PM_{2.5}$ samples collected at three sampling sites (urban, suburban-highway and rural) in the Atlanta area. Chapter 2 described the development of a method measuring 28 PAHs and methyl-substituted PAHs (Me-PAH) on $PM_{2.5}$ samples using a novel direct elution extraction method followed by isotope dilution gas chromatography/high resolution mass spectrometry (GC/HRMS) analysis. The method was then used to measure archived $PM_{2.5}$ samples collected in Atlanta during 2003-2004 periods, which was described in Chapter 3. In-depth analyses were carried out on seasonal and spatial variation of PAH concentrations, correlations of PAHs to other air pollutants (e.g. $PM_{2.5}$, OC, EC and potassium ion), and potential markers for specific sources (such as retene as an indicator for biomass burning and methyl phenanthrenes for petroleum sources). The second part of the thesis focused on biomonitoring. Chapter 4 introduced a method that was developed to measure 24 urinary biomarkers – OH-PAH metabolites in human, using enzymatic de-conjugation, automated liquid-liquid extraction, evaporation and derivatization, and isotope dilution GC/HRMS determination. A study was then designed and carried out to study intra- and inter-personal variability of these urinary biomarkers in a group of non-occupational exposed volunteers and conduct sample size calculations utilizing the study findings, which comprised Chapter 5. Finally in Chapter 6, a study was designed and conducted to evaluate non-occupational exposure to atmospheric PAHs

through both personal air sampling and biomonitoring. Given below are conclusions from individual chapters in this dissertation.

Determination of 28 PAHs and Methyl-PAHs in Air Particulate Matter Samples using Direct Elution and Isotope Dilution GC/MS

We developed a method for measuring particle-bound PAHs in PM samples using direct elution and GC/HRMS determination. The extraction method is by far the most time-efficient method requiring the least amount of labor. The instrument analysis using isotope dilution GC/MS operated at 10,000 resolution gave high accuracy, precision and sensitivity, which enables measuring PAHs on low-flow PM samples. The recovery of this method was evaluated by calculating the spiked isotopically labeled internal standards. The accuracy of the method was evaluated by measuring $PM_{2.5}$-bound PAHs from NIST reference material RM8785 and determined concentrations were consistent with the reference value. This method was used to analyze $PM_{2.5}$ samples collected from three sites in the metropolitan Atlanta area and again the results were consistent with an earlier investigation studying PAH concentrations on $PM_{2.5}$ samples from the same locations using ASE. Distinct seasonal and site variations were observed in both studies, which were discussed in detail in Chapter 3.

Characterization of $PM_{2.5}$-bound Polycyclic Aromatic Hydrocarbons in Atlanta

Concentrations, seasonal and spatial variations of atmospheric particle-bound PAHs at three sites in and near Atlanta were reported in two studies, first a pilot study followed by an expanded study. We found strong seasonal variation of PAHs, with highest levels during the winter months. PAHs were correlated with $PM_{2.5}$, OC, EC and K^+ and the correlations have a temperature-dependency. $PM_{2.5}$-bound PAH concentrations were more elevated at the highway-impacted suburban site, followed by the urban site, and were the lowest at the rural site. Relative concentration of methylated

phenanthrenes to its parent compound phenanthrene suggest that this can be used as a crude indicator for petrogenic sources, with fuel evaporative sources playing a relatively larger role during the summer. Interestingly, retene was noted in both studies for its unique profiles compared to other PAHs. It was found to be a better indicator than potassium ion of forest burning events in which brush and leaves are the predominant fuel. These results have implications for PM source apportionment methodologies as well as for gaining a better understanding of ambient PAH levels in the southeastern United States.

Measurement of Urinary Mono-Hydroxy Polycyclic Aromatic Hydrocarbons Using Automated Liquid-Liquid Extraction and Isotope Dilution GC/HRMS

We have developed a method to measure 24 urinary OH-PAH metabolites, utilizing enzymatic hydrolysis, automated liquid-liquid extraction, and GC/HRMS. The sample preparation method is highly automated and required a minimum of manual labor, which contributes to high throughput desired for conducting large biomonitoring studies. De-conjugation kinetics was studied and all parameters were optimized to give the highest yield. The method was among one of the most sensitive reported with its limits and detection at low parts per trillion levels. It has been used to analyze samples from a number of studies including the National Health and Nutritional Examination Survey, an on-going survey reports biannually the level of biomarkers in a statistically representative sample of the U.S. population..

Variability of Urinary Polycyclic Aromatic Hydrocarbon Metabolite Levels in Adults

Levels of urinary PAH metabolites in a group of non-occupationally exposed non-smoking adults varied widely both within-subject and between-subjects. There was also considerable temporal variation within a day that far exceeded the between-day variation.

Creatinine adjustment is crucial for spot samples and first morning urine sampling but not for the 24-hour voids. Taking 24-hour void specimens, though burdensome on the study subjects with potential non-compliance, was the most reliable and reproducible sampling practice, and it also requires the fewest number of subjects in epidemiological studies, less than half of what would be required if spot urine samples were taken. Hence, it is of utmost importance that these considerations are taken into account when planning an epidemiological study since investigators will need to balance the additional labor of collecting 24-hour void samples against the costs of recruiting and analyzing more than twice as many samples to reach the same statistical power.

Assessment of Non-occupational Exposure to Polycyclic Aromatic Hydrocarbons through Personal Air Sampling and Urinary Biomonitoring

We studied exposure to PAHs on 8 non-smoking subjects with no known occupational exposure through both personal air sampling and biomonitoring of urinary OH-PAH metabolites. Concentrations of 28 PAHs and Me-PAHs varied largely, in personal air samples collected at different locations/activities within each subjects, and among samples collected in the 8 households. Smaller PAHs such as naphthalene primarily came from indoor sources while larger PAHs with 4 or more aromatic rings originated from outdoor sources such as traffic emission. Our results stressed the importance of personalized exposure approaches in environmental epidemiological studies. Urinary biomonitoring can capture both inhalation and ingestion exposure, although the urinary metabolite levels alone can only give an indication on overall exposure levels and can not distinguish exposure routes. By combining personal air sampling and urinary biomonitoring, we were able to deduce exposure sources and we found that inhalation is the major route for naphthalene exposure while ingestion is the main route of exposure for phenanthrene and pyrene in this group of non-occupationally exposed reference population.

Future Directions

PAHs in Ambient Air

PAHs in ambient air were studied in two sections in this thesis. First, $PM_{2.5}$-bound PAHs in the Atlanta area were investigated through two segments in Chapter 3, i.e. a pilot study on daily samples taken at three stationary sites in one summer month (June 2004) and one winter month (December 2003), followed by an extended study measuring PAHs in $PM_{2.5}$ samples collected in all 12 months during 2004 (5 samples/site/month). Second, in Chapter 6, combined gas phase and particle phase PAHs were measured in personal air samples taken by 8 subjects while at home, at work, and during commuting (driving or jogging through traffic). Due to the small volumes of air sampled on each personal air samples (0.12 – 1.60 m^3/sample), we were not able to analyze the filter and sorbents separately to obtain information on gaseous and particulate PAHs, and results from the personal air samples were a combination of the gaseous and particulate phases. In both sections, valuable information was obtained regarding the concentration, pattern and profile of PAHs in the ambient air. From environmental epidemiology point of view, it would be very useful to get an understanding on how pollutant levels from stationary monitoring sites compared to those from personal level and how well the air monitoring results from routine monitoring sites represent actual personal exposure. In this thesis, however, we could not make such direct comparison on PAHs in air samples collected at three sites in Atlanta to the personal air samples collected by subjects living in the Atlanta area, because particle phase PAHs were studied in the ambient air study whereas the data available from personal air samples were a combination of gas phase and particle phase. Therefore, one future direction would be carrying out a study that collect separate particle and gas phase air samples from both stationary sites and from personal levels, and compare concentrations of gaseous and particular PAHs in central sites to those at personal exposure levels, to better understand exposure to ambient PAHs. When

designing such a study, one thing to keep in mind is to increase the personal air sampling time or flow rate in order to increase the air volume and pollutant mass sampled and therefore, increase the detection frequency of the pollutants on the personal air samples.

An interesting point that this thesis discovered was the potential of retene as a marker for biomass burning. We found that retene did not have the same site variation and was not well correlated to the other PAHs, indicating its unique source, formation and environmental fate. Concentrations of retene at all three sites peaked in March, 2004, coinciding with the highest amount of prescribed and un-planned fires in the state of Georgia. December was the month with the second highest retene concentrations, which was consistent with elevated residential wood burning in that month. In comparison, potassium, a biomass burning marker used in source apportionment, was elevated in December but not in March, which lead to the conclusion that retene might be a better marker for general biomass burning, while potassium might be a better marker for wood burning. However, retene is not a common analyte measured in air samples. Therefore, it is worthy to take on further research on retene, and confirm its association with biomass burning. In addition, levoglucosan, a breakdown product from thermal decomposition of cellulose in plants and vegetations (Fraser and Lakshmanan, 2000), has also been proposed as a biomass combustion marker in aerosols. Thus, it would be interesting to study in depth retene, levoglucosan and PAHs on the specificity and sensitivity of these compounds as biomass burning markers.

Biomonitoring

Urinary OH-PAH metabolites have been established as biomarkers for exposure to PAHs. However, there are several limitations on using such approach for exposure assessment. Metabolites of large PAHs with 4 or more aromatic rings have been reported to be excreted primarily through feces, instead of urine (ATSDR, 1995); thus they are mostly undetectable in urine samples. Therefore, assessing exposure to the larger PAHs

using urinary biomonitoring can be misleading due to the alternative excretion routes. In addition, OH-PAHs are only one group of metabolites resulted from PAH exposure; additional possible markers include dihydrodiols in urine (Grimmer et al., 1993), DNA/protein adducts in blood and/or tissue (Paleologo et al., 1992; Lodovici et al., 1998), and parent PAHs in breast milk (Grova et al., 2002). Specifically, PAH adducts have been reported to have long half-lives up to 120 days, and thus information on the adducts enables assessment of long term exposure (Needham et al., 2005). Furthermore, PAHs undergo relatively fast metabolism rate and the OH-PAH metabolites are usually excreted within hours after exposure; thus, OH-PAH levels reflect recent exposure to PAHs, instead of long-term exposure. Lastly, biomarker levels reflect exposure from all routes, and cannot distinguish specific sources. In the interest of exposure assessment, especially in occupational groups and/or sensitive demographic groups such as children, it is necessary and important to obtain source-specific information. Therefore, developing source specific-biomarker in biomonitoring studies is of utmost interest. One possibility would be using available sources-specific markers such as retene and studying the potential of retene metabolites as biomarkers for inhalation exposure to biomass burning. In summary, future development on biomonitoring of human exposure to PAHs can be extended on the following directions, i) study the potential of alternative biomarkers, such as urinary dihydrodiols and DNA/hemoglobin adducts; ii) study the potential of alternative matrices, including blood, breast milk and feces; iii) develop biomarkers for source-specific or route-specific exposure scenarios.

Assessing Human Exposure to PAHs through Personal Air Sampling and Biomonitoring

We designed and conducted a study to assess non-occupational exposure to atmospheric PAHs through simultaneous personal air sampling and biomonitoring. The study gave rise to valuable information on exposure of a group of non-smoking subjects

with no known occupational exposure to PAHs. However, we did not observe a direct causal effect between inhalation exposure to traffic emissions and biomarker concentrations. This is mostly due to the low-level inhalation exposure that the 8 volunteers in this study were subjected to and the small number of subjects enrolled. The highest potential inhalation event in this study was a 140-mile or 2-hour long driving trip from Atlanta to a mountainous rural area in the northern part of the state. We only observed slight increase on the levels of the fluorene metabolite, but not on the rest of the OH-PAHs. The driving trip, although long compared to routine commuting and thus regarded as a high exposure scenario, potentially is not as a high exposure as we interpreted, because the subject was in a car with vent closed and was driving in mostly rural areas with assumed light traffic. Unfortunately, this hypothesis cannot be confirmed since the subject did not take any air samples while traveling. Among the rest of the subjects with up to 2-hour/day accumulative commuting, we also did not observe consistent increase in biomarker levels. Concentrations of small PAHs such as naphthalene and fluorene were not elevated in driving air, which explained the metabolites of those PAHs were not increased following driving. On the other hand, pyrene levels in personal air samples taken while commuting were higher than at home or at work. However, the main exposure route for pyrene in this group of subjects was through dietary ingestion, not inhalation; therefore, we could not link the 1-PYR levels with inhalation exposure either. On the other hand, it is still of great interest to study exposure of citizens to urban air pollution caused by traffic, especially in metropolitan areas such as Atlanta. When planning future studies, instead of taking on reference populations with low exposure, researchers should focus on specific occupations or demographic groups, such as roadway workers and motorcycle patrol policemen who are exposure consistently to traffic emissions. In addition, the study design can be simplified to have only two exposure scenarios, i.e. workdays with high exposure and off-work days (e.g. weekends) with limited exposure. One thing that we learned from this study is that

ingestion can be a dominant route, and therefore, diet should be strictly controlled and kept consistent in the future studies. Further, with a more simple study design and thus less burdensome on study subjects, more subjects should be included in such a study to increase statistical power desired to distinguish various exposure scenarios.

APPENDIX A

STUDY DESIGN AND INSTRUCTION FOR THE PERSONAL AIR SAMPLING AND BIOMONITORING STUDY

This Appendix includes the participant instructions in the study discussed in Chapter 6 that titled, "Assessment of Human Exposure to Polycyclic Aromatic Hydrocarbons Employing Personal Air Sampler and Urinary PAH Biomonitoring".

Instructions for Study Participation

Total study duration: 2 weeks (Monday through Sunday)

Sampling days: 4 days/week, i.e. Tuesday, Wednesday, Saturday and Sunday. In total, there will be 8 sampling days in this study (Table A.1). Non sampling days: Monday, Thursday and Friday. In total, there will be 6 non-sampling days in this study.

Table A.1 Study design for the personal air sampling and biomonitoring study

No.	Week	Day	Driving	PAH-containing food
1	Week 1	Tuesday-Wednesday	High	Low
2	Week 1	Saturday-Sunday	Low	Low
3	Week 2	Tuesday-Wednesday	High	High
4	Week 2	Saturday-Sunday	Low	High

During the 8-day sample collection period

- Eat only the foods that are on the list of food that we recommend.

- Collect a ~60 mL urine sample into urine cups every time you urinate. Estimate and record the total volume of urine excreted each time.
- Carry a portable air pump connected to an air sampling tube at all time. Change the sampling tubes four times a day, and record the sampling duration and time of change.
- Complete the *Daily Activity Log* (*Log*). Note on the Table at what time you visited the restroom and the total volume of urine excreted, time of air sampler change, duration of air sampling, as well as driving and dietary information.
- Every Wednesday and Thursday morning, we will collect your coolers with samples and any waste items, and give you 2 other coolers (one for air samplers and one for urine samples) with fresh cold packs and one-day supplies. For the weekends, we will give you two-day supplies on Friday afternoon, collect samples and waste items on Monday morning.

During the 6 non-sampling days

- No urine and air samples will be collected
- We will not provide food. But please avoid BBQ, grilled, smoked food, and fast-food hamburger. If you eat those foods, please note the type and amount of food, and time of the intake on the questionnaire sheet.

Urine Sampling Instruction

Instruction for urine sample collection, transport and storage

1. Collect all urine excreted in a disposable graduated beaker
2. Label a urine specimen cup with labels that we provided
3. Read and record the total volume and time of urination on the *Log*, with the correct sample number from the label (e.g. S1-U01).

4. Pour ~ 60 mL of urine into the specimen cup and cap the cup.
5. Put the sample cup in the cooler
6. Discard the rest of urine, and seal the beaker in a 12 x 12 Zip-loc bag
7. If you prefer to use gloves for this procedure, please put the used gloves in the Zip-loc bag
8. We will collect the samples and wastes on Wednesday, Thursday, and Monday morning

Air Sampling Instruction

During the 8-day sample collection period

- Please collect personal air samples continuously for the 9 days.
- During weekdays, please change air samplers 4 time a day, at the following intervals
    - In the morning before leaving home for work (e.g. 7 am)
    - In the morning after arriving work (e.g. 8 am)
    - In the afternoon before leaving work (e.g. 5 pm)
    - In the afternoon after arriving home (e.g. 6 pm)
- During the weekends
    - Avoid driving
    - If you will be driving (minimum 30 min), changing the air samplers before and after driving
    - If you will be driving for less than 30 minutes, do not change the air sampler
    - Otherwise, change air samplers at the same time points as the weekday

Instruction for personal air sample collection, transport and storage

1. Read the time of air sampling from the pump timer for the existing tube (e.g. S1-A01) and record it on the Log.
2. Label a new air sample tube with labels that we provided (e.g. S1-A02), take off caps from both ends.
3. Turn off the pump (this will reset the timer), disconnect the existing tube (e.g. S1-A01) from the pump, cap the tube from both ends, and place it in a cooler.
4. Connect the new tube (e.g. S1-A02) to the pump, turn on the pump, and the time will start from zero minute.
5. Record time of air sampler change with the corresponding air sample number.
6. We will collect the samples and wastes on Wednesday, Thursday, and Monday morning.

Instructions on Dietary Intake

Recommended food for sampling days (Tue, Wed, Sat, Sun).

- Beverages: Yes Water, juice, milk, and soda (limited)
  No *Coffee*
- Snacks: Yes Assorted nuts, most diary products, non-chocolate candies, assorted non-chocolate cookies, jelly, egg
  No *Yogurt (flavored/frozen), chocolates, pies, cottage cheese*
- Fruits and Vegetables:
  Yes Cantaloupe, Cole slaw, strawberry, grapefruit, beans and peas
  No *most greens*
- Breakfast: Yes Blueberry muffin, corn muffin, whole oat cereal, corn flakes, bran cereal, bread (not toasted)
  No *Grits, oatmeal, cooked cereal, toasted food*

- Lunch (See below)
- Dinner: Yes Pre-packaged (preferred) or home-cooked roasted or strewed meat, steamed rice and vegetable

  No *Grilled, barbecued, smoked, and deep fried food*

Table A.2 Lunch recommendation during study period.

Meals	Low PAH-containing diet (week 1)		High PAH-containing diet (week 2)	
	Type	**Portion**	**Type**	**Portion**
Lunch	Pan fried burger from McDonald's or equivalents	112 g	Grilled burger from Burger King or equivalents	176 g
	Potato chip	42 g	Fries	102 g

During non-sampling days (Mon, Thu, Fri)

Avoid BBQ, grilled, smoked food, and fast food hamburgers. If you eat those foods, please note the type and amount of the food, and time of intake on the questionnaire.

APPENDIX B

PAH CONCENTRATIONS IN INDIVIDUAL HOMES AND CORRELATION MATRICES IN AIR SAMPLES

This Appendix gives the concentration of 28 PAHs and Me-PAHs in personal air samples taken at 8 individual homes in the study discussed in Chapter 6. The study was titled, "Assessment of Human Exposure to Polycyclic Aromatic Hydrocarbons Employing Personal Air Sampler and Urinary PAH Biomonitoring". Correlation matrices among PAH compounds with over 50% detection frequency are also given in this appendix according to the following categories.

- All air samples
- Air samples taken at home
- Air samples taken at work
- Air samples taken while driving
- Air samples taken while jogging
- Air samples taken in individual homes

Table B.1 Table B.1 Median and quartile concentrations (ng/m3) of PAHs in personal air samples taken at 8 individual homes

PAH	Median and quartile concentrations (ng/m^3)							
	S11	S12	S13	S14	S15	S16	S17	S18
naphthalene	1633 (925-1987)	5030 (3642-6458)	206 (157-267)	715 (546-786)	1164 (854-1288)	300 (291-388)	136 (50-176)	17176 (13382-20210)
2-methylnaphthalene	553 (383-685)	594 (573-655)	108 (78-141)	565 (535-787)	695 (586-803)	170 (111-186)	63 (24-76)	66 (44-78)
1-methylnaphthalene	224 (148-252)	290 (252-317)	54 (38-82)	248 (215-268)	283 (157-428)	78 (60-89)	20 (10-30)	31 (22-38)
biphenyl	137 (53-498)	97 (76-151)	53 (31-61)	42 (33-45)	40 (32-49)	21 (18-27)	18 (13-19)	25 (17-35)
acenaphthylene	1.3 (1-1.9)	3.3 (3.1-3.6)	1.3 (1.1-2)	0.6 (0.5-0.9)	2.1 (1.6-2.3)	0.8 (0.7-0.9)	0.5 (0.4-0.7)	0.3 (0.2-0.4)
acenaphthene	6.5 (5.1-7)	22.2 (19.7-23.9)	5.4 (4.6-7.4)	2.2 (2.0-2.5)	8.4 (6.8-9.2)	3.6 (2.7-4.1)	3.2 (3-3.9)	1.7 (1.4-2.1)
fluorene	9.8 (8.5-14)	21.9 (18.9-25)	4.4 (3.6-6.1)	3.4 (3.0-4.0)	21.5 (19.8-26.6)	5.9 (4.9-6.7)	3.8 (2.6-4.2)	4 (3-4.4)
dibenzothiophene	2.1 (1.2-2.5)	2.9 (2.8-3.3)	1.3 (1-1.7)	2.2 (1.8-2.5)	1.8 (1.6-2)	1.2 (1-1.4)	1.1 (0.9-1.2)	4 (2.9-4.5)
phenanthrene	14.5 (9.9-17.9)	15.9 (14.2-16.7)	6.8 (5.8-9.4)	6 (4.9-6.4)	12.4 (10.9-14.3)	5.7 (4.6-6)	5.9 (4.7-6.6)	15.6 (10.9-22.1)
anthracene	0.7 (0.5-1)	1.0 (0.9-1.1)	0.5 (0.3-0.5)	0.4 (0.3-0.7)	1.2 (1.1-1.5)	0.4 (0.3-0.4)	0.1 (0.1-0.4)	0.7 (0.4-0.8)
3-methylphenanthrene	2.9 (2-3.7)	2.9 (2.7-3.3)	1.5 (1.1-1.7)	1.9 (1.6-2.3)	2.6 (2.3-2.9)	2.3 (2-2.8)	1 (0.6-2.3)	10 (7.6-11.4)
2-methylphenanthrene	3.7 (2.4-4.6)	3.4 (3.1-3.8)	1.6 (1.3-2)	2.1 (1.8-2.7)	2.7 (2.5-3.1)	2.8 (2.5-3.1)	1.2 (0.7-3.1)	12.3 (8.9-14.1)
9-methylphenanthrene	2.5 (2-3.4)	2.6 (2.3-2.9)	1.3 (1.1-1.6)	2.4 (2.2-3.1)	2.5 (2.2-2.9)	2.6 (1.9-2.8)	0.9 (0.6-1.4)	15.2 (11.5-18)
1-methylphenanthrene	1.8 (1.3-2.2)	1.6 (1.4-1.7)	0.9 (0.8-1.1)	1.4 (1.2-1.8)	1.5 (1.3-1.8)	1.5 (1.2-1.8)	0.6 (0.4-1)	8.9 (6.1-10)

Table B.1 (Continued)

PAH	Median and quartile concentrations (ng/m^3)							
	S11	S12	S13	S14	S15	S16	S17	S18
fluoranthene	1.14 (0.87-1.34)	0.49 (0.45-0.63)	0.68 (0.53-0.78)	0.23 (0.15-0.31)	0.42 (0.38-0.45)	0.24 (0.21-0.29)	0.56 (0.37-0.69)	0.17 (0.12-0.26)
pyrene	0.7 (0.45-0.88)	0.45 (0.4-0.52)	0.54 (0.44-0.62)	0.21 (0.17-0.32)	0.34 (0.32-0.4)	0.71 (0.6-0.74)	0.27 (0.19-0.49)	0.12 (0.08-0.14)
retene	0.25 (0.2-0.3)	0.25 (0.21-0.34)	0.28 (0.24-0.37)	0.14 (0.12-0.24)	0.24 (0.21-0.32)	0.15 (0.12-0.22)	0.17 (0.16-0.28)	0.17 (0.14-0.23)
benzo(c)phenanthrene	0.02 (0.02-0.04)	0.02 (0.02-0.03)	0.02 (0.02-0.03)	0.02 (0.02-0.02)	0.02 (0.02-0.02)	0.03 (0.03-0.04)	0.04 (0.03-0.05)	0.03 (0.02-0.03)
benzo(a)anthracene	0.03 (0.02-0.04)	0.02 (0.02-0.03)	0.06 (0.04-0.08)	0.03 (0.02-0.04)	0.02 (0.02-0.03)	0.03 (0.02-0.04)	0.04 (0.03-0.05)	0.03 (0.02-0.03)
chrysene	0.06 (0.04-0.07)	0.05 (0.04-0.06)	0.12 (0.09-0.16)	0.06 (0.04-0.06)	0.04 (0.03-0.06)	0.06 (0.05-0.08)	0.05 (0.05-0.05)	0.04 (0.03-0.05)
benzo(b)fluoranthene	0.05 (0.03-0.09)	0.05 (0.03-0.07)	0.14 (0.08-0.21)	0.06 (0.05-0.12)	0.05 (0.03-0.06)	0.05 (0.03-0.1)	0.05 (0.04-0.09)	0.04 (0.03-0.08)
benzo(k)fluoranthene	0.03 (0.02-0.05)	0.02 (0.02-0.03)	0.05 (0.03-0.08)	0.03 (0.02-0.04)	0.02 (0.02-0.02)	0.03 (0.03-0.04)	0.05 (0.04-0.05)	0.03 (0.02-0.03)
benzo(e)pyrene	0.04 (0.03-0.09)	0.04 (0.04-0.07)	0.11 (0.07-0.18)	0.05 (0.05-0.08)	0.04 (0.03-0.06)	0.05 (0.03-0.08)	0.07 (0.04-0.1)	0.04 (0.03-0.06)
benzo(a)pyrene	0.04 (0.03-0.07)	0.04 (0.02-0.06)	0.09 (0.06-0.17)	0.05 (0.04-0.08)	0.03 (0.02-0.05)	0.05 (0.03-0.07)	0.06 (0.04-0.1)	0.03 (0.03-0.05)
perylene	0.02 (0.02-0.04)	0.02 (0.02-0.03)	0.03 (0.02-0.04)	0.02 (0.02-0.03)	0.02 (0.02-0.02)	0.03 (0.02-0.03)	0.04 (0.03-0.05)	0.03 (0.02-0.03)
indeno(123-cd)pyrene	0.06 (0.04-0.11)	0.07 (0.04-0.1)	0.13 (0.08-0.17)	0.06 (0.05-0.09)	0.04 (0.04-0.07)	0.05 (0.04-0.11)	0.08 (0.05-0.15)	0.06 (0.03-0.09)
dibenz(a,h)anthracene	0.02 (0.02-0.04)	0.02 (0.02-0.03)	0.03 (0.02-0.03)	0.02 (0.02-0.02)	0.02 (0.02-0.02)	0.03 (0.02-0.03)	0.04 (0.03-0.05)	0.03 (0.02-0.04)
benzo(ghi)perylene	0.08 (0.05-0.17)	0.1 (0.05-0.12)	0.18 (0.13-0.26)	0.07 (0.05-0.1)	0.05 (0.04-0.07)	0.06 (0.04-0.12)	0.07 (0.04-0.16)	0.05 (0.03-0.09)

Table B.2 Correlation matrix for PAHs in all personal air samples (n = 171)

	NAPH	2-MNap	1-MNap	BIPH	ANYL	ANAP	FLUO	DBT	PHEN	ANTH	3-MPhe	2-MPhe	9-MPhe	1-MPhe	FLR	PYR	RET[1]
NAPH		-0.05	-0.04	0.00	-0.08	0.01	0.03	0.58	0.38	0.06	0.87	0.86	0.91	0.89	-0.17	-0.15	-0.10
2-MNap	-0.05		0.95[2]	0.31	0.23	0.44	0.64	0.31	0.35	0.10	0.03	0.02	-0.02	-0.02	0.01	0.01	0.10
1-MNap	-0.04	0.95		0.26	0.24	0.49	0.66	0.31	0.34	0.10	0.04	0.03	-0.03	-0.03	-0.03	-0.02	0.06
BIPH	0.00	0.31	0.26		0.09	0.26	0.33	0.14	0.24	0.09	0.03	0.05	-0.01	-0.01	0.08	0.05	0.05
ANYL	-0.08	0.23	0.24	0.09		0.38	0.28	0.31	0.38	0.64	0.04	0.02	-0.07	-0.04	0.52	0.56	0.45
ANAP	0.01	0.44	0.49	0.26	0.38		0.78	0.39	0.55	0.12	0.06	0.07	-0.06	-0.04	0.24	0.21	0.36
FLUO	0.03	0.64	0.66	0.33	0.28	0.78		0.34	0.59	0.25	0.12	0.11	0.00	0.01	0.11	0.05	0.23
DBT	0.58	0.31	0.31	0.14	0.31	0.39	0.34		0.69	0.32	0.76	0.76	0.70	0.71	0.12	0.13	0.25
PHEN	0.38	0.35	0.34	0.24	0.38	0.55	0.59	0.69		0.36	0.60	0.60	0.45	0.48	0.42	0.28	0.58
ANTH	0.06	0.10	0.10	0.09	0.64	0.12	0.25	0.32	0.36		0.18	0.16	0.09	0.11	0.23	0.17	0.22
3-MPhe	0.87	0.03	0.04	0.03	0.04	0.06	0.12	0.76	0.60	0.18		0.99	0.96	0.97	0.00	0.01	0.14
2-MPhe	0.86	0.02	0.03	0.05	0.02	0.07	0.11	0.76	0.60	0.16	0.99		0.96	0.97	0.00	-0.01	0.13
9-MPhe	0.91	-0.02	-0.03	-0.01	-0.07	-0.06	0.00	0.70	0.45	0.09	0.96	0.96		1.00	-0.11	-0.07	0.01
1-MPhe	0.89	-0.02	-0.03	-0.01	-0.04	-0.04	0.01	0.71	0.48	0.11	0.97	0.97	1.00		-0.07	-0.04	0.06
FLR	-0.17	0.01	-0.03	0.08	0.52	0.24	0.11	0.12	0.42	0.23	0.00	0.00	-0.11	-0.07		0.86	0.71
PYR	-0.15	0.01	-0.02	0.05	0.56	0.21	0.05	0.13	0.28	0.17	0.01	-0.01	-0.07	-0.04	0.86		0.69
RET	-0.10	0.10	0.06	0.05	0.45	0.36	0.23	0.25	0.58	0.22	0.14	0.13	0.01	0.06	0.71	0.69	
average r	0.25	0.21	0.16	0.12	0.25	0.27	0.28	0.43	0.46	0.20	0.36	0.36	0.29	0.31	0.18	0.17	0.25

1. PAHs larger than retene were not included due to their low detection frequencies.
2. Correlation coefficients over 0.5 are underlined.

Table B.3 Correlation matrix for PAHs in air samples taken at homes (n = 91)

	NAPH	2-MNap	1-MNap	BIPH	ANYL	ANAP	FLUO	DBT	PHEN	ANTH	3-MPhe	2-MPhe	9-MPhe	1-MPhe	FLR	PYR	RET
NAPH		-0.20	-0.19	-0.08	-0.15	-0.03	-0.06	0.78	0.58	0.18	0.94	0.93	0.93	0.92	-0.21	-0.32	-0.11
2-MNap	-0.20		0.94	0.27	0.44	0.41	0.66	0.23	0.36	0.42	-0.16	-0.17	-0.21	-0.21	0.22	0.09	0.24
1-MNap	-0.19	0.94		0.19	0.51	0.49	0.68	0.24	0.37	0.44	-0.15	-0.16	-0.21	-0.20	0.18	0.09	0.24
BIPH	-0.08	0.27	0.19		0.18	0.23	0.28	0.05	0.28	0.04	-0.06	-0.04	-0.11	-0.10	0.47	0.26	0.21
ANYL	-0.15	0.44	0.51	0.18		0.83	0.76	0.14	0.41	0.67	-0.18	-0.19	-0.28	-0.28	0.33	0.29	0.41
ANAP	-0.03	0.41	0.49	0.23	0.83		0.80	0.30	0.47	0.41	-0.10	-0.10	-0.20	-0.20	0.28	0.25	0.31
FLUO	-0.06	0.66	0.68	0.28	0.76	0.80		0.23	0.57	0.63	-0.05	-0.07	-0.16	-0.15	0.26	0.21	0.33
DBT	0.78	0.23	0.24	0.05	0.14	0.30	0.23		0.80	0.34	0.81	0.81	0.77	0.77	0.04	-0.17	0.03
PHEN	0.58	0.36	0.37	0.28	0.41	0.47	0.57	0.80		0.59	0.64	0.65	0.55	0.55	0.45	0.13	0.24
ANTH	0.18	0.42	0.44	0.04	0.67	0.41	0.63	0.34	0.59		0.20	0.19	0.13	0.13	0.15	-0.01	0.19
3-MPhe	0.94	-0.16	-0.15	-0.06	-0.18	-0.10	-0.05	0.81	0.64	0.20		0.99	0.98	0.98	-0.11	-0.21	-0.08
2-MPhe	0.93	-0.17	-0.16	-0.04	-0.19	-0.10	-0.07	0.81	0.65	0.19	0.99		0.98	0.98	-0.07	-0.19	-0.07
9-MPhe	0.93	-0.21	-0.21	-0.11	-0.28	-0.20	-0.16	0.77	0.55	0.13	0.98	0.98		1.00	-0.22	-0.31	-0.14
1-MPhe	0.92	-0.21	-0.20	-0.10	-0.28	-0.20	-0.15	0.77	0.55	0.13	0.98	0.98	1.00		-0.19	-0.29	-0.13
FLR	-0.21	0.22	0.18	0.47	0.33	0.28	0.26	0.04	0.45	0.15	-0.11	-0.07	-0.22	-0.19		0.63	0.47
PYR	-0.32	0.09	0.09	0.26	0.29	0.25	0.21	-0.17	0.13	-0.01	-0.21	-0.19	-0.31	-0.29	0.63		0.47
RET	-0.11	0.24	0.24	0.21	0.41	0.31	0.33	0.03	0.24	0.19	-0.08	-0.07	-0.14	-0.13	0.47	0.47	
average r	0.24	0.21	0.22	0.13	0.24	0.26	0.31	0.38	0.48	0.29	0.28	0.28	0.22	0.22	0.17	0.06	0.16

Table B.4 Correlation matrix for PAHs in air samples taken at work (n = 27)

	NAPH	2-MNap	1-MNap	BIPH	ANYL	ANAP	FLUO	DBT	PHEN	ANTH	3-MPhe	2-MPhe	9-MPhe	1-MPhe	FLR	PYR	RET
NAPH		0.72	0.74	0.60	0.67	0.55	0.64	0.50	0.61	0.69	0.59	0.56	0.55	0.61	0.18	0.39	0.44
2-MNap	0.72		0.98	0.73	0.80	0.72	0.78	0.58	0.67	0.71	0.61	0.60	0.56	0.65	0.38	0.62	0.63
1-MNap	0.74	0.98		0.72	0.79	0.67	0.75	0.55	0.66	0.78	0.63	0.61	0.55	0.67	0.35	0.59	0.60
BIPH	0.60	0.73	0.72		0.56	0.76	0.76	0.67	0.71	0.40	0.68	0.69	0.67	0.68	0.37	0.53	0.55
ANYL	0.67	0.80	0.79	0.56		0.71	0.80	0.62	0.62	0.75	0.57	0.59	0.57	0.69	0.32	0.65	0.67
ANAP	0.55	0.72	0.67	0.76	0.71		0.97	0.74	0.82	0.34	0.57	0.62	0.56	0.63	0.51	0.68	0.82
FLUO	0.64	0.78	0.75	0.76	0.80	0.97		0.79	0.81	0.49	0.63	0.67	0.61	0.68	0.47	0.69	0.83
DBT	0.50	0.58	0.55	0.67	0.62	0.74	0.79		0.82	0.40	0.76	0.77	0.79	0.84	0.67	0.80	0.73
PHEN	0.61	0.67	0.66	0.71	0.62	0.82	0.81	0.82		0.40	0.76	0.79	0.69	0.83	0.81	0.83	0.82
ANTH	0.69	0.71	0.78	0.40	0.75	0.34	0.49	0.40	0.40		0.48	0.47	0.40	0.56	0.13	0.38	0.39
3-MPhe	0.59	0.61	0.63	0.68	0.57	0.57	0.63	0.76	0.76	0.48		0.99	0.96	0.96	0.53	0.77	0.58
2-MPhe	0.56	0.60	0.61	0.69	0.59	0.62	0.67	0.77	0.79	0.47	0.99		0.94	0.96	0.56	0.79	0.63
9-MPhe	0.55	0.56	0.55	0.67	0.57	0.56	0.61	0.79	0.69	0.40	0.96	0.94		0.94	0.45	0.75	0.54
1-MPhe	0.61	0.65	0.67	0.68	0.69	0.63	0.68	0.84	0.83	0.56	0.96	0.96	0.94		0.64	0.86	0.68
FLR	0.18	0.38	0.35	0.37	0.32	0.51	0.47	0.67	0.81	0.13	0.53	0.56	0.45	0.64		0.85	0.75
PYR	0.39	0.62	0.59	0.53	0.65	0.68	0.69	0.80	0.83	0.38	0.77	0.79	0.75	0.86	0.85		0.87
RET	0.44	0.63	0.60	0.55	0.67	0.82	0.83	0.73	0.82	0.39	0.58	0.63	0.54	0.68	0.75	0.87	
average r	0.57	0.67	0.66	0.63	0.65	0.67	0.71	0.69	0.73	0.48	0.69	0.70	0.66	0.74	0.50	0.69	0.66

Table B.5 Correlation matrix for PAHs in air samples taken while driving (n = 27)

	NAPH	2-MNap	1-MNap	BIPH	ANYL	ANAP	FLUO	DBT	PHEN	ANTH	3-MPhe	2-MPhe	9-MPhe	1-MPhe	FLR	PYR	RET
NAPH		0.11	0.16	0.30	0.36	-0.01	0.19	0.34	0.09	0.48	0.24	0.26	0.31	0.28	-0.16	-0.19	-0.11
2-MNap	0.11		0.93	0.16	0.39	0.33	0.41	0.08	0.29	0.05	0.15	0.17	0.12	0.10	0.21	0.10	0.21
1-MNap	0.16	0.93		0.21	0.46	0.32	0.36	0.09	0.26	0.07	0.17	0.18	0.08	0.11	0.13	0.05	0.12
BIPH	0.30	0.16	0.21		0.72	0.17	0.36	0.33	0.29	0.60	0.26	0.26	0.34	0.37	0.46	0.47	0.32
ANYL	0.36	0.39	0.46	0.72		0.28	0.36	0.25	0.29	0.62	0.21	0.20	0.23	0.26	0.33	0.34	0.21
ANAP	-0.01	0.33	0.32	0.17	0.28		0.59	0.38	0.70	0.03	0.41	0.44	0.36	0.41	0.57	0.40	0.70
FLUO	0.19	0.41	0.36	0.36	0.36	0.59		0.42	0.87	0.53	0.65	0.66	0.51	0.57	0.50	0.17	0.57
DBT	0.34	0.08	0.09	0.33	0.25	0.38	0.42		0.49	0.33	0.78	0.82	0.93	0.89	0.11	0.06	0.32
PHEN	0.09	0.29	0.26	0.29	0.29	0.70	0.87	0.49		0.32	0.67	0.69	0.53	0.60	0.55	0.26	0.74
ANTH	0.48	0.05	0.07	0.60	0.62	0.03	0.53	0.33	0.32		0.36	0.36	0.38	0.39	0.12	-0.04	0.04
3-MPhe	0.24	0.15	0.17	0.26	0.21	0.41	0.65	0.78	0.67	0.36		0.97	0.88	0.93	0.20	0.08	0.50
2-MPhe	0.26	0.17	0.18	0.26	0.20	0.44	0.66	0.82	0.69	0.36	0.97		0.90	0.93	0.24	0.07	0.46
9-MPhe	0.31	0.12	0.08	0.34	0.23	0.36	0.51	0.93	0.53	0.38	0.88	0.90		0.97	0.17	0.09	0.42
1-MPhe	0.28	0.10	0.11	0.37	0.26	0.41	0.57	0.89	0.60	0.39	0.93	0.93	0.97		0.24	0.13	0.51
FLR	-0.16	0.21	0.13	0.46	0.33	0.57	0.50	0.11	0.55	0.12	0.20	0.24	0.17	0.24		0.87	0.77
PYR	-0.19	0.10	0.05	0.47	0.34	0.40	0.17	0.06	0.26	-0.04	0.08	0.07	0.09	0.13	0.87		0.63
RET	-0.11	0.21	0.12	0.32	0.21	0.70	0.57	0.32	0.74	0.04	0.50	0.46	0.42	0.51	0.77	0.63	
average r	0.17	0.24	0.23	0.35	0.34	0.38	0.48	0.41	0.48	0.29	0.47	0.48	0.45	0.48	0.33	0.22	0.40

Table B.6 Correlation matrix for PAHs in air samples taken while jogging (n = 8)

	NAPH	2-MNap	1-MNap	BIPH	ANYL	ANAP	ANTH	FLR	PYR	RET	BCP	BAA	CHRY	BBF	BKF	BEP	BAP	PER	IP	DBA	BP
NAPH		0.52	-0.11	-0.22	0.53	0.39	0.45	0.32	0.38	0.77	-0.57	0.03	0.12	0.02	0.01	0.08	-0.01	-0.19	0.06	-0.41	0.15
2-MNap	0.52		0.56	0.52	0.90	0.71	0.76	0.16	0.16	0.35	-0.37	-0.07	-0.01	-0.06	-0.05	0.00	-0.09	-0.30	-0.04	-0.41	0.10
1-MNap	-0.11	0.56		0.87	0.30	0.78	0.69	-0.25	-0.35	0.20	0.22	-0.53	-0.51	-0.48	-0.49	-0.49	-0.50	-0.52	-0.48	-0.35	-0.38
BIPH	-0.22	0.52	0.87		0.31	0.74	0.74	0.08	-0.02	0.03	0.09	-0.22	-0.18	-0.14	-0.17	-0.17	-0.17	-0.21	-0.15	-0.08	-0.07
ANYL	0.53	0.90	0.30	0.31		0.56	0.61	0.14	0.19	0.24	-0.56	0.02	0.10	0.06	0.05	0.12	0.02	-0.22	0.07	-0.34	0.19
ANAP	0.39	0.71	0.78	0.74	0.56		0.97	0.21	0.14	0.62	-0.09	-0.23	-0.15	-0.16	-0.19	-0.15	-0.21	-0.31	-0.14	-0.25	-0.01
ANTH	0.45	0.76	0.69	0.74	0.61	0.97		0.38	0.32	0.59	-0.19	-0.08	0.02	0.00	-0.03	0.01	-0.05	-0.19	0.02	-0.17	0.15
FLR	0.32	0.16	-0.25	0.08	0.14	0.21	0.38		0.98	0.25	-0.05	0.84	0.87	0.85	0.84	0.85	0.83	0.76	0.87	0.63	0.89
PYR	0.38	0.16	-0.35	-0.02	0.19	0.14	0.32	0.98		0.20	-0.16	0.86	0.91	0.88	0.86	0.89	0.87	0.77	0.90	0.60	0.92
RET	0.77	0.35	0.20	0.03	0.24	0.62	0.59	0.25	0.20		-0.12	-0.18	-0.14	-0.20	-0.19	-0.18	-0.23	-0.28	-0.16	-0.34	-0.09
BCP	-0.57	-0.37	0.22	0.09	-0.56	-0.09	-0.19	-0.05	-0.16	-0.12		0.11	-0.03	0.05	0.12	0.00	0.07	0.30	0.03	0.49	-0.01
BAA	0.03	-0.07	-0.53	-0.22	0.02	-0.23	-0.08	0.84	0.86	-0.18	0.11		0.98	0.98	1.00	0.98	0.99	0.96	0.98	0.83	0.95
CHRY	0.12	-0.01	-0.51	-0.18	0.10	-0.15	0.02	0.87	0.91	-0.14	-0.03	0.98		0.99	0.99	1.00	0.99	0.92	1.00	0.78	0.98
BBF	0.02	-0.06	-0.48	-0.14	0.06	-0.16	0.00	0.85	0.88	-0.20	0.05	0.98	0.99		0.99	1.00	1.00	0.95	1.00	0.84	0.98
BKF	0.01	-0.05	-0.49	-0.17	0.05	-0.19	-0.03	0.84	0.86	-0.19	0.12	1.00	0.99	0.99		0.99	0.99	0.96	0.99	0.85	0.97
BEP	0.08	0.00	-0.49	-0.17	0.12	-0.15	0.01	0.85	0.89	-0.18	0.00	0.98	1.00	1.00	0.99		0.99	0.93	1.00	0.80	0.99
BAP	-0.01	-0.09	-0.50	-0.17	0.02	-0.21	-0.05	0.83	0.87	-0.23	0.07	0.99	0.99	1.00	0.99	0.99		0.96	1.00	0.85	0.97
PER	-0.19	-0.30	-0.52	-0.21	-0.22	-0.31	-0.19	0.76	0.77	-0.28	0.30	0.96	0.92	0.95	0.96	0.93	0.96		0.94	0.94	0.89
IP	0.06	-0.04	-0.48	-0.15	0.07	-0.14	0.02	0.87	0.90	-0.16	0.03	0.98	1.00	1.00	0.99	1.00	1.00	0.94		0.83	0.99
DBA	-0.41	-0.41	-0.35	-0.08	-0.34	-0.25	-0.17	0.63	0.60	-0.34	0.49	0.83	0.78	0.84	0.85	0.80	0.85	0.94	0.83		0.77
BP	0.15	0.10	-0.38	-0.07	0.19	-0.01	0.15	0.89	0.92	-0.09	-0.01	0.95	0.98	0.98	0.97	0.99	0.97	0.89	0.99	0.77	
average r	0.12	0.17	-0.09	0.08	0.17	0.16	0.25	0.52	0.52	0.06	-0.03	0.46	0.48	0.48	0.47	0.48	0.46	0.40	0.49	0.34	0.52

Table B.7 Correlation matrix for PAHs in air samples taken at S11's home (n = 8)

	NAPH	2-MNap	1-MNap	BIPH	ANYL	ANAP	FLUO	DBT	PHEN	ANTH	3-MPhe	2-MPhe	9-MPhe	1-MPhe	FLR	PYR	RET
NAPH		0.86	0.78	0.12	-0.11	0.77	0.60	0.76	0.82	-0.16	0.76	0.77	0.75	0.70	0.82	0.65	0.29
2-MNap	0.86		0.90	0.24	-0.02	0.93	0.79	0.81	0.85	-0.17	0.80	0.82	0.80	0.75	0.94	0.75	0.36
1-MNap	0.78	0.90		-0.01	0.02	0.86	0.63	0.94	0.91	-0.14	0.93	0.94	0.95	0.91	0.93	0.80	0.35
BIPH	0.12	0.24	-0.01		-0.20	0.38	0.61	-0.08	0.01	-0.32	-0.17	-0.11	-0.14	-0.17	0.10	0.13	0.25
ANYL	-0.11	-0.02	0.02	-0.20		0.06	0.16	0.05	0.13	0.93	0.16	0.13	0.02	0.03	0.04	0.15	0.39
ANAP	0.77	0.93	0.86	0.38	0.06		0.92	0.84	0.89	-0.09	0.80	0.84	0.79	0.78	0.93	0.76	0.45
FLUO	0.60	0.79	0.63	0.61	0.16	0.92		0.60	0.71	0.01	0.55	0.61	0.53	0.52	0.75	0.62	0.49
DBT	0.76	0.81	0.94	-0.08	0.05	0.84	0.60		0.97	-0.08	0.97	0.98	0.97	0.97	0.91	0.76	0.33
PHEN	0.82	0.85	0.91	0.01	0.13	0.89	0.71	0.97		0.02	0.94	0.95	0.92	0.92	0.93	0.73	0.34
ANTH	-0.16	-0.17	-0.14	-0.32	0.93	-0.09	0.01	-0.08	0.02		0.02	-0.01	-0.13	-0.11	-0.12	-0.13	0.11
3-MPhe	0.76	0.80	0.93	-0.17	0.16	0.80	0.55	0.97	0.94	0.02		0.99	0.98	0.97	0.92	0.82	0.44
2-MPhe	0.77	0.82	0.94	-0.11	0.13	0.84	0.61	0.98	0.95	-0.01	0.99		0.97	0.97	0.94	0.83	0.44
9-MPhe	0.75	0.80	0.95	-0.14	0.02	0.79	0.53	0.97	0.92	-0.13	0.98	0.97		0.99	0.91	0.83	0.40
1-MPhe	0.70	0.75	0.91	-0.17	0.03	0.78	0.52	0.97	0.92	-0.11	0.97	0.97	0.99		0.89	0.81	0.40
FLR	0.82	0.94	0.93	0.10	0.04	0.93	0.75	0.91	0.93	-0.12	0.92	0.94	0.91	0.89		0.84	0.48
PYR	0.65	0.75	0.80	0.13	0.15	0.76	0.62	0.76	0.73	-0.13	0.82	0.83	0.83	0.81	0.84		0.81
RET	0.29	0.36	0.35	0.25	0.39	0.45	0.49	0.33	0.34	0.11	0.44	0.44	0.40	0.40	0.48	0.81	
average r	0.57	0.65	0.67	0.04	0.12	0.68	0.57	0.67	0.69	-0.03	0.68	0.69	0.66	0.65	0.70	0.63	0.39

Table B.8 Correlation matrix for PAHs in air samples taken at S12's home (n = 13)

	NAPH	2-MNap	1-MNap	BIPH	ANYL	ANAP	FLUO	DBT	PHEN	ANTH	3-MPhe	2-MPhe	9-MPhe	1-MPhe	FLR	PYR	RET
NAPH		0.45	0.28	0.12	0.55	0.67	0.73	0.29	0.51	0.29	0.21	0.15	0.09	0.04	0.23	0.26	0.39
2-MNap	0.45		0.86	0.47	0.70	0.72	0.68	0.34	0.55	0.34	0.39	0.44	0.26	0.30	0.34	0.17	0.24
1-MNap	0.28	0.86		0.28	0.74	0.66	0.56	0.50	0.58	0.49	0.43	0.49	0.36	0.35	0.49	0.31	0.40
BIPH	0.12	0.47	0.28		0.30	0.30	0.18	0.20	0.22	-0.28	0.56	0.61	0.38	0.47	0.37	0.21	-0.11
ANYL	0.55	0.70	0.74	0.30		0.84	0.83	0.82	0.87	0.62	0.71	0.74	0.60	0.64	0.61	0.51	0.73
ANAP	0.67	0.72	0.66	0.30	0.84		0.80	0.57	0.69	0.39	0.41	0.46	0.19	0.25	0.34	0.22	0.44
FLUO	0.73	0.68	0.56	0.18	0.83	0.80		0.68	0.90	0.63	0.56	0.53	0.38	0.43	0.55	0.45	0.60
DBT	0.29	0.34	0.50	0.20	0.82	0.57	0.68		0.90	0.60	0.85	0.84	0.75	0.77	0.82	0.76	0.77
PHEN	0.51	0.55	0.58	0.22	0.87	0.69	0.90	0.90		0.75	0.76	0.74	0.64	0.67	0.79	0.70	0.74
ANTH	0.29	0.34	0.49	-0.28	0.62	0.39	0.63	0.60	0.75		0.31	0.30	0.37	0.33	0.46	0.42	0.64
3-MPhe	0.21	0.39	0.43	0.56	0.71	0.41	0.56	0.85	0.76	0.31		0.98	0.93	0.95	0.88	0.83	0.67
2-MPhe	0.15	0.44	0.49	0.61	0.74	0.46	0.53	0.84	0.74	0.30	0.98		0.90	0.95	0.86	0.76	0.64
9-MPhe	0.09	0.26	0.36	0.38	0.60	0.19	0.38	0.75	0.64	0.37	0.93	0.90		0.97	0.85	0.88	0.75
1-MPhe	0.04	0.30	0.35	0.47	0.64	0.25	0.43	0.77	0.67	0.33	0.95	0.95	0.97		0.82	0.81	0.70
FLR	0.23	0.34	0.49	0.37	0.61	0.34	0.55	0.82	0.79	0.46	0.88	0.86	0.85	0.82		0.93	0.70
PYR	0.26	0.17	0.31	0.21	0.51	0.22	0.45	0.76	0.70	0.42	0.83	0.76	0.88	0.81	0.93		0.80
RET	0.39	0.24	0.40	-0.11	0.73	0.44	0.60	0.77	0.74	0.64	0.67	0.64	0.75	0.70	0.70	0.80	
average r	0.33	0.45	0.49	0.27	0.68	0.50	0.59	0.65	0.69	0.42	0.65	0.65	0.58	0.59	0.63	0.56	0.57

Table B.9 Correlation matrix for PAHs in air samples taken at S13's home (n = 12)

	NAPH	2-MNap	1-MNap	BIPH	ANYL	ANAP	FLUO	DBT	PHEN	ANTH	3-MPhe	2-MPhe	9-MPhe	1-MPhe	FLR	PYR	RET
NAPH		0.69	0.57	0.51	0.69	0.66	0.59	0.73	0.69	0.66	0.75	0.70	0.64	0.57	0.49	0.73	0.20
2-MNap	0.69		0.97	0.91	0.96	0.94	0.94	0.93	0.95	0.52	0.92	0.95	0.84	0.84	0.94	0.97	0.32
1-MNap	0.57	0.97		0.92	0.93	0.93	0.94	0.91	0.94	0.48	0.89	0.92	0.83	0.84	0.94	0.93	0.29
BIPH	0.51	0.91	0.92		0.88	0.88	0.89	0.90	0.88	0.44	0.91	0.89	0.88	0.88	0.87	0.84	0.21
ANYL	0.69	0.96	0.93	0.88		0.93	0.89	0.93	0.93	0.55	0.90	0.92	0.86	0.83	0.91	0.94	0.36
ANAP	0.66	0.94	0.93	0.88	0.93		0.98	0.98	0.99	0.71	0.95	0.95	0.93	0.93	0.94	0.94	0.41
FLUO	0.59	0.94	0.94	0.89	0.89	0.98		0.94	0.97	0.64	0.92	0.91	0.90	0.93	0.96	0.92	0.40
DBT	0.73	0.93	0.91	0.90	0.93	0.98	0.94		0.98	0.73	0.98	0.97	0.96	0.93	0.88	0.92	0.33
PHEN	0.69	0.95	0.94	0.88	0.93	0.99	0.97	0.98		0.71	0.96	0.94	0.92	0.91	0.92	0.95	0.38
ANTH	0.66	0.52	0.48	0.44	0.55	0.71	0.64	0.73	0.71		0.70	0.65	0.73	0.68	0.49	0.54	0.50
3-MPhe	0.75	0.92	0.89	0.91	0.90	0.95	0.92	0.98	0.96	0.70		0.96	0.96	0.93	0.85	0.90	0.26
2-MPhe	0.70	0.95	0.92	0.89	0.92	0.95	0.91	0.97	0.94	0.65	0.96		0.89	0.87	0.89	0.92	0.31
9-MPhe	0.64	0.84	0.83	0.88	0.86	0.93	0.90	0.96	0.92	0.73	0.96	0.89		0.98	0.81	0.81	0.24
1-MPhe	0.57	0.84	0.84	0.88	0.83	0.93	0.93	0.93	0.91	0.68	0.93	0.87	0.98		0.85	0.79	0.20
FLR	0.49	0.94	0.94	0.87	0.91	0.94	0.96	0.88	0.92	0.49	0.85	0.89	0.81	0.85		0.92	0.39
PYR	0.73	0.97	0.93	0.84	0.94	0.94	0.92	0.92	0.95	0.54	0.90	0.92	0.81	0.79	0.92		0.34
RET	0.20	0.32	0.29	0.21	0.36	0.41	0.40	0.33	0.38	0.50	0.26	0.31	0.24	0.20	0.39	0.34	
average r	0.62	0.85	0.83	0.79	0.84	0.88	0.86	0.87	0.88	0.61	0.86	0.85	0.82	0.81	0.82	0.84	0.32

Table B.10 Correlation matrix for PAHs in air samples taken at S14's home (n = 14)

	NAPH	2-MNap	1-MNap	BIPH	ANYL	ANAP	FLUO	DBT	PHEN	ANTH	3-MPhe	2-MPhe	9-MPhe	1-MPhe	FLR	PYR	RET
NAPH		0.56	0.59	0.28	-0.08	0.53	0.76	0.68	0.62	0.34	0.54	0.50	0.50	0.50	0.32	0.36	-0.04
2-MNap	0.56		0.92	0.13	-0.25	0.39	0.81	0.73	0.55	0.10	0.33	0.41	0.43	0.45	0.03	0.10	0.45
1-MNap	0.59	0.92		0.25	-0.20	0.29	0.80	0.69	0.57	0.05	0.34	0.42	0.41	0.42	0.11	0.16	0.55
BIPH	0.28	0.13	0.25		0.02	0.04	0.35	0.33	0.44	0.54	0.57	0.45	0.47	0.53	-0.17	-0.19	0.00
ANYL	-0.08	-0.25	-0.20	0.02		0.10	-0.11	-0.52	-0.30	0.30	-0.42	-0.45	-0.46	-0.44	0.42	0.46	0.16
ANAP	0.53	0.39	0.29	0.04	0.10		0.73	0.30	0.51	0.68	0.07	0.02	0.03	0.05	0.57	0.56	0.06
FLUO	0.76	0.81	0.80	0.35	-0.11	0.73		0.74	0.79	0.56	0.48	0.50	0.50	0.53	0.31	0.32	0.25
DBT	0.68	0.73	0.69	0.33	-0.52	0.30	0.74		0.82	0.22	0.81	0.84	0.85	0.85	0.05	0.03	-0.02
PHEN	0.62	0.55	0.57	0.44	-0.30	0.51	0.79	0.82		0.48	0.71	0.72	0.71	0.76	0.33	0.30	-0.05
ANTH	0.34	0.10	0.05	0.54	0.30	0.68	0.56	0.22	0.48		0.28	0.17	0.18	0.24	0.23	0.20	-0.14
3-MPhe	0.54	0.33	0.34	0.57	-0.42	0.07	0.48	0.81	0.71	0.28		0.97	0.97	0.97	-0.02	-0.07	-0.20
2-MPhe	0.50	0.41	0.42	0.45	-0.45	0.02	0.50	0.84	0.72	0.17	0.97		0.99	0.98	-0.01	-0.02	-0.09
9-MPhe	0.50	0.43	0.41	0.47	-0.46	0.03	0.50	0.85	0.71	0.18	0.97	0.99		0.99	-0.06	-0.09	-0.18
1-MPhe	0.50	0.45	0.42	0.53	-0.44	0.05	0.53	0.85	0.76	0.24	0.97	0.98	0.99		-0.08	-0.09	-0.16
FLR	0.32	0.03	0.11	-0.17	0.42	0.57	0.31	0.05	0.33	0.23	-0.02	-0.01	-0.06	-0.08		0.97	0.20
PYR	0.36	0.10	0.16	-0.19	0.46	0.56	0.32	0.03	0.30	0.20	-0.07	-0.02	-0.09	-0.09	0.97		0.29
RET	-0.04	0.45	0.55	0.00	0.16	0.06	0.25	-0.02	-0.05	-0.14	-0.20	-0.09	-0.18	-0.16	0.20	0.29	
average r	0.44	0.39	0.40	0.25	-0.11	0.31	0.52	0.46	0.50	0.28	0.40	0.40	0.39	0.41	0.20	0.21	0.07

Table B.11 Correlation matrix for PAHs in air samples taken at S15's home (n = 12)

	NAPH	2-MNap	1-MNap	BIPH	ANYL	ANAP	FLUO	DBT	PHEN	ANTH	3-MPhe	2-MPhe	9-MPhe	1-MPhe	FLR	PYR	RET
NAPH		0.75	0.64	0.40	0.77	0.79	0.81	0.83	0.71	0.70	0.72	0.66	0.74	0.74	0.28	0.37	0.36
2-MNap	0.75		0.88	0.78	0.87	0.93	0.84	0.89	0.92	0.76	0.86	0.73	0.76	0.76	0.58	0.61	0.60
1-MNap	0.64	0.88		0.65	0.77	0.84	0.72	0.72	0.76	0.52	0.78	0.71	0.65	0.64	0.38	0.34	0.31
BIPH	0.40	0.78	0.65		0.81	0.83	0.69	0.71	0.85	0.64	0.68	0.46	0.50	0.53	0.88	0.86	0.76
ANYL	0.77	0.87	0.77	0.81		0.96	0.91	0.91	0.88	0.64	0.87	0.74	0.76	0.78	0.60	0.62	0.54
ANAP	0.79	0.93	0.84	0.83	0.96		0.94	0.96	0.94	0.72	0.93	0.81	0.84	0.85	0.65	0.67	0.63
FLUO	0.81	0.84	0.72	0.69	0.91	0.94		0.98	0.92	0.74	0.95	0.89	0.92	0.92	0.57	0.60	0.53
DBT	0.83	0.89	0.72	0.71	0.91	0.96	0.98		0.93	0.78	0.94	0.86	0.92	0.92	0.57	0.64	0.63
PHEN	0.71	0.92	0.76	0.85	0.88	0.94	0.92	0.93		0.83	0.90	0.77	0.83	0.83	0.78	0.79	0.70
ANTH	0.70	0.76	0.52	0.64	0.64	0.72	0.74	0.78	0.83		0.62	0.47	0.61	0.61	0.67	0.70	0.62
3-MPhe	0.72	0.86	0.78	0.68	0.87	0.93	0.95	0.94	0.90	0.62		0.95	0.96	0.96	0.53	0.56	0.56
2-MPhe	0.66	0.73	0.71	0.46	0.74	0.81	0.89	0.86	0.77	0.47	0.95		0.97	0.95	0.34	0.37	0.39
9-MPhe	0.74	0.76	0.65	0.50	0.76	0.84	0.92	0.92	0.83	0.61	0.96	0.97		0.99	0.43	0.49	0.52
1-MPhe	0.74	0.76	0.64	0.53	0.78	0.85	0.92	0.92	0.83	0.61	0.96	0.95	0.99		0.46	0.53	0.57
FLR	0.28	0.58	0.38	0.88	0.60	0.65	0.57	0.57	0.78	0.67	0.53	0.34	0.43	0.46		0.96	0.83
PYR	0.37	0.61	0.34	0.86	0.62	0.67	0.60	0.64	0.79	0.70	0.56	0.37	0.49	0.53	0.96		0.92
RET	0.36	0.60	0.31	0.76	0.54	0.63	0.53	0.63	0.70	0.62	0.56	0.39	0.52	0.57	0.83	0.92	
average r	0.64	0.78	0.64	0.69	0.78	0.83	0.81	0.82	0.83	0.66	0.80	0.69	0.74	0.75	0.60	0.63	0.59

Table B.12 Correlation matrix for PAHs in air samples taken at S16's home (n = 9)

	NAPH	2-MNap	1-MNap	BIPH	ANYL	ANAP	FLUO	DBT	PHEN	ANTH	3-MPhe	2-MPhe	9-MPhe	1-MPhe	FLR	PYR	RET
NAPH		0.88	0.88	0.25	0.70	0.68	0.81	0.74	0.61	0.78	0.34	0.28	0.51	0.46	0.40	-0.15	0.09
2-MNap	0.88		0.95	0.39	0.62	0.75	0.83	0.80	0.58	0.67	0.08	0.02	0.25	0.16	0.09	-0.32	0.15
1-MNap	0.88	0.95		0.29	0.73	0.81	0.87	0.82	0.66	0.73	0.13	0.09	0.34	0.20	0.22	-0.25	0.23
BIPH	0.25	0.39	0.29		0.33	0.32	0.22	0.32	0.04	0.49	0.41	0.47	0.23	0.15	-0.35	0.01	-0.19
ANYL	0.70	0.62	0.73	0.33		0.91	0.89	0.89	0.87	0.83	0.67	0.61	0.75	0.59	0.48	0.05	0.15
ANAP	0.68	0.75	0.81	0.32	0.91		0.95	0.96	0.80	0.69	0.45	0.40	0.60	0.42	0.40	-0.08	0.24
FLUO	0.81	0.83	0.87	0.22	0.89	0.95		0.96	0.88	0.73	0.36	0.29	0.56	0.45	0.43	-0.27	0.22
DBT	0.74	0.80	0.82	0.32	0.89	0.96	0.96		0.87	0.66	0.47	0.38	0.63	0.51	0.44	-0.08	0.33
PHEN	0.61	0.58	0.66	0.04	0.87	0.80	0.88	0.87		0.62	0.46	0.32	0.59	0.53	0.49	-0.12	0.28
ANTH	0.78	0.67	0.73	0.49	0.83	0.69	0.73	0.66	0.62		0.50	0.48	0.47	0.35	0.11	-0.19	-0.31
3-MPhe	0.34	0.08	0.13	0.41	0.67	0.45	0.36	0.47	0.46	0.50		0.96	0.90	0.86	0.55	0.54	0.10
2-MPhe	0.28	0.02	0.09	0.47	0.61	0.40	0.29	0.38	0.32	0.48	0.96		0.88	0.82	0.51	0.49	0.06
9-MPhe	0.51	0.25	0.34	0.23	0.75	0.60	0.56	0.63	0.59	0.47	0.90	0.88		0.96	0.81	0.43	0.38
1-MPhe	0.46	0.16	0.20	0.15	0.59	0.42	0.45	0.51	0.53	0.35	0.86	0.82	0.96		0.81	0.39	0.38
FLR	0.40	0.09	0.22	-0.35	0.48	0.40	0.43	0.44	0.49	0.11	0.55	0.51	0.81	0.81		0.40	0.59
PYR	-0.15	-0.32	-0.25	0.01	0.05	-0.08	-0.27	-0.08	-0.12	-0.19	0.54	0.49	0.43	0.39	0.40		0.38
RET	0.09	0.15	0.23	-0.19	0.15	0.24	0.22	0.33	0.28	-0.31	0.10	0.06	0.38	0.38	0.59	0.38	
average r	0.52	0.43	0.48	0.21	0.63	0.58	0.57	0.61	0.53	0.48	0.49	0.44	0.58	0.50	0.40	0.08	0.19

Table B.13 Correlation matrix for PAHs in air samples taken at S17's home (n = 6)

	NAPH	2-MNap	1-MNap	BIPH	ANYL	ANAP	FLUO	DBT	PHEN	ANTH	3-MPhe	2-MPhe	9-MPhe	1-MPhe	FLR	PYR	RET
NAPH		0.96	0.75	0.78	0.68	0.22	0.87	-0.39	-0.64	0.42	-0.94	-0.92	-0.89	-0.92	-0.81	-0.90	-0.91
2-MNap	0.96		0.90	0.88	0.72	0.45	0.94	-0.14	-0.40	0.63	-0.89	-0.88	-0.80	-0.84	-0.68	-0.80	-0.94
1-MNap	0.75	0.90		0.90	0.72	0.70	0.85	0.22	0.01	0.82	-0.64	-0.64	-0.53	-0.57	-0.36	-0.48	-0.80
BIPH	0.78	0.88	0.90		0.70	0.66	0.86	0.05	-0.15	0.73	-0.59	-0.57	-0.48	-0.52	-0.31	-0.49	-0.70
ANYL	0.68	0.72	0.72	0.70		0.34	0.57	-0.17	-0.30	0.27	-0.51	-0.47	-0.37	-0.43	-0.23	-0.37	-0.51
ANAP	0.22	0.45	0.70	0.66	0.34		0.63	0.77	0.57	0.91	-0.13	-0.15	0.09	0.01	0.21	0.00	-0.38
FLUO	0.87	0.94	0.85	0.86	0.57	0.63		0.08	-0.24	0.77	-0.84	-0.84	-0.69	-0.75	-0.59	-0.76	-0.92
DBT	-0.39	-0.14	0.22	0.05	-0.17	0.77	0.08		0.94	0.63	0.36	0.32	0.52	0.46	0.56	0.47	0.10
PHEN	-0.64	-0.40	0.01	-0.15	-0.30	0.57	-0.24	0.94		0.41	0.63	0.60	0.72	0.70	0.76	0.73	0.40
ANTH	0.42	0.63	0.82	0.73	0.27	0.91	0.77	0.63	0.41		-0.38	-0.40	-0.24	-0.29	-0.11	-0.25	-0.62
3-MPhe	-0.94	-0.89	-0.64	-0.59	-0.51	-0.13	-0.84	0.36	0.63	-0.38		1.00	0.96	0.98	0.92	0.97	0.96
2-MPhe	-0.92	-0.88	-0.64	-0.57	-0.47	-0.15	-0.84	0.32	0.60	-0.40	1.00		0.95	0.98	0.92	0.97	0.97
9-MPhe	-0.89	-0.80	-0.53	-0.48	-0.37	0.09	-0.69	0.52	0.72	-0.24	0.96	0.95		0.99	0.98	0.95	0.88
1-MPhe	-0.92	-0.84	-0.57	-0.52	-0.43	0.01	-0.75	0.46	0.70	-0.29	0.98	0.98	0.99		0.97	0.97	0.92
FLR	-0.81	-0.68	-0.36	-0.31	-0.23	0.21	-0.59	0.56	0.76	-0.11	0.92	0.92	0.98	0.97		0.95	0.82
PYR	-0.90	-0.80	-0.48	-0.49	-0.37	0.00	-0.76	0.47	0.73	-0.25	0.97	0.97	0.95	0.97	0.95		0.89
RET	-0.91	-0.94	-0.80	-0.70	-0.51	-0.38	-0.92	0.10	0.40	-0.62	0.96	0.97	0.88	0.92	0.82	0.89	
average r	-0.17	-0.06	0.12	0.11	0.04	0.31	0.00	0.30	0.29	0.20	0.12	0.12	0.19	0.17	0.25	0.18	0.01

Table B.14 Correlation matrix for PAHs in air samples taken at S18's home (n = 10)

	NAPH	2-MNap	1-MNap	BIPH	ANYL	ANAP	FLUO	DBT	PHEN	ANTH	3-MPhe	2-MPhe	9-MPhe	1-MPhe	FLR	PYR	RET
NAPH		0.93	0.84	-0.23	-0.16	0.66	0.82	0.82	0.83	0.60	0.91	0.85	0.82	0.78	0.50	0.23	-0.14
2-MNap	0.93		0.92	-0.28	-0.06	0.71	0.94	0.87	0.89	0.78	0.90	0.87	0.84	0.78	0.68	0.38	-0.08
1-MNap	0.84	0.92		-0.18	0.30	0.82	0.88	0.78	0.75	0.67	0.82	0.81	0.74	0.69	0.63	0.50	-0.07
BIPH	-0.23	-0.28	-0.18		0.13	-0.11	-0.25	-0.08	-0.10	-0.43	-0.01	0.00	0.04	0.13	-0.41	-0.22	0.17
ANYL	-0.16	-0.06	0.30	0.13		0.50	0.04	-0.03	-0.14	0.05	-0.18	-0.11	-0.19	-0.19	0.11	0.42	-0.22
ANAP	0.66	0.71	0.82	-0.11	0.50		0.80	0.79	0.70	0.65	0.67	0.72	0.64	0.63	0.43	0.37	-0.46
FLUO	0.82	0.94	0.88	-0.25	0.04	0.80		0.88	0.88	0.73	0.85	0.85	0.80	0.76	0.73	0.51	-0.09
DBT	0.82	0.87	0.78	-0.08	-0.03	0.79	0.88		0.97	0.73	0.89	0.93	0.92	0.90	0.48	0.20	-0.43
PHEN	0.83	0.89	0.75	-0.10	-0.14	0.70	0.88	0.97		0.78	0.87	0.87	0.88	0.84	0.56	0.20	-0.33
ANTH	0.60	0.78	0.67	-0.43	0.05	0.65	0.73	0.73	0.78		0.59	0.62	0.62	0.54	0.53	0.11	-0.40
3-MPhe	0.91	0.90	0.82	-0.01	-0.18	0.67	0.85	0.89	0.87	0.59		0.98	0.97	0.96	0.36	0.11	-0.11
2-MPhe	0.85	0.87	0.81	0.00	-0.11	0.72	0.85	0.93	0.87	0.62	0.98		0.99	0.98	0.33	0.10	-0.20
9-MPhe	0.82	0.84	0.74	0.04	-0.19	0.64	0.80	0.92	0.88	0.62	0.97	0.99		0.99	0.28	0.01	-0.22
1-MPhe	0.78	0.78	0.69	0.13	-0.19	0.63	0.76	0.90	0.84	0.54	0.96	0.98	0.99		0.20	-0.04	-0.22
FLR	0.50	0.68	0.63	-0.41	0.11	0.43	0.73	0.48	0.56	0.53	0.36	0.33	0.28	0.20		0.83	0.11
PYR	0.23	0.38	0.50	-0.22	0.42	0.37	0.51	0.20	0.20	0.11	0.11	0.10	0.01	-0.04	0.83		0.28
RET	-0.14	-0.08	-0.07	0.17	-0.22	-0.46	-0.09	-0.43	-0.33	-0.40	-0.11	-0.20	-0.22	-0.22	0.11	0.28	
average r	0.57	0.63	0.62	-0.11	0.02	0.53	0.63	0.60	0.59	0.45	0.60	0.60	0.57	0.55	0.40	0.25	-0.15

APPENDIX C

OH-PAH CONCENTRATIONS FROM INDIVIDUAL SUBJECTS IN THE PERSONAL AIR SAMPLING AND BIOMONITORING STUDY

This Appendix gives the concentration profiles of 9 major detectable OH-PAH metabolites measured in 8 participants in the study discussed in Chapter 6. The study was titled, "Assessment of Human Exposure to Polycyclic Aromatic Hydrocarbons Employing Personal Air Sampler and Urinary PAH Biomonitoring". The concentrations were all creatinine-adjusted to correct for urine dilution, and the unit for the concentrations is ng/g creatinine. The 9 OH-PAHs are listed below.

- 1-NAP: 1-naphthol
- 2-NAP: 2-naphthol
- 9-FLUO: 9-hydroxyfluorene
- 3-FLUO: 3-hydroxyfluorene
- 2-FLUO: 2-hydroxyfluorene
- 3-PHEN: 3-hydroxyphenanthrene
- 1-PHEN: 3-hydroxyphenanthrene
- 2-PHEN: 3-hydroxyphenanthrene
- 1-PYR: 1-hydroxypyrene

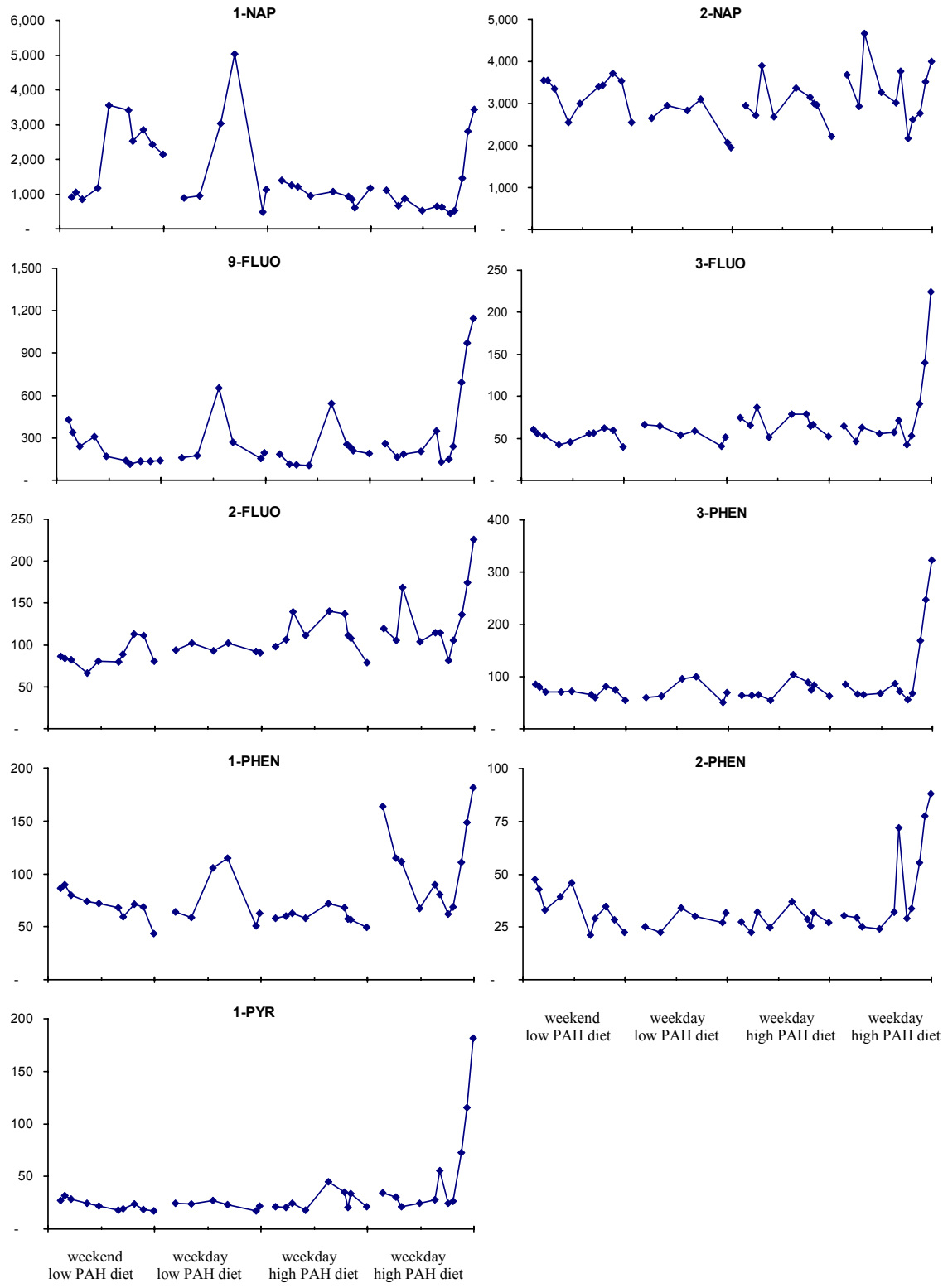

Figure C.1 Concentrations (ng/g creatinine) of 9 OH-PAH metabolites in subject S11 over 8 days

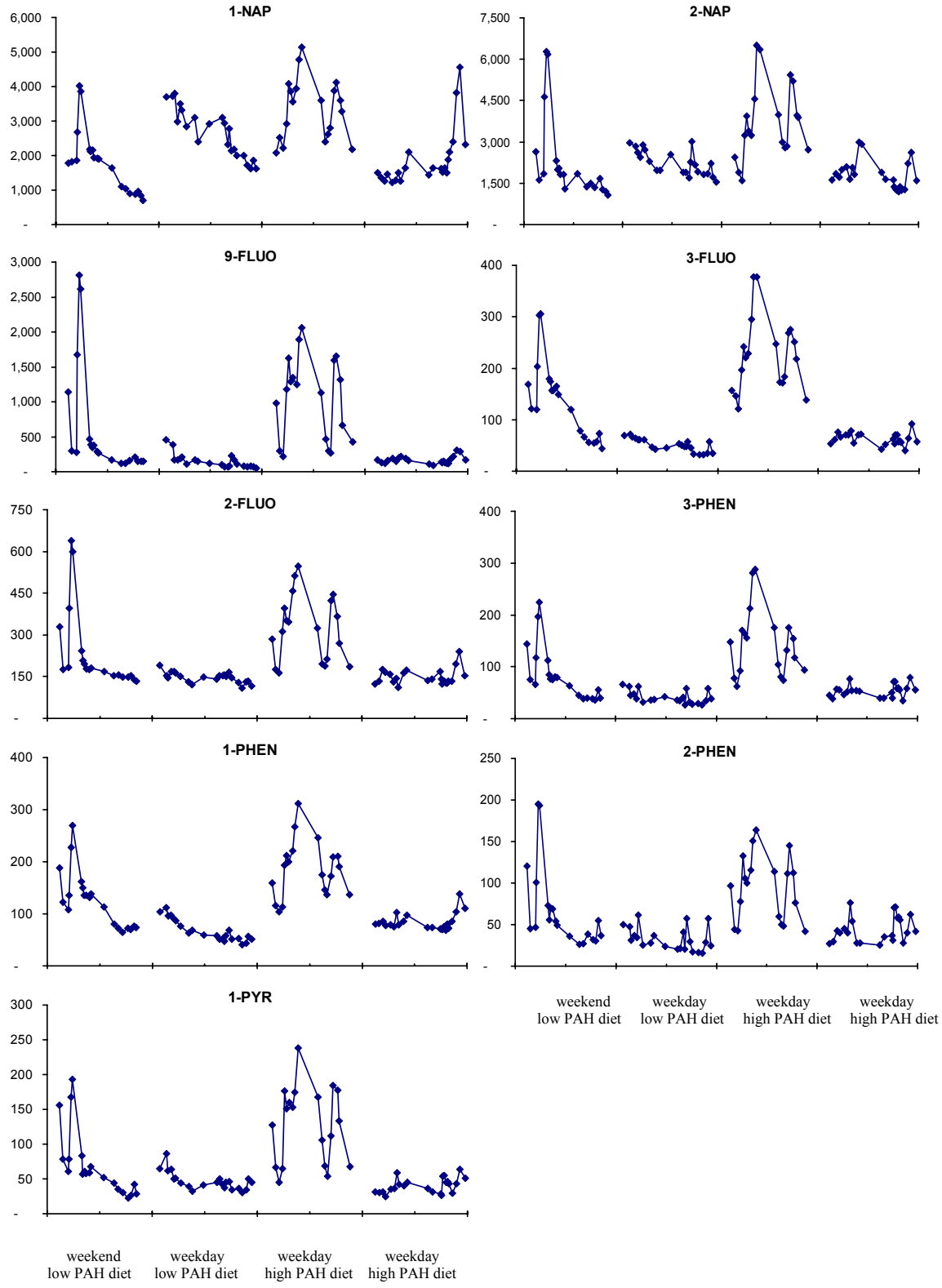

Figure C.2 Concentrations (ng/g creatinine) of 9 OH-PAH metabolites in subject S12 over 8 days

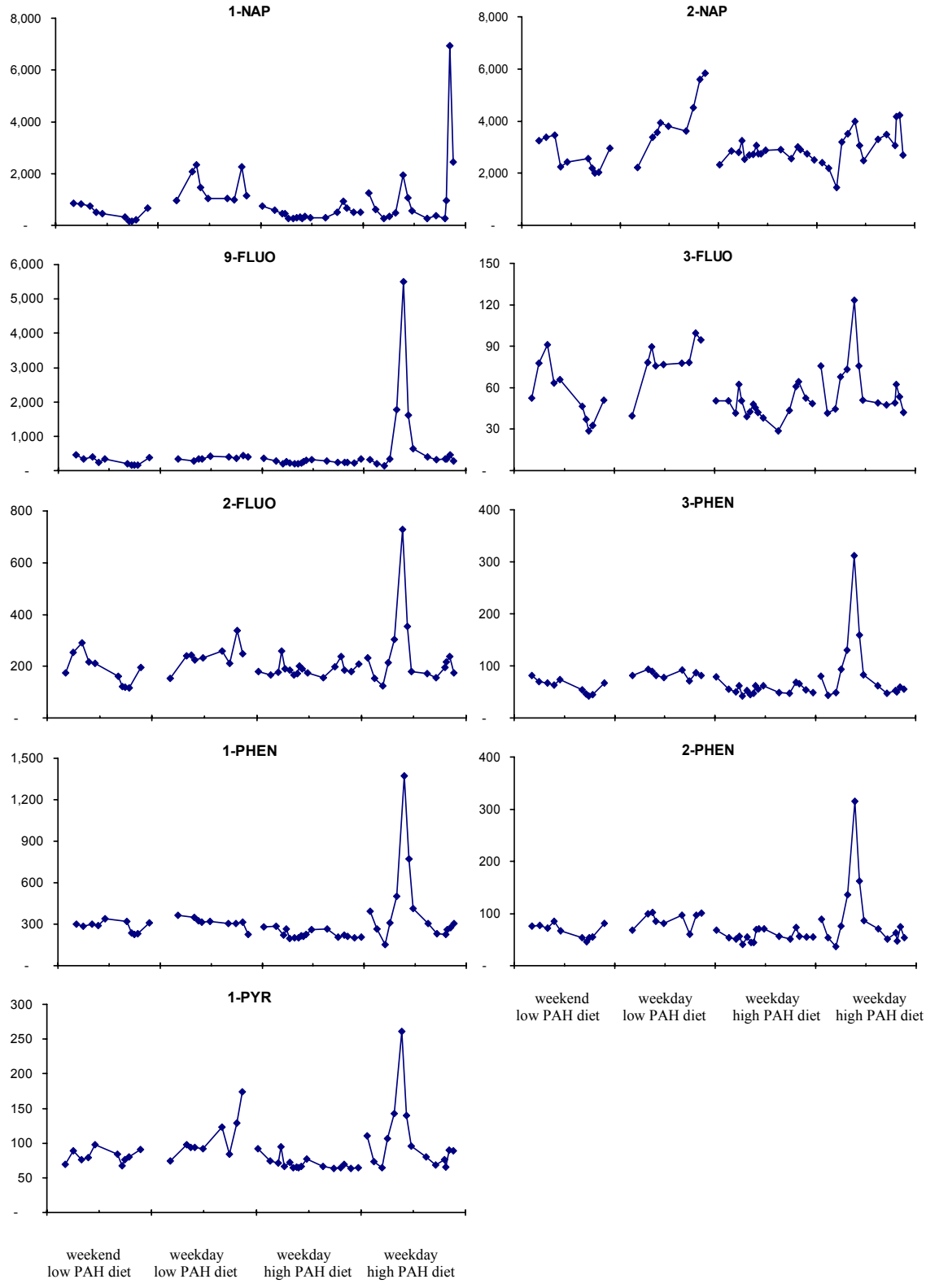

Figure C.3 Concentrations (ng/g creatinine) of 9 OH-PAH metabolites in subject S13 over 8 days

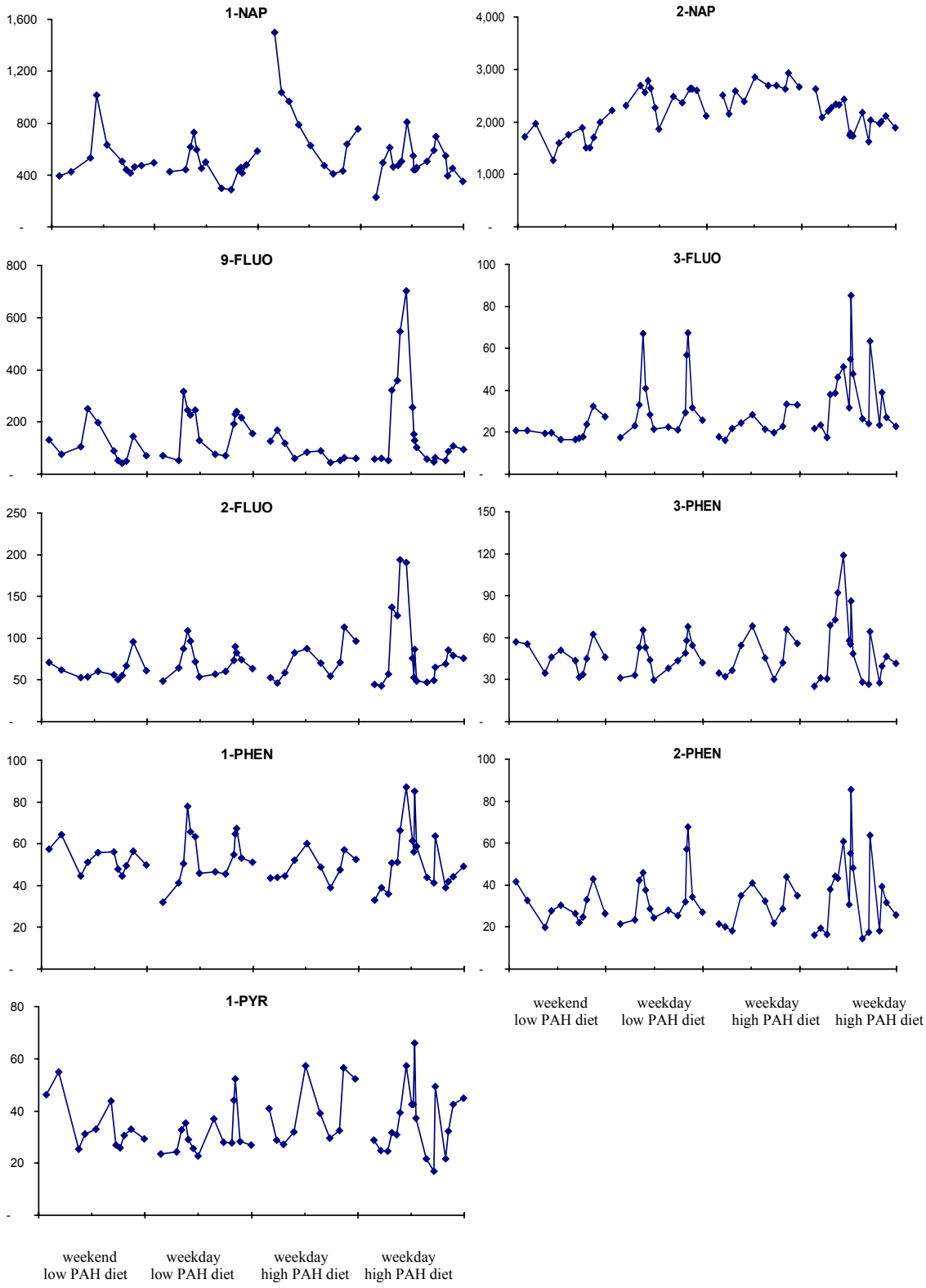

Figure C.4 Concentrations (ng/g creatinine) of 9 OH-PAH metabolites in subject S14 over 8 days

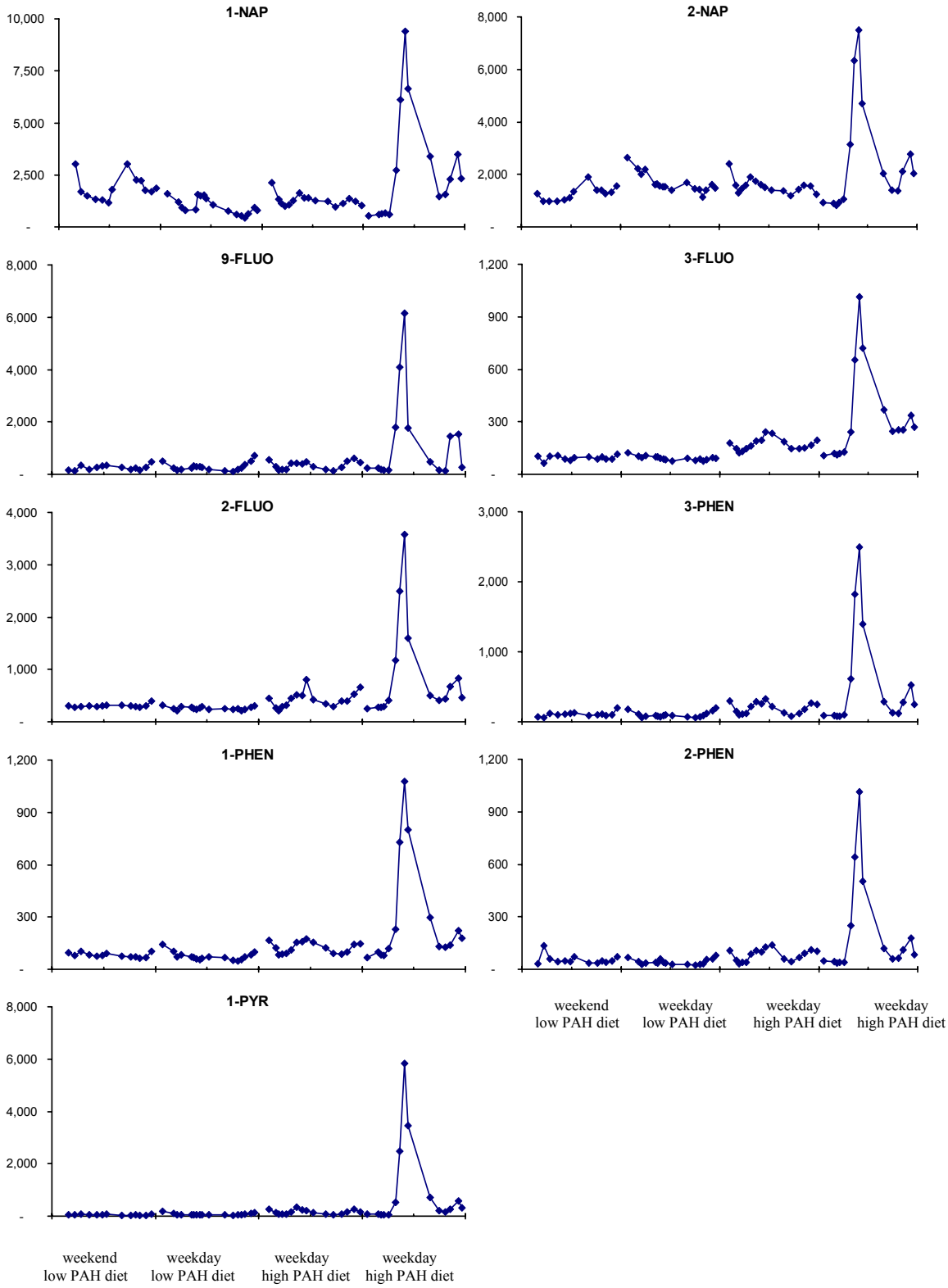

Figure C.5 Concentrations (ng/g creatinine) of 9 OH-PAH metabolites in subject S15 over 8 days

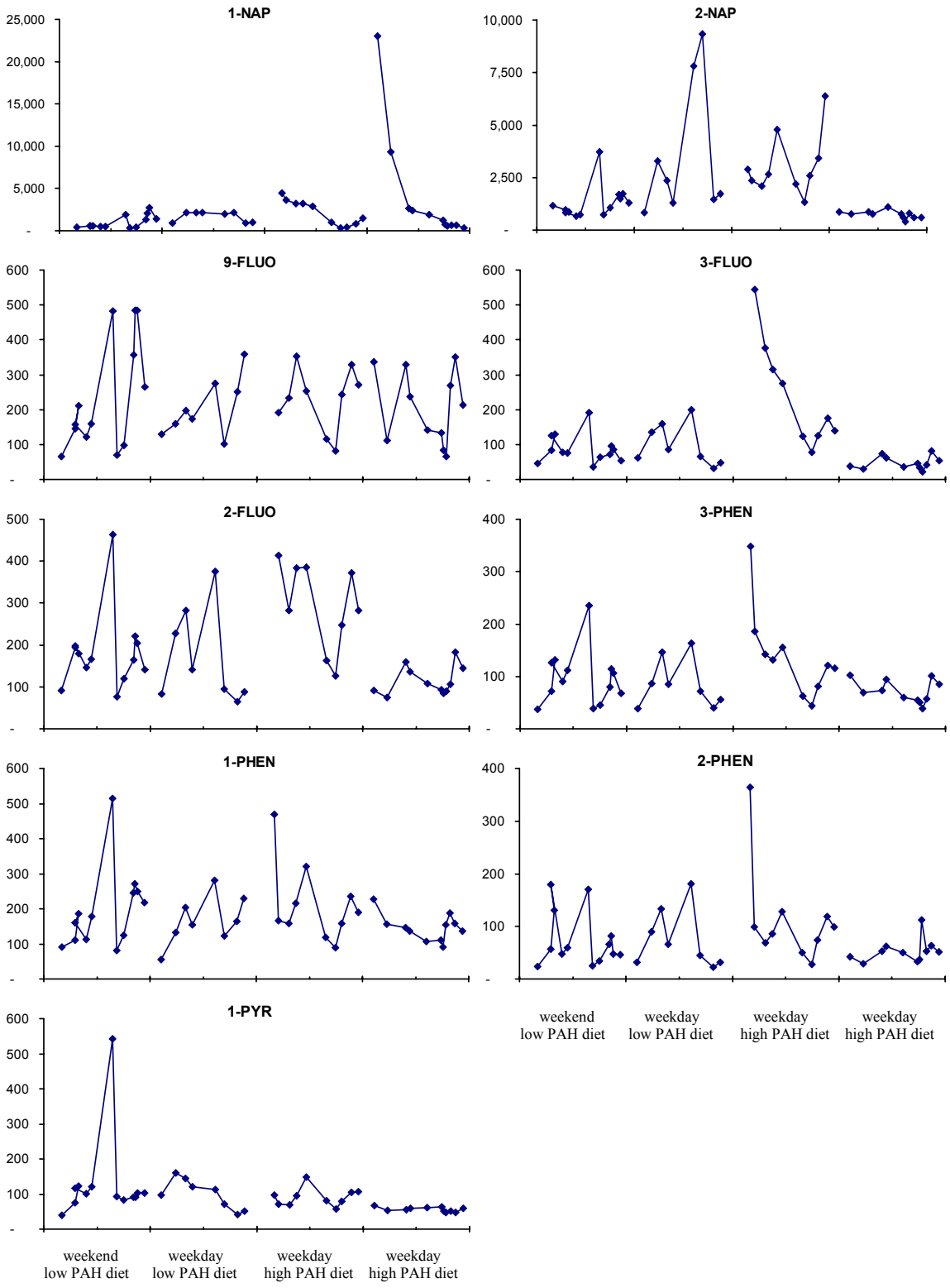

Figure C.6 Concentrations (ng/g creatinine) of 9 OH-PAH metabolites in subject S16 over 8 days

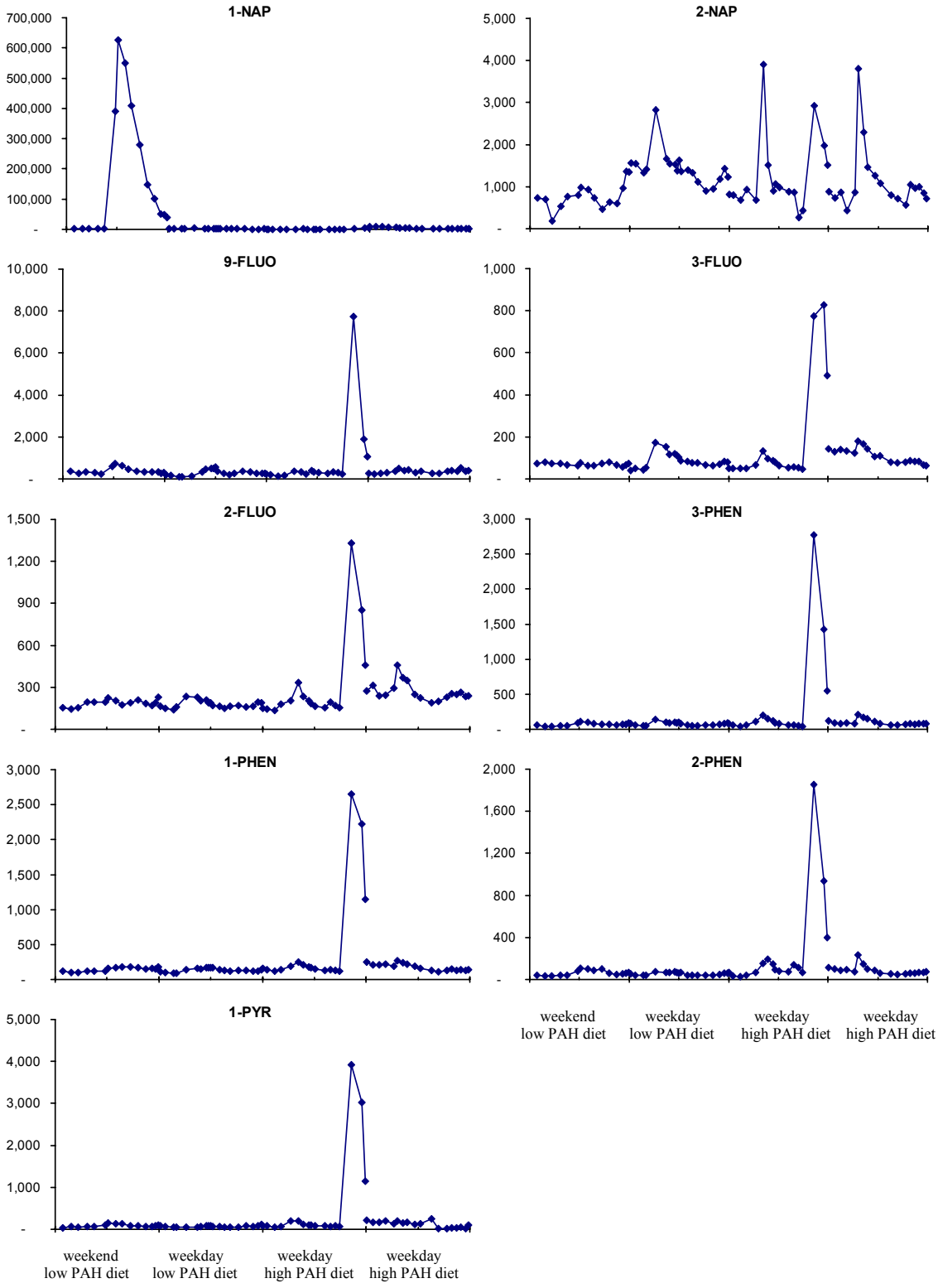

Figure C.7 Concentrations (ng/g creatinine) of 9 OH-PAH metabolites in subject S17 over 8 days

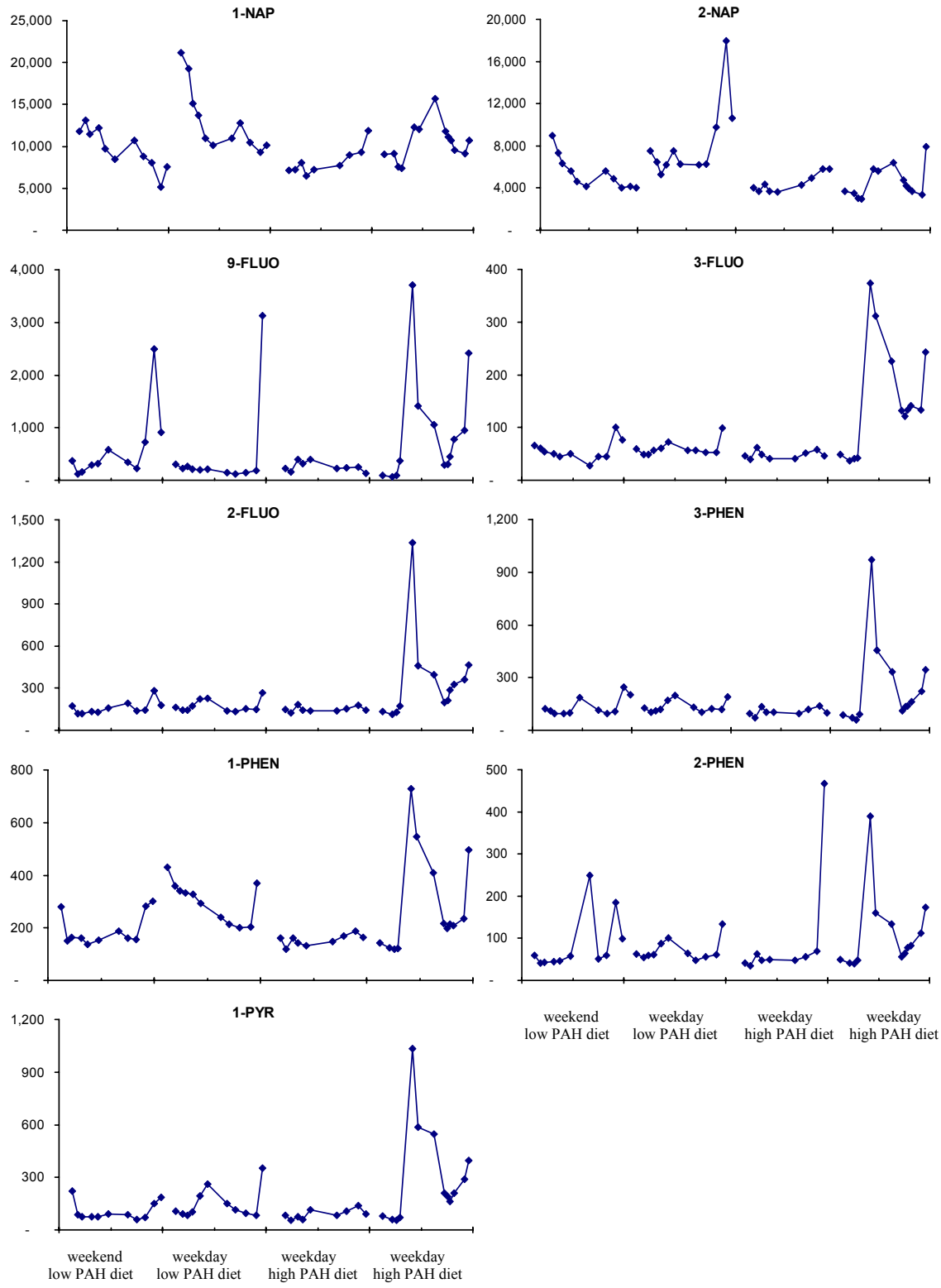

Figure C.8 Concentrations (ng/g creatinine) of 9 OH-PAH metabolites in subject S18 over 8 days

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VITA

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Zheng (Jane) Li was born in Hunan province, P.R. China. She received a B.S. in Chemistry from Wuhan University, P.R. China in 1996 and a M.S. in Chemistry with focus on environmental and analytical chemistry from Mississippi State University in 2001. She started working as a research chemist at the Centers for Disease Control and Prevention (CDC) in April, 2001. In the fall of 2003, while remaining working at the CDC, Jane started pursuing a Ph.D. degree on environmental engineering, a field that she has always been fascinated about, at the Georgia Institute of Technology (GT). Under the advisement of Professor Jim Mulholland at the GT and Dr. Andreas Sjodin at the CDC, she studied polycylic aromatic hydrocarbons (PAH) in ambient air and exposure assessment of human exposure to PAHs. While she is not working on her research, Jane enjoys traveling and learning about different cultures. Jane is the proud wife of Jon Morgan and proud mom of son Kaidon.