URINARY 1-HYDROXYPYRENE IN NON-SMOKERS: A BIOMARKER FOR COKE SMOKE EXPOSURE AND GENERAL URBAN PAH EXPOSURE

by

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A dissertation submitted to the

School of Public Health

University of Medicine and Dentistry of New Jersey and

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

University of Medicine and Dentistry of New Jersey

School of Public Health

Written under the direction of

Junfeng (Jim) Zhang, Ph.D.

and approved by

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New Brunswick, New Jersey

January, 2008

ABSTRACT OF THE DISSERTATION

Urinary 1-hydroxypyrene in Nonsmokers: A Biomarker for Coke Smoke Exposure and General Urban PAH Exposure

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This dissertation research examined the validity of urinary 1-OHP as a biomarker of PAH for coke production workers and non-coke oven workers in Anshan City, China. It has been examined whether a first morning urine sample can be used to reflect daily average exposure. Results show that intra-individual differences ranged from 40% to 62% between first morning voids and 24-hour urine composite urine samples. Coke workers showed larger intra-individual differences than non-coke oven workers. Creatinine adjustment of 1-OHP in urine reduced intra-individual difference by approximately 10% for both coke and non-coke oven workers. Despite significant intra-individual difference, a high overall correlation (r=0.76) was observed between first morning and 24-hour average 1-OHP concentrations. Significant effect of season on the association between first morning and 24-hour 1-OHP in urine has been observed. Creatinine adjustment did not improve overall correlation between 1-OHP concentrations in first morning voids and 24-hour composite urine samples.

An exposure-biomarker relationship between PAH exposure and urinary 1-OHP concentration has been determined with a wide range of personal inhalation exposure,

suggesting a threshold value for this exposure-biomarker relationship at 49 ng/m^3 for personal air pyrene and 20 ng/m^3 for personal air BaP. The corresponding urinary 1-OHP concentration was 0.46 μmol/(mol creatinine). However, no significant exposure-biomarker relationship was observed for dietary intake. With inhalation exposure, an increase of 1-OHP concentration by 1 μmol/(mol creatinine) predicted an increase in personal air concentration of BaP by 0.12 μg/m^3 and 0.13 μg/m^3 in males and females, respectively.

In addition, reproducibility of urinary 1-OHP has been demonstrated over 6 to 9 months. Intra-class correlation coefficient for urinary 1-OHP was similar to those for personal air and dietary intakes of pyrene. Within-person variances were larger than between-person variances in all measured media. Urinary 1-OHP had the highest variance ratio (within- to between-person), suggesting that urinary 1-OHP may be less accurate to estimate PAH exposure than personal air and dietary intake. No significant effects of work type and season were found on intra-class correlation in urinary 1-OHP, personal air concentration of pyrene, and dietary pyrene intake.

Acknowledgements

First of all, I would like to express my great appreciation to Dr. Junfeng (Jim) Zhang for his continuous direction, help, and financial support. His constant encouragement was the driving force throughout my graduate study. I also wish to thank the members of my dissertation committee, Drs. Pamela Ohman-Strickland, Dana Barr, and Mark Robson, who has shared their research resources and insightful comments.

I appreciated the help provided by Dr. George Rhoads, Dr. Duan Xiaoli, Dr. Lin Zhang, on the manuscripts' reviews and comments. I would also like to thank the research staffs and subjects in China for their help on the study. I am very grateful to Dr. Lin Zhang and Dr. Weili Liu for their help on the experiment design and data interpretation. I would like to thank my lab colleagues, Dr. Jason Herrington, Jicheng, Jean, Chen, for their pleasant cooperation and sharing the joy of working during this research. I would like to acknowledge the UMDNJ-SPH staffs, Margaret Mitchell, Tina Greco, and Diane Hammer for their administrative support. In addition, I would like to thank my EOHSI fellows, Dr. Changho Yu, Dr. Kyunghwa Jung, Dr. Jaymin Kwon, Kunning, Xiangmei, for their sincere friendship.

I would like to thank the National Cancer Institute for funding the Biomarker Validation for Cancer Risk under 1R21-CA94743-01.

Dedication

To my loving wife Heyreoun An, daughter

and Parents, Woonghee Han, Yoonsik Lee, Keumsok An, and Soonhwa Lee

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Chapter 1

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of hydrocarbons fused with two or more aromatic rings. PAHs are ubiquitous in the environment. Sources of PAHs include industrial combustion, environmental tobacco smoke (ETS), pyrolysis, vehicle exhaust, and cooking. Routes of PAH exposure include inhalation, ingestion, and dermal contact. Some components of PAHs have been known to cause cancer to animals and humans (IARC 1984; IARC 1985). Measurement of PAH concentrations in environmental media (e.g., air, food) even at personal levels can not determine internal doses that may be measured using biomarkers. Among various biomarkers, urinary 1-hydroxypyrene (1-OHP) has been suggested as a surrogate to estimate internal doses for total PAH exposure since the 1980s. As a major metabolite of pyrene, it is eliminated in urine (20%) and feces (80%), and its total excretion reflects 90% of pyrene metabolism (Jongeneelen et al. 1986). In addition, studies have established correlations between urinary 1-OHP and PAH/pyrene in the air. Measurement of PAHs and/or their metabolites in human fluids may provide more direct information towards understanding PAH exposure and health effects. The aims of the present dissertation research were (1) to examine whether first morning urine can represent 24-hour composite urine specimens; (2) to quantify relationships between PAH inhalation exposure and urinary 1-hydroxypyrene (1-OHP) concentration; and (3) to evaluate seasonal and between-person

variability of urinary 1-OHP. The following section summarizes a literature review on PAH exposure, PAH health effects, exposure assessment with urinary 1-OHP, in the context of the scope for the current dissertation study.

1.1 Health Effects of Polycyclic Aromatic Hydrocarbons

The health effect of PAH containing materials was first observed in the eighteenth century. The carcinogenicity of PAHs was first reported when scrotal cancer in chimney sweeps resulted from the exposure to soot and elevated incidences of skin cancer were observed for workers in the coal tar industry (Melicow 1975). In the early 20th century, it is well known that coal tar, pitch, and soot are carcinogenic to human (Dipple et al. 1985). More sophisticated animal studies have found carcinogenic effect of PAHs associated with the fraction of compounds containing 4 to 7 aromatic rings (Grimmer et al. 1984; Grimmer et al. 1983). The International Agency for Research on Cancer (IARC) has evaluated several PAH-containing materials and selected PAHs (IARC 1983). Among these individual PAHs, benzo[a]pyrene (BaP) has been used as an indicator of carcinogenic PAHs. The carcinogenicity of individual PAHs is summarized in Table 1.1.

Thus, a number of studies have investigated elevated various cancer risks associated with occupational exposures to PAHs. For example, Reid and Buck (1956) observed increased lung cancer risk (relative risk=1.4) for coke oven workers in United Kingdom. In China, lung cancer risk for coke oven workers was 2.6 (relative risk). The highest lung cancer risk was observed in top of oven workers (Wu 1988). Chau et al (1993) investigated lung cancer risk in 536 retired coke oven plant workers . The authors found increased lung cancer risk for coke oven workers and those who worked near coke

ovens workers as well. Increased skin cancer risks were observed for workers, exposed to PAH containing tar, pitch, soot, and mineral oil. Gallagher et al (1996) reported increased basal cell carcinoma risk in Canadians exposed to petroleum products. In addition, various PAH exposure sources (coal tar, diesel exhaust, oil soot, aluminum smelting, and gas station) have been associated with increased rates of bladder cancer (Baxter and McDowall 1986; Bonassi et al. 1989; Schumacher et al. 1989; Steineck et al. 1990a; Steineck et al. 1990b). Table 1.2. summarizes exposures to PAH and various cancer in humans.

In addition, there is an emerging concern that PAH exposure may cause other adverse health effects (e.g., respiratory, cardiovascular, neurological, and reproductive effect) (Northridge et al. 1999; Perera et al. 2005; Perera et al. 2006; Riedl and Diaz-Sanchez 2005). Jedrychowski et al. (2003)reported that pregnant women's exposure to PAH was significantly higher in the heating season and high prenatal exposure to airborne PAH and $PM_{2.5}$ led to a reduction in birth weight, length and head circumference. Previous studies have shown that reduced birth size can adversely affect children's learning ability and increase their risk of health problems. The investigators also found that high prenatal exposure to PAHs was linked to significantly lower scores on tests of infant intelligence at two years of age (Jedrychowski et al. 2003). Prenatal exposure to PAHs and second-hand smoke are observed increasing children's respiratory symptoms and likelihood of developing asthma (Miller et al. 2004).

Table 1. 1. The degree of evidence for carcinogenicity of selected PAHs in experimental animals and overall evaluation of carcinogenicity to humans.

PAH	Number of rings	IARC classification	
		Humans	Animals
Anthracence	3	3	I
Phenanthrene	3	3	I
Chrysene	4	3	L
Benz[a]anthracene	4	2A	S
Pyrene	4	3	I
BaP	5	2A	S
Dibenz[a,h]anthracene	5	2A	S
Benzo[ghi]perylene	6	3	I

2A, probably carcinogenic to humans; 3, not classifiable; I, inadequate eveidence; L, limited evidence; S, sufficient evidence.
Source : Adapted from IARC (1983)

Table 1. 2. Health effect of PAH exposure in industrial workers and general population.

Author	Country	Follow-up[a]	Study population	Exposure	Observed cases	RR[b]	CI[c]
Reid and Buck 1956	UK	1950-54	8,000	Coke oven	14	1.4	0.8-2.3
Sakabe et al 1975	Japan	1949-73	2,178	Coke oven	15	1.3	0.7-2.1
Davies 1977	UK	1954-65	610	Coke oven	8	0.8	0.4-1.6
Hurley et al 1983	UK	1966-80	3,952	Coke oven	167	1.2	1.0-1.4
Wu 1988	China	1971-82	21,995	Coke oven	93	2.6	2.1-3.1
Swanen et al 1991	Netherland	1954-84	5,639	Coke oven	62	1.3	1.0-1.7
Franco et al 1993	Italy	1960-90	538	Coke oven	19	1.7	1.0-2.7
Chau et al 1993	France	1963-87	536	Coke oven	2	1.8	0.2-6.3
Costantino et al 1995	USA/Canada	1951-82	15,818	Coke oven	255	2.0	1.6-2.3
Bye et al 1998	Norway	1964-88	888	Coke oven	7	0.8	0.3-1.7

[a]Follow-up outcome as death except Bye et al (incidence).
[b]RR, relative risk.
[c]CI, confidence interval
Source : Adapted from Bosetti et al (2007)

1.2 External Measurement of PAH Exposure

Humans can be exposed to PAH through inhalation of air, ingestion of food, and dermal contacts in occupational and environmental settings. In earlier assessments of PAH exposure, indirect or direct exposure assessment methodology has been used. Most studies have considered airborne exposure in occupational settings where inhalation was the primary route of entry of PAH into the body. Samples were collected at fixed sites or near to persons' breathing zones with air pumps. Results from a large number of studies at various workplaces found large variations in PAH concentrations even at similar or identical workplaces (Boogaard and van Sittert 1995; Grimmer et al. 1993; IARC 1984; Kuljukka et al. 1997; Lin et al. 2006; Lu et al. 2002; Mielynska et al. 1997; Pan et al. 1998; Pyy et al. 1997; Siwinska et al. 2004). In addition, PAH profiles vary not only from one emission source to another, but also within a workplace. For example, the proportion of pyrene in different working environments varies from 2% to 20% of total PAHs (Ovrebo et al. 1995c).

Unlike occupational settings, ambient air, water, food, and environmental tobacco smoke may be more relevant to PAH exposure in the general, non-occupationally exposed population. Presently, benzo[a]pyrene concentrations in the air are generally less than 10 ng/m^3 in ambient air of rural or even urban living areas in European countries (Jacob and Seidel 2002). Elevated concentrations may be expected in areas with dense vehicle traffic, in road tunnels or in area near to industrial emissions (Cocco et al. 2007; Merlo et al. 1998; Tsai et al. 2004). Food appears to be the main source of PAH intake for the general population (Chuang et al. 1999; Scherer et al. 2000; Viau et al. 2002).

Dietary PAH intake has been estimated on the basis of the measurement of PAH concentrations in food products. These reported PAH concentration range normally combined with food consumption diary (Scherer et al. 2000). The method, however, inaccurately estimates dietary PAH intake because food intake per person is certainly the heterogeneous array of cooking styles and foodstuffs used (dell'Omo and Lauwerys 1993). To overcome the disadvantages, several studies used "duplicate diet" method to estimate dietary PAH intake for non-occupationally exposed population (Lioy and Greenberg 1990; Viau et al. 2002; Viau et al. 2004b).

The Total Human Environmental Exposure Study (THEES), a multimedia study of human exposure to BaP, was conducted in a rural town, Phillipsburg, NJ. Lioy et al. (1988) measured BaP concentrations in air and food. In comparing the inhalation and ingestion routes in each home, the authors found that potential PAH intakes were similar in each medium. Because PAHs are present not only in ambient air, but also in other environmental media, mainly the diet and tobacco smoke, PAH measurements in a single media can only produce measures of PAH exposure when other routes of exposure prove to be negligible. To get a more accurate total exposure, all routes of PAH exposure must be considered. However, it is difficult to obtain all samples from personal air, diet, and skin since it is labor-intensive, inconvenient, time consuming, and the measurements are costly. The best approach to assess total PAH exposure, thus, appears to be the one that can measure total intakes/uptakes through the use of reliable and effective biomarkers of PAH exposure.

1.3 Biomarkers of PAHs

Biomarkers are usually classified into three categories: biomarkers of exposure, biomarkers of biological or health effects, and biomarkers of susceptibility. Exposure biomarkers can be either a marker of internal dose or a marker of effective dose. The exogenous compound or its metabolites in the body may serve as either of these two types of biomarkers. Their presence confirms that the compound has entered the body and possibly establishes a relation between environmental exposures and those measured in the appropriate biological medium.

Effects biomarkers represent the interaction between the pollutant and the human body. These markers can be measured by a biochemical alteration of a physiologic disorder, with or without clinical expression. Susceptibility biomarkers define the degree of an individual's sensitivity to an external exposure to pollutant. Biomarker studies can potentially fill in the links between pollutant sources and their health effects (Figure 1.1).

Biomonitoring of exposure to PAHs has been widely used since the 1980s. Biomarker of PAH exposure can be used as a means of assessing exposure to these compounds. However, in order to have valid PAH biomarkers for wide application in human populations, a number of factors should be considered: inter- and intra-individual variation, sensitivity, and specificity of the analytical measurement methods. From this perspective, the markers that have been explored include urinary PAH metabolites (markers of internal dose), leukocyte DNA adducts (markers of biological effective dose), genetic polymorphisms of cytochrome P4501A1 and glutathione S-transferase M1 (markers of susceptibility) (Pan et al, 1998). Although these studies of PAH biomarkers present useful data for better understanding of mechanisms of toxicity and particularly

carcinogenicity of PAHs (Bouchard and Viau, 1996; Jongeneelen et al, 1987), they have not contributed, so far, to the identification and quantification of cancer risk or other health risks (Boffetta et al, 1997; Jongeneelen, 2001).

Among these potential biomarkers, a drawback with DNA adduct measurements is that it cannot accurately determine the relationship between adduct measurement and PAH exposure (Angerer et al. 1997a). Protein adducts of PAH have been measured in hemoglobin and serum albumin for various occupational and environmental exposures (Helleberg and Tornqvist 2000; Hemminki et al. 1994; Nielsen et al. 1996). Although the relationship between PAH exposure and protein adducts were found in some studies, the correlations were weak. Thus, the protein adducts may not be specific markers to PAH exposure. The analysis of urinary metabolites has been widely used as PAH exposure biomarkers since the determination of hydroxylated PAHs in urine is specific and sensitive to PAH exposure; and urine collection can be readily achieved (Angerer et al. 1997b; Buckley et al. 1995; Elovaara et al. 2003; Gardiner et al. 1992; Jacob et al. 1989; Jongeneelen 1994; Jongeneelen 1997; Jongeneelen 2001; Jongeneelen et al. 1985; Jongeneelen et al. 1988; Jongeneelen et al. 1986; Kanoh et al. 1993; Wu et al. 2002; Zhao et al. 1990).

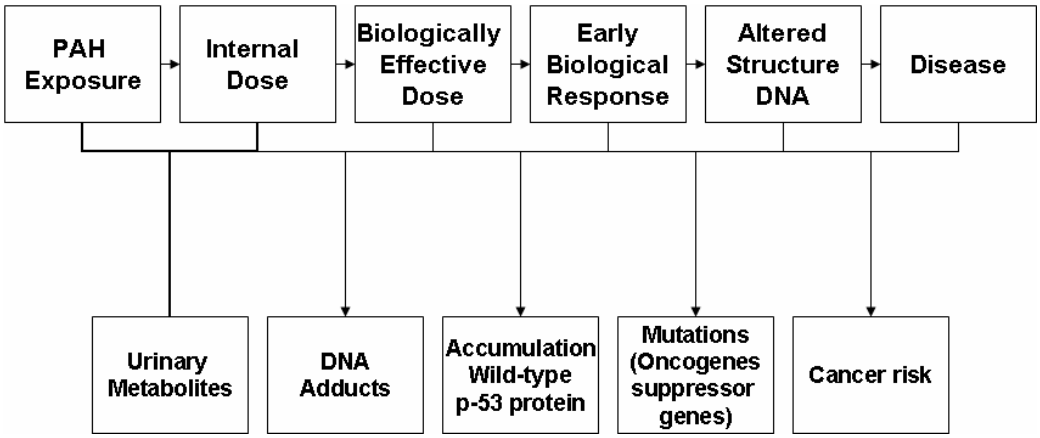

Figure 1. 1. Exposure continuum (Adapted from Godchalk et al. 2003).

1.4 Urinary Metabolites as PAH Biomarkers

The measurement of PAH metabolites in urine has been proposed as a means of assessing recent exposure to these compounds. PAH metabolites in urine may reflect a more accurate estimation of the quantity of the actual PAH intake of an individual compared to external measurements since it estimates the internal dose from exposure through several routes including both dermal contact and dietary PAH uptake (Jacob and Seidel 2002). For reliable biomonitoring of PAH metabolites in urine, the metabolites need to be present at concentrations higher than the analytical detection limit.

A number of urinary metabolites of PAHs have been investigated to assess recent exposure to these compounds. Jongeneleen et al. (1984) measured 3-hydroxybenzo[a]pyrene (3-OHBaP) in rat urine after oral administation of BaP given in different dose levels. The investigators found that the urinary concentration of 3-OHBaP was 2500-fold lower than that of 1-OHP in psoriatic patients during dermal coal tar treatment. Thus they suggested that measurement of 1-OHP in urine would be more sensitive assay to measure exposure to PAHs. The metabolism of benzo[*a*]pyrene (BaP) has been extensively studied in the literature. IARC summarized that BaP is metabolized initially by the microsomal cytochrome P-450 monooxygenase system to several arene oxides, which may rearrange spontaneously to phenols, undergo hydration to the corresponding trans- dihydrodiols, or react covalently with glutathione, either spontaneously or in a reaction catalyzed by glutathione-S-transferases. One of the phenolic metabolites, 6-hydroxybenzo[*a*]pyrene, is further oxidized to the 1,6-, 3,6-, or 6,12-quinones. The phenols, quinones, and dihydrodiols can be detoxified by conjugation to glucuronides and sulfate esters and the quinones can also form glutathione

conjugates. In addition to conjugation, the dihydrodiols undergo further oxidative metabolism. Benzo[*a*]pyrene 7,8- dihydrodiol is in part oxidized to the 7,8-diol-9,10-epoxide, a compound considered to be the ultimate carcinogenic metabolite of BaP (IARC, 1983). Hepatobiliary excretion and elimination in the feces is the primary route in which metabolites of benzo[*a*]pyrene are excreted (U.S. EPA, 1991). Two weeks following inhalation exposure to radiolabeled benzo[*a*]pyrene for 30 minutes, most of the radioactivity was recovered in the feces of rats (Sun et al., 1982). Similarly, essentially all of the radioactivity was recovered in the feces of mice that had been treated topically with radiolabeled benzo[*a*]pyrene (Heidelberger and Weiss, 1951).

Urinary 1-OHP, a major metabolite of pyrene that is excreted in urine, is one of the most commonly used biomarkers of PAH exposure among a variety of urinary PAH metabolites. Pyrene is one of the most abundant airborne PAHs present in the urban atmosphere and occupational settings. Pyrene undergoes simple metabolism to 1-OHP that can be readily measured in excreted urine. Since pyrene is typically present in the PAH-mixture regardless of source, this marker of pyrene exposure has also been regarded as a biomarker of exposure to PAHs in general. To date, a large number of papers are available reporting urinary 1-OHP concentrations in occupationally exposed workers. Elevated concentrations of 1-OHP in urine have been observed in workers occupationally exposed to PAH concentrations. (Wu et al, 1998; Mumford et al, 1995; Karahalil et al, 1998; Øvrebø et al, 1995). A selection of data is summarized in Table 1.3 These results indicate that considerable variability occurs at different worksites although a strong association was observed between urinary 1-OHP and PAH exposure.

A number of studies have also reported urinary 1-OHP concentrations in environmentally exposed individuals. (Merlo et al. 1998; Siwinska et al. 1999; Zhao et al. 1992) (Hummelen et al, 1993; Øvrebø et al, 1995). However, it is still unkown whether urinary 1-OHP can serve as a valid PAH exposure biomarker when exposure occurs at low, environmental levels. Some studies have reported an association between urinary 1-OHP and traffic-related PAH exposure in urban area (Chuang et al. 1999; Cocco et al. 2007; Fiala et al. 2001; Lai et al. 2004; Tsai et al. 2004; Zwirner-Baier and Neumann 1999) whereas others have not observed an association in the general population (Merlo et al. 1998; Sorensen et al. 2003). Diet is considered one of the confounding factors for the relationship between urinary 1-OHP and PAH inhalation exposure in general population. Although diet has been considered a major PAH source to affect baseline urinary 1-OHP, urinary 1-OHP is not believed as a reliable biomarker of dietary PAH intake because of large inter-individual variation (Buckley et al. 1995; Buratti et al. 2000; Strickland and Kang 1999; Strickland et al. 1996; Strickland and Groopman 1995; Vanrooij et al. 1994; Viau et al. 2002). Thus, identifying the relative contribution of several potential sources of variability (e.g. laboratory, exposure, or biological differences) is the focus of much of the current research in this area (Strickland and Kang 1999).

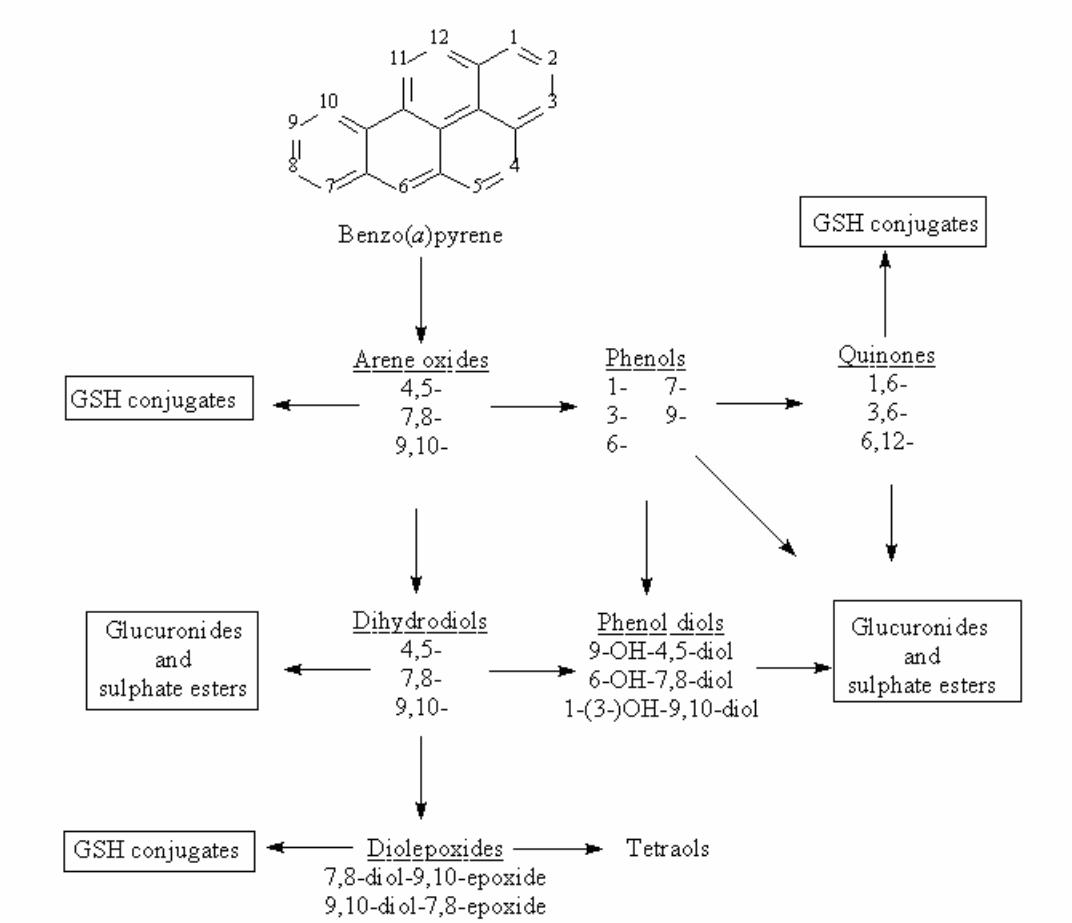

Figure 1. 2. Benzo[a]pyrene as a model of polycyclic aromatic hydrocarbons metabolism (IARC, 1983).

Table 1. 3. Urinary concentrations of 1-hydroxypyrene reported in previous studies.

Work place	Country	BaP ($\mu g/m^3$)	1-OHP ($\mu mol/mol$)	Reference
Coke plant	Finland	0.3 – 2.5	0.2-0.6	(Pyy et al. 1997)
Coke plant	China	0.89-4.3	4.0-12.0	(Pan et al. 1998)
Coke plant	Taiwan	105.1[a]	9.7±21.6	(Wu et al. 2002)
Coke plant	Poland	0.31-1.51	2.4-15.8	(Ovrebo et al. 1995b)
Coke plant	UK	0.79-2.14	1.9-2.6	(Unwin et al. 2006)
Anode plant	Europe	2.39	4.8-24.6	(Petry et al. 1996)
Coke plant	NA	NA	6.9±7.2	(Pavanello et al. 2004)
Coke plant	NA	2.77	2.5	(Marczynski et al. 2002)
Creosote	NA	NA	1.63	(Viau et al. 1995)
Paving work	Uklaine	NA	0.2-0.7	(McClean et al. 2004)
Urban	Taiwan	NA	0.26	(Cocco et al. 2007)
Urban	Taiwan	NA	0.34-0.69	(Mucha et al. 2006)
Highway toll booth	Taiwan	NA	0.91-3.02	(Tsai et al. 2004)
Bus garage	Poland	NA	0.10-0.15	(Kuusimaki et al. 2004)
Urban	Italy	42-64.5[b]	0.36-0.49	(Siwinska et al. 1999)
Traffic Police	NA	0.05-4.22[b]	0.07-0.27	(Merlo et al. 1998)
Urban	NA	NA	0.07-0.12	(Viau et al. 1995)
Urban	Czech		0.06-0.12	(Fiala et al. 2001)

[a] Benzene soluble
[b] ng/m^3
NA : Not available

1.5 PAH Biomarker Validation Study

The PAH biomarker validation study, on which this dissertation research is based, was designed to evaluate the validity of using two urinary markers of PAH exposure, 1-hydroxypyrene and 9-hydroxybenzo[*a*]pyrene, for predicting benzo[a]pyrne (BaP) exposure in the context of quantifying cancer risk.

Through collaboration with China National Environmental Monitoring Center in Beijing (CNEMC) and Anshan Environmental Monitoring Station in Anshan City, China, we recruited 100 human subjects based on the criteria as follow:

Category 1: subjects in a suburban district with typical ambient BaP level of 0.7-5.7 ng/m^3;

Category 2: subjects in an urban district with typical ambient BaP level of 20-60 ng/m^3;

Category 3: subjects working in the An-Shan Iron-Steel Factory but not directly exposed to coke oven emissions, with typical BaP level of 30-600 ng/m^3; and

Category 4: subjects working in the factory and directly exposed to coke oven emissions, with typical BaP level of 9000-154000 ng/m^3.

These subjects were recruited from An-Shan City, located in Northeast of China (Figure 1.2). The city has the largest coke plant in China, the An-Shan Iron-Steel Company. In general, the coke oven factory uses coke as the main fuel in iron-making blast furnaces. The process typically extracts iron from iron ore obtained from the destructive distillation of coking coals at temperatures of more than 1,000 degrees in

Celcius. PAHs and other toxic chemicals are generated during the process. The coke plant was built in 1918 and has 17 coke-ovens and more than 5,000 employees. Over 1,000 workers have been exposed to coke-oven emissions while working at the top, the middle, and the bottom of the oven. A significantly increased risk of lung cancer has also been reported in the workers (Pan et al, 1998). Investigators in China and CNEMC have been involved in PAH monitoring in this plant and in An-Shan City as well. Ambient BaP levels observed in urban districts of the city are typical for many polluted cities in China, but higher than typical levels measured in US and other developed countries.

The study subjects were healthy adults aged 23 to 51 years. More details on subjects' characteristics can be found in Table 2.1 of Chapter 2. Current cigarette smokers, those who have been regular smokers within the previous year, pregnant women and those with diseases and additional physical disabilities that precluded wearing a personal sampler, were not eligible for participation. Those who were suspected to have regular dermal exposure to PAHs were not eligible for participation because dermal exposure can be a significant contributor to total PAH exposure. This has been demonstrated in workers having dermal contact with used engine oil and with chimney soot (IARC, 1985).

The study consisted of two sets of repeated measurements for each subject. Each set of the measurement included: (1) 24-h personal breathing-zone concentrations of gas-phase PAHs, (2) 24-h personal breathing-zone concentrations of particle-phase PAHs, (3) 24-h dietary PAH intakes, (4) biomarker concentrations in first morning urine, and (5) biomarker concentrations in 24-h composite urine. All these measurements were made within the same 24-h period. For each subject, two repeated measurements were

separated by at least three months, maximizing the representativeness of everyday exposure within the scope and budget limit of the proposed study.

Personal air sampling was achieved through the use of a sampling pump. Collected samples were separately extracted for gaseous and particle PAH. Dietary sampling was conducted by a "Duplicate Diet" method that required participants to set aside the same amount of their meals and snacks. All food items taken within the 24-h monitoring period were sampled. Participants were asked to keep a daily diary of the food consumed including approximate amounts and method of preparation. For urine samples, a first morning void and all urine voids within the 24-hour monitoring period were collected for each subject. The subjects were provided with opaque plastic containers sufficient for collecting all urine voids.

My role in the biomarker validation study included: (1) preparation of air, food, and urine samples for chemical analysis; (2) analytical analysis of personal air and food PAH by HPLC with fluorescence detection; (3) constructing the study database, and data linkage between PAH/ biomarker and questionnaire with the help of Dr. Lin Zhang, Dr. Weili Liu, and Ms. Chen Zhang; (4) and preparing graphs and tables for the final report that was submitted to the funding agency-National Cancer Institute. Although the biomarker validation study produced an extensive database of personal PAH and polycyclic aromatic compound (PAC) exposure and 15 different PAH metabolites in urine; only personal PAH exposure and 1-OHP in urine data were used in this dissertation research.

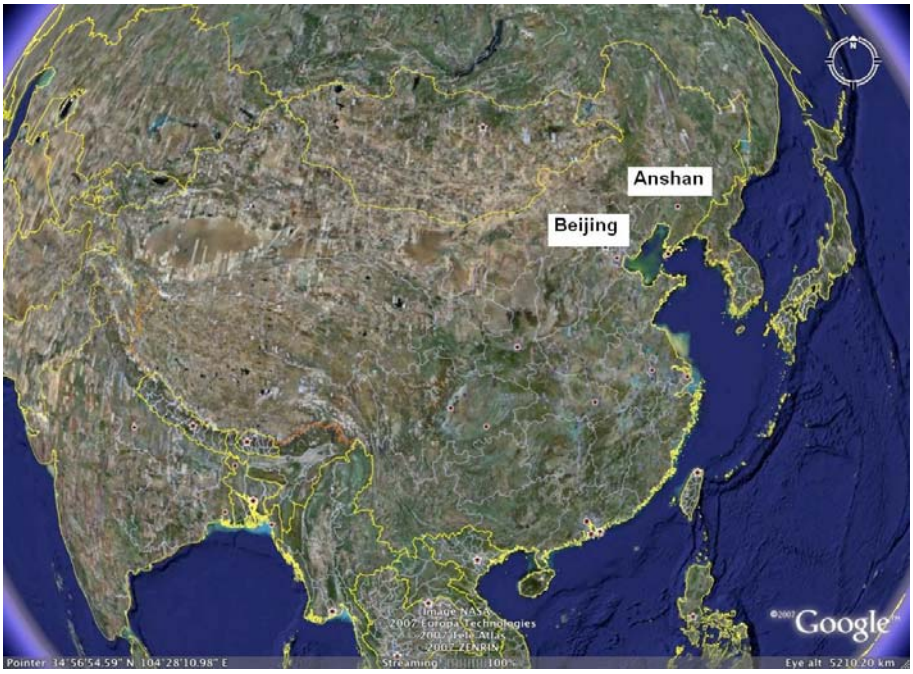

Figure 1. 3. Geographical location of Anshan city in China (source, http://www,google.com).

1.6 Dissertation Hypotheses and Specific Aims

The overall goal of this dissertation research is to examine the validity of urinary 1-OHP as a biomarker of PAH for coke production workers and non-coke oven workers. To achieve the overall goal, differences of 1-OHP levels in first morning urine and 24-hour composite voids will be examined to evaluate inter and intra-individual comparison (Aim 1); The usability of urinary 1-OHP will be examined in predicting personal air pyrene/BaP concentrations (Aim 2); and seasonal variability and between- and within-person variances will be investigated to evaluate the temporal reproducibility of urinary 1-OHP measurements (Aim 3).

Aim 1. In studies investigating PAH exposures in occupational or environmental settings, both 24-hour and spot (often first morning) void urine samples have been used to measure daily urinary 1-OHP concentrations. A number of studies have used spot urine samples to estimate occupational and environmental exposures to PAHs (Bouchard et al. 2001; Fiala et al. 2001). In previous studies concerning occupational exposure, spot urine samples were collected to examine differences between before and after a work shift (Kim et al. 2005; Unwin et al. 2006; Wu et al. 1998a). It seems reasonable to compare urine samples collected before and after a work shift because the half-life of 1-OHP in urine ranges from 4-35 hour. Conveniently, first morning urine samples were often used to assess PAH exposures in environmental settings (Chuang et al. 1999; Motykiewicz et al. 1998; Mucha et al. 2006). However, it would be ideal to use 24-hour urine void samples to estimate daily representative PAH exposure. Hence, it still remains

unknown as to how well the first morning urinary 1-OHP concentrations can reflect the daily average 1-OHP concentrations.

Hypothesis 1: 1-OHP concentrations in first morning urine samples are not significantly different from 1-OHP concentrations in 24 hour urine samples.

In Chapter 2, I will present the results from 1-OHP measurements in first morning urine and in 24-hour urine voids from 50 coke oven workers and 50 non-coke oven workers in Anshan city, China. I will compare urinary 1-OHP concentration differences between first morning urine and 24-hour urine voids within the same subject. I will examine association of urinary 1-OHP between first morning urine and 24-hour urine voids within wide range of PAH exposure.

Aim 2. Since epidemiologic studies of cancer mortality related to average urinary marker concentration are insufficient, and the current risk assessment frame work is based up on air concentrations, it is necessary to "translate" urinary marker concentrations to air concentrations also known as reverse dosimetry. A number of studies have found a linear relationship between urinary 1-OHP concentration and occupational PAH exposure, however, no significant linear relationship was found between urinary 1-OHP concentrations and environmental PAH exposure. Under this aim, quantitative relationships will be examined between urinary 1-OHP concentrations and PAH inhalation exposure with a wide range of personal air concentrations. The following hypothesis will be tested.

Hypothesis 2: Urinary 1-OHP concentrations are correlated with pyrene and BaP exposure estimated as personal air measurement concentrations in occupational and environmental settings.

In Chapter 3, I will evaluate relationships between personal air and dietary pyrene/BaP exposure and urinary 1-OHP concentrations. I will estimate "threshold" personal air PAH concentration above which urinary 1-OHP may be used to predict personal air PAH concentration.

Aim 3. For a biomarker to be useful for estimating cancer risk, this marker should reflect people's average (steady) exposures over time. To test the reliability of urinary 1-OHP as a biomarker of PAH exposure, the following hypothesis will be tested.

Hypothesis 3: Within-person variation in urinary 1-OHP concentrations over a few months is small relative to between-person variation in occupational and environmental settings.

In Chapter 4, I will evaluate the relationship between measurements made in a winter and a summer for each of the study subjects. I will estimate the reproducibility of repeated samples for personal air pyrene, dietary pyrene intake, and urinary 1-OHP using variance component models.

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Chapter 2

1-Hydroxypyrene Concentrations in First Morning Voids and 24-hour Composite Urine: Intra- and Inter-person Comparisons*

2.1 Abstract

Urinary 1-hydroxypyrene (1-OHP) has been suggested as an exposure biomarker for polycyclic aromatic hydrocarbons (PAHs). However, it remains unknown whether a first morning urine sample can be used to reflect average exposure. In this paper, we examine intra-individual differences and inter-individual associations between first morning voids and 24-hour composite urine samples. The analysis was performed using data collected from 100 adults who had a wide range of PAH exposure because of differences in their occupation, e.g.., coke oven workers vs. non-coke-oven workers. For each subject, all the urine voids within each of two 24-hour measurement periods were collected. Results showed a significant (40% to 62%) intra-individual difference between first morning voids and 24-hour urinary 1-OHP concentrations (in ng per ml urine). Creatinine adjustments of 1-OHP concentrations (in μmol per mol urinary creatinine) reduced the intra-individual difference by approximately 10%. Across all the subjects, a high overall correlation (r=0.76) was observed between first morning and 24-hour average 1-OHP concentrations. Work environment and sampling season were found to significantly affect the relationship between first morning and 24-hour 1-OHP concentrations. An increase of 1 ng/mL of first morning urinary 1-OHP predicted an

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* *This chapter has been accepted by Journal of Exposure Science and Environmental Epidemiology for publication.*

increase of 0.5 ng/mL and 0.25 ng/mL of 24-hour urinary 1-OHP for coke oven workers and non-coke oven workers, respectively. Data collected in a winter season showed a higher correlation between first morning and 24-hour concentrations than data collected in a fall season. Creatinine adjustments did not significantly improve overall correlations between concentrations of 1-OHP in first morning void and 24-hour urines but increased total variances for concentration of 1-OHP in 24-hour urines explained by first morning urinary 1-OHP levels in coke oven workers.

2.2 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are present ubiquitously in the environment. Due to their adverse health effects including carcinogenicity, it is important to assess PAH exposures in both occupational and environmental settings. The use of biomarkers, especially noninvasive biomarkers such as urinary metabolites, can be effective means of exposure assessment. Among various potential urinary biomarkers , 1-hydroxypyrene (1-OHP), a metabolite of pyrene, has been considered an appropriate biomarker for exposures to PAH (Angerer et al. 1997a; Jongeneelen 1994; Jongeneelen 2001; Keimig et al. 1983; Serdar et al. 2003). Previous studies indicated that pyrene was rapidly distributed, metabolized, and eliminated from the body and 1-OHP in urine represented a constant fraction (2%) of total pyrene intake in urine and faeces (Bouchard et al. 1998). The half life of urinary 1-OHP excretion ranges from 4 to 35 hours (Boogaard and van Sittert 1994; Jongeneelen et al. 1990) and the excretion declines to near baseline concentrations within 48 hours following an exposure event (Buckley and Lioy 1992). Thus, urinary 1-OHP may be used to assess individuals' recent PAH exposure. In addition, the potential exists that chronic exposure to PAHs (e.g., via meat consumption, smoke inhalation) may be deposited in adipose tissues without being metabolized immediately and may allow for a low-level steady state excretion (Madhavan and Naidu 1995; Somogyi and Beck 1993).

In studies investigating PAH exposures in occupational or environmental settings, both 24-hour and spot (often first morning) void urine samples have been used to measure daily urinary 1-OHP concentrations. In theory, data from 24-hour composite urine samples may represent daily PAH exposure more reliably than data derived from

spot urine samples. However, compliance of 24-hour urine collection may be difficult to obtain because of forgetfulness, inconvenience, misplacement of samples, lack of container capacity, contamination during the storage, or loss of urine (Garde et al. 2004). In contrast, collecting spot urine samples is substantially more convenient. Hence, a number of studies have used first morning urine samples to estimate occupational and environmental exposures to PAHs (Bouchard et al. 2001; Fiala et al. 2001). In previous studies concerning occupational exposure, spot urine samples were collected to examine differences between before and after a work shift (Kim et al. 2005; Unwin et al. 2006; Wu et al. 1998a). First morning urine samples were often used to assess PAH exposures in environmental settings (Chuang et al. 1999; Motykiewicz et al. 1998; Mucha et al. 2006). However, it still remains unknown as to how well the first morning urinary 1-OHP concentrations can reflect the daily average 1-OHP concentrations.

Concentrations of 1-OHP in both first morning and 24-hour composite urine samples may also be influenced by a number of factors including urine output rate or dilution (Hinwood et al. 2002). Since there can be large variations in urine volume output rate both across individuals and within a given individual during a day, various adjustment techniques for urinary 1-OHP concentrations have been proposed to account for the dilution factors and to obtain more reliable estimates of exposure to PAH (Viau et al. 2004a). Among those, creatinine adjustment has been commonly used to correct urine dilution by dividing 1-OHP concentrations by urinary creatinine concentrations using similar concentration units. Creatinine excretion, however, also varies with many factors such as intake of meat, diurnal variation, age, sex, and other factors (Barr et al. 2005;

Boeninger et al. 1993). The creatinine adjustment method, therefore, is still controversial as to whether it actually reduces the measurement variability because of urine dilution.

In this paper, we examine intra-individual differences and inter-individual associations between first morning and 24-hour urinary 1-hydroxypyrene (1-OHP) concentrations with 100 subjects who had a wide range of PAH exposure from different occupations (coke oven workers vs. non-coke oven workers). Seasonal effect is investigated on intra and inter-individual associations between the first morning and 24-hour urine voids. We also examine whether creatinine adjustment can reduce the inter- and/or intra-individual differences between the first morning urine and 24-hour composite urine measurements.

2.3 Methods

2.3.1 Study participants

Data used for the present analysis were collected as part of a larger exposure biomarker study conducted in An-Shan, China, where a coke plant was built in 1918 with 17 coke-ovens and more than 5,000 employees. High levels of PAHs, regularly monitored as benzo[a]pyrene (BaP) in the air, have been found with varying concentrations at different locations of the plant. Typical BaP air levels ranged from 9 - 154 μg/m3 based on historical data. Over 1,000 workers have been exposed to coke-oven emissions while working at the top, the middle, and the bottom of the oven (Pan et al. 1998). During 2002-2003, 50 non-smoking coke oven workers at various job sites from this factory were recruited to participate in the exposure biomarker study. Another 50

non-smoking adults were recruited through advertisement in several community activity centers. These 50 subjects were urban residents who were not directly exposed to coke emissions. Subjects at each group were not matched by age and sex but within the defined age range for both coke-oven workers and non-workers (see below).

All potential subjects were interviewed to ascertain their eligibility for the study; and each of them was administered a screening questionnaire. The exclusion criteria include the following: younger than 20 years or older than 55 years, having routine dermal contact with engine oil or chimney soot, having been a smoker during the past 12 months, being pregnant, having conditions or physical disabilities that prevented them from wearing a personal sampler weighing ~2 kg . Those who met the recruitment criteria were thoroughly explained the study protocol and any potential risk of participation, and were allowed to ask and have answered any questions they might have before they signed informed consent documents. Institutional Review Board (IRB) approval was obtained before the commencement of the subject recruitment and renewed approval was maintained throughout the entire course of the study including the data analysis phase. All subjects who participated in the study provided written, informed consent. Table 2.1 summarizes the demographic characteristics of the study participants by occupational group (coke oven workers vs. non-coke oven workers). There were no statistical significant age differences between coke-oven workers and non-coke oven workers ($p=0.498$). The subjects ranged in age from 23 to 51 years of age and the average age was 36 years. The coke oven workers were heavier and taller than the non-coke oven workers ($p<0.001$). The mean body mass index (BMI) for the coke oven workers was higher than that for the non-coke oven workers ($p<0.001$).

2.3.2 Sample Collection and Analysis

The urine collection and measurement protocol consisted of two sets of repeated measurements for each subject. Each set of the measurements included: (1) 1-OHP concentrations in the first morning urine, and (2) 1-OHP concentrations in a 24-hour composite urine. This protocol required subjects to work as usual during a 24-hour sampling period. Each participant was asked to collect all urine voids (with entire volume) within the 24-hour monitoring period until the next morning void using opaque plastic bottles provided along with a container (cooler with dry ice).

In the laboratory, the first morning urine bottle was separated from the bottles containing all the other urine voids. Except the first morning urine, all of the remaining urine samples were mixed to obtain a 24-hour composite urine sample. When a participant worked a night-shift, a worker's first morning urine was actually collected in the late morning or early afternoon, but it was still the first urine void after they awakened. Each subject's urinary 1-OHP was measured in winter and in the summer or fall to maximize the representation of everyday exposure and to determine if seasonal variation existed.

All urine samples were stored at -20°C and protected from light in 500-ml amber glass containers to prevent decomposition of hydroxyl metabolites of PAHs. For sample analysis, stored samples were slowly thawed and mixed in the laboratory. A small aliquot (~ 1ml) was used for creatinine measurements using a standard assay kit (Sigma Chemicals, St. Louis, MO). Briefly, the thawed urine samples were diluted approximately 40- fold with water. The 0.5mL of diluted urine samples were transferred into 5mL cuvet.

3mL of alkaline picrate solution (Sigma-Aldrich; Picric acid, approximately 0.6%, sodium borate and surfactant) was added to each cuvet. Each cuvet was covered with a lid, mixed well, and allowed to sit for 10-12 minutes. The cuvets were inserted into the autosampler of the spectrophotometer (Genesys 10, Thermo Fisher Scientific, Inc., Waltham, MA) set at 500 nm of wavelength. The absorbance of the sample was recorded and the concentrations were calculated using standard calibration plots. As expected, the creatinine concentrations in the first morning urine samples in each group were higher than 24-hour urine samples. For coke oven workers, median of creatinine concentration was 103 mg/dL and 93.3 mg/dL for first morning urines and 24-hour urine samples, respectively. For non-coke oven workers, median of creatinine concentration was 100 mg/dL for first morning urine and 93.3 mg/dL for 24-hour urine samples. The comparison between the morning urines and 24-hour voids was marginal or nominally significant for coke oven workers ($p=0.07$) and for non-coke oven workers ($p=0.08$), respectively. In addition, no significant differences in urinary creatinine concentrations were found between the two occupational groups in either the first morning urine samples ($p=0.36$) or the 24-hour urine samples ($p=0.74$) (Table 2.1).

Another 10mL aliquot of urine sample was aliquoted from the urine sample for the preparation of urinary hydroxylated PAHs. Then the urine was adjusted to a pH=5 with 1N hydrochloric acid and 0.1 M acetate buffer (pH=5) was added to a final volume of 30 mL. This mixture was incubated overnight (16 hr) with 20 µl of glucoronidase-arylsulphatase (114400 β-Glucuronidase units/ml and 3290 Sulfatase units/ml, Sigma-Aldrich CO.) at 37°C in an electronically controlled rotary shaking bath. After the enzymatic hydrolysis, a sample enrichment and purification cartridge, packed with C18

reversed-phase liquid chromatographic material (Sep-Pak C18 cartridge, Waters) was used for sample extraction. The cartridge was first primed with 5 mL of methanol followed by 10 mL distilled water. The treated urine was filtered, then passed through the cartridge at a flow rate of 1 mL/min. After the cartridge was washed with 3 ml distilled water and 3 ml of 50% methanol in water, the final elution was performed with 10 ml methanol. The eluate was further concentrated by evaporating the methanol solution down to 1 mL; and the vials were wrapped with aluminum foil and stored in a freezer at -7°C or lower. Sample analysis was preformed using an HPCL-fluorescence technique as described below. A Waters HPLC system (600E) and a Waters 470 fluorescence detector with a LC-18 Supelco Sil 250×4.6 mm column (Supelco, Bellefonte, PA) were used in the analysis. The HPLC analysis was performed in 25 min using a mixture of methanol and water (71%:29% by volume) as the isocratic mobile phase. Excitation and emission wavelengths for the fluorescence detector were 242 nm and 388 nm, respectively. The method detection limit of 1-OHP was 0.02 ng/mL. Extraction recovery of urinary 1-OHP was 74.1% at 2.5 ng/ml of urine. Repeated analyses of same samples showed an average relative standard deviation (RSD) of 8.3%.

2.3.3 Statistical Analysis

The effects of work environment, seasonal variation on the association between the first morning and 24-hour urinary 1-OHP concentrations were examined. Work environment was stratified as coke factory and non-coke factory. The sampling periods were used to study potential seasonal variation. The sampling periods were winter (December, 2002) and summer/fall (August to October, 2003).

Normality tests for 1-OHP concentrations in both the first morning urines and 24-hour composite urines were performed using the Shapiro-Wilk test. Descriptive statistics were performed to calculate geometric means (GM) and 95% confidence intervals (CI) for urinary 1-OHP concentrations, because the concentration data did not follow a normal distribution. We defined intra-individual difference of 1-OHP concentrations in urine as the difference between the first morning and 24-hour urine measurement for each subject within a day. Whether the intra-individual difference was significantly different from zero was tested using a Wilcoxon sign rank test. We also calculated the RSD between the first morning urinary 1-OHP concentrations and 24-hour urinary 1-OHP concentrations among individuals.

Spearman rank correlation coefficients were used to determine the strength of the association between the first morning and 24-hour urinary 1-OHP concentrations. A linear regression model was used to determine whether the first morning urinary 1-OHP concentrations can predict 24-hour urinary 1-OHP concentrations. Both the first morning and 24-hour urinary 1-OHP concentrations were log-transformed to improve the variance because the data were not normally distributed. In the initial model, the dependent variable was log-transformed 24-hour urinary 1-OHP concentration; the independent variable was first morning urinary 1-OHP concentration. Analyses were performed separately by work environment because of unequal variances for coke oven workers and non workers. Sampling period (season) and sex were used as independent variables and an interaction term was included in the regression model. Age and BMI were used as covariates in the regression model. For example, the linear regression model investigating the effect of season on the association between log-transformed 24-hour urinary 1-OHP

concentrations and log-transformed first morning urinary 1-OHP concentrations was the following:

$$\text{Log}\,[1\text{-OHP}_{24}] = \beta_0 + \beta_1\,\text{Log}[1\text{-OHP}_M] + \beta_2\,(\text{season})$$
$$+ \beta_3\;\text{Log}[1\text{-OHP}_M] \times (\text{season}) + \text{covariates} + \varepsilon$$

The level of statistical significance for all analyses was determined at $\alpha = 0.05$. We also constructed linear regression models with original data. The log-transformed model results were compared to the results using non log-transformed original data. We reported results using original data because there were no differences for estimation of regression coefficients and variance explanation (r^2) compared to the results from the log-transformed analysis

2.4 Results

2.4.1 Summary of the data

One hundred pairs of the first morning urine and 24-hour urine voids were collected during each sampling season. All values were included in the analysis of data distribution. Urinary 1-OHP concentrations for both sample collection type are summarized in Table 2.2. The data distributions were positively skewed. The medians of the first morning urinary 1-OHP concentrations and 24-hour urinary 1-OHP concentrations were 0.85 ng/mL and 0.77 ng/mL, respectively. The medians of first morning urinary 1-OHP concentrations were always higher than 24-hour urinary 1-OHP concentrations regardless of work environment and sampling periods.

We also used creatinine data to check the validity of the first morning urine samples. According to the WHO guidelines for urine sampling in occupational monitoring, if a spot urine sample has creatinine concentration > 300 mg/dL or < 30 mg/dL it should be considered invalid or should be resampled (WHO 1996). Seven first morning urine samples were out of this range and thus were excluded from data analysis. These excluded samples included 4 non-coke oven workers (all <30 mg/dL) and 3 coke oven workers (all > 300 mg/dL). The fraction of invalid urine samples in total samples was 3.5 %.

2.4.2 Comparison of urinary 1-OHP concentration

Table 2.3 summarizes results from a comparison between the first morning and 24-hour urinary 1-OHP concentrations. Significant intra-individual differences were found at the individual level using the pooled data from all the subjects (p=0.008). The median difference between paired 1-OHP concentrations was 0.76 ng/mL and the first morning urinary 1-OHP concentrations were 51% higher than 24-hour urinary 1-OHP concentrations. Among 193 paired data, 126 (65%) had higher 1-OHP concentrations (ng/mL) in the first morning urine voids than the 24-hour composite urine samples. Stratified analyses indicated a significant intra-individual difference by work environments. In the coke oven workers, intra-individual differences in median urinary 1-OHP concentrations were also significant (median difference = 1.56 ng/ml or 48 %, p<0.001), with 64 out of 97 paired samples (66%), had higher first morning urinary 1-OHP concentrations than 24-hour urinary 1-OHP concentrations. In the non-coke oven workers, the median difference of 1-OHP concentration between two sample types were -

0.04 ng/mL and the first morning urinary 1-OHP concentration was 54% higher than 24-hour urinary 1-OHP concentration. Sixty-two out of 96 paired samples (65%) had higher 1-OHP concentrations in the first morning urine voids than 24-hour urine samples. A seasonal effect was observed for the intra-individual difference between the first morning and 24-hour urinary 1-OHP concentrations. The samples collected in the winter did not show statistically significant intra-individual difference between the sample types although the first morning urinary 1-OHP concentrations were higher than paired 24-hour urinary 1-OHP concentrations (median = 0.92 ng/mL or 40%) . For the summer/fall season, first morning urinary 1-OHP concentrations were higher than 24-hour urinary 1-OHP concentrations (median = 0.60 ng/mL or 62%).

Results showed that creatinine adjustment reduced the intra-individual difference from 51% to 42% for the pooled data (all subjects), although the difference remained statistically significant between creatinine-adjusted first morning and 24-hour urinary 1-OHP concentrations. The creatinine adjustment reduced the intra-individual difference from 48% to 38% in coke-workers and from 54% to 46% in non-coke oven workers. The adjustment reduced the intra-individual difference from 40% to 30% and from 62% to 55% for the winter and the summer/fall seasons, respectively (see Table 2.3).

We also analyzed the degree of agreement between the first morning and 24-hour urinary 1-OHP using a Bland-Altman plot (Figure 2.1). The plot showed a high degree of agreement with 96% of samples within the limits of agreement as determined by 2 standard deviations (SD). In total, 8 out of 193 data points were outside of ± 2SD. Three data points for the non-coke oven workers were outside this agreement range, with two data points showing higher concentrations in 24-hour urines. Five coke oven workers

were outside the agreement range, four of whom had 1-OHP concentrations higher in the first morning urines.

2.4.3 Linear Regression Analyses

The Spearman correlation matrix shows a strong correlation between first-morning and 24-hour average 1-OHP concentrations using the pooled data (r= 0.76, 95% CI= 0.70~0.82) (Figure 2.2). Table 2.4 shows results from the linear regression models evaluating the predictability of first-morning urinary 1-OHP concentration for 24-hour average concentration.

Work environment and seasonal effects were observed in the relationship between the first morning and 24-hour urinary 1-OHP concentrations. Among the 50 coke oven workers, 22% of the total variance for 24-hour 1-OHP concentration was explained by first morning urine. The linear regression model resulted in a significant regression coefficient of 0.48 (95% CI: 0.28~0.35). When examining the effect of season on the association between 24-hour and first morning 1-OHP concentrations, we found that total variance explanation by first morning urine in winter was higher than that in summer. The effect of sex was not found to influence the relationship between 1-OHP in first morning and 24-hour urines.

In the non-coke oven workers, first morning urinary 1-OHP concentrations only contributed 7% of the total variance for 24-hour urinary 1-OHP concentrations. The regression coefficient for 1-OHP in 24-hour urine regressed on 1-OHP in first morning urine was 0.36 (95% CI: 0.09~0.62). The concentration of 1-OHP in the first morning urine in winter was only a significant predictor for the concentration of 1-OHP in the 24-

hour samples (Table 2.4). Sex was not a significant effect on the association between 1-OHP concentration between first morning and 24-hour urine voids.

For inter-individual comparisons, regression coefficients using creatinine-adjusted concentrations were not significantly different from those using the unadjusted concentration data. However, total variances for 24-hr urine explained by the first morning urine were increased by approximately 16% in coke oven workers (Table 2.4). In non-coke oven workers, total variance for creatinine adjusted 24-hour urine was not improved compared with creatinine unadjusted regression models.

2.5 Discussion

In the present analysis, we observed an intra-individual difference between 1-OHP concentration in first morning urine and 1-OHP concentration in 24-hour urine voids for coke oven workers and non-coke oven workers. First morning urinary 1-OHP concentrations were 51% and 42% higher than 24-hour urinary 1-OHP concentrations for unadjusted and creatinine-adjusted concentrations, respectively. This was consistent with the general thought that urine from a first morning void is more concentrated (Que Hee 1993). We could not find previous work addressing intra-individual differences between first-morning and 24-hour average urinary concentrations for 1-OHP, but there appear to be published studies reporting intra-individual concentration differences for other urinary metabolites within a day. Kissel et al (2005) reported that the percent deviations from weighted daily average urines ranged from 37% to 63% for metabolites of various organophosphorus pesticides in first morning urine samples. Scher et al (2006) examined the ratio between first morning voids and 24-hour urine samples for chlorpyrifos from

farmers and their children. The authors reported that geometric mean of the ratio ranged from 1.1 to 1.7.

Previous measurements of coke oven workers showed that intra-individual variations varied from 14% to 41% using 24-hour urine samples during 4 consecutive days (Grimmer et al. 1993). Obrevo et al (1995c) studied intra-individual variations in aluminum workers during 2.5 years. The authors found that more than half of the workers had CV < 50%. In our study, we found that two thirds of subjects had CV < 50%. Siwinska (1998) measured the first morning urinary 1-OHP concentrations during 6 consecutive days. They reported more than half of the subjects had CV> 50% for intra-individual variations.

We found a positive association between first morning and 24-hour urinary 1-OHP concentrations. Urinary 1-OHP concentration of 1 ng/mL in the first morning void was equivalent to 0.5 ng/mL in the 24-hour composite urine. First morning urines explained 22% and 7% of total variances in 24-hour urine voids for coke oven workers and non-coke oven workers, respectively. Although preceding inhalation exposure in work place has a positive effect on urinary 1-OHP (McClean et al. 2004), it is likely that these two specimens did not reflect the same exposure scenario. The first morning urines mainly represents the metabolism that has taken place during the night while the 24-hour composite urine also includes urine collected in closer vicinity to the exposure known to have occurred.

Associations in winter for both coke oven workers and non-coke oven workers were stronger than that in summer; and the predictability of first-morning 1-OHP concentration for 24-hour average 1-OHP concentration was not demonstrated in summer

for non-coke oven worker subjects. The lack of association may be attributable to several factors. One possible explanation is the relatively narrow range of 1-OHP concentrations across these subjects. The narrow ranges of urinary 1-OHP concentration for both types of urine samples may prevent a meaningful analysis of the association. Another explanation is that no linear association may exist at concentrations that are close to "background" values. Sex was not a significant predictor between first morning urine and 24-hour composite urine. The fact may relate to high variability in 1-OHP excretion rate among female compared to male. In addition, the relatively small number of subjects evaluated or the statistical methods used may account for the inability to detect significant effect of sex in urinary 1-OHP levels in present study.

Urinary creatinine concentrations were commonly used to adjust dilution in spot urine samples and to determine whether a spot urine sample is valid. Ninety seven percent of collected urine samples were valid in this study. The portion of invalid samples was small comparing to a previous study that the rate of outside the criteria range was approximately 13%, measured in mostly workers (Viau et al. 2004a).

The use of creatinine adjustment underlies the assumption that creatinine excretion is proportional to the excretion of an analyte (e.g., 1-OHP) at corresponding time intervals. However, it has been argued because of intra- and inter-individual variations in the excretion of creatinine (Boeninger et al. 1993; Hines et al. 2003). In addition, creatinine adjustment for urinary 1-OHP concentration were controversial since some studies showed little effect of creatinine adjustment on the relationship between airborne pyrene and urinary 1-OHP (Kuljukka et al. 1997; Levin et al. 1995). Other researchers have also reported large variation in creatinine excretion in people (Barr et al.

2005; Kissel et al. 2005). The results showed that creatinine adjustment reduced variability of 1-OHP concentrations between the first morning and 24-hour urine voids (7% to 10% decrease in intra-individual difference). The findings in this study may be explained by the parallelism between the urinary excretion of 1-OHP and creatinine, as suggested by Viaul et al (2004a) that creatinine adjustment may be useful when the metabolite urinary excretion kinetics parallels that of creatinine.

The effect of creatinine adjustment did not appear to significantly improve correlations between first morning and 24-hour 1-OHP concentrations across all the subjects of this study. Hinwood et al (2002) investigated the effect of creatinine adjustment for urinary inorganic arsenic concentrations. They concluded that there was little benefit in adjusting with creatinine for urinary inorganic arsenic concentrations. The correlation coefficient for unadjusted spot and unadjusted 24-hour urinary inorganic arsenic concentrations was 0.65 but that for adjusted spot and adjusted 24-hour urinary inorganic arsenic concentrations was 0.64. Results from our study supported their findings that creatinine adjustment did not significantly improve the degree of agreement, or the correlation between 1-OHP in first morning urines and in 24-hour urines. However, the adjustment appeared to reduce deviations between first morning measurements and 24-hour average concentrations. In addition, we found that creatinine adjustments increased total variances for 24-hr urines explained by first morning urines in coke oven workers.

The data are limited to first morning urine and 24-hour composite urine. We did not measure 1-OHP concentrations in multiple spot urine samples within a day and during several days. No concentration data were available on the individual urine

samples that were combined to form the 24-hour void specimen. Thus, the variability and creatinine adjustment effect among repeated spot urine samples are unknown and could not be examined with the data from the present study.

In conclusion, first morning urine 1-OHP was substantially correlated with 24-hour urinary 1-OHP and appears to discriminate occupationally exposed workers from those not so exposed. Creatinine adjustments of 1-OHP concentrations reduced the intra-individual difference between first morning and 24-hour composite urine measurements by approximately 10% but did not significantly improve overall correlations between the two measurements.

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Table 2. 1. Characteristics of subjects by occupational group.

	Mean(SD)		
	Non-coke oven workers	Coke workers	*p* value[c]
Number of subjects	50	50	
Female	26	9	
Age (yr)	36.2 (7.2)	35.6 (8.1)	0.498
Height (cm)	165.9 (6.9)	170.3 (7.3)	<0.001
Weight (kg)	62.1 (10.9)	70.0 (12.6)	<0.001
BMI	22.4 (2.7)	24.0 (3.4)	<0.001
Creatinine (mg/dL)[a]			
First morning urines	100 (1.70)	103 (1.74)	0.362
24-hour urines	93.3 (1.51)	93.3 (1.58)	0.738
p value [b]	0.069	0.079	

[a] For each subject, two repeated measurements were used to calculate the overall mean. Geometric means (GM) and geometric standard deviations (GSD) were calculated for creatinine concentrations.

[b] Creatinine difference between first morning and 24-hour urine in non-coke oven workers and coke oven workers were tested using Wilcoxon singed rank test.

[c] Creatinine difference between non-coke oven workers and coke oven workers were tested using Wilcoxon-Mann-Whitney tests.

Table 2. 2. Weighted quantiles (95% Confidence intervals) of urinary 1-hydroxypyrene concentrations for the study participants (2002-2003).

	First morning urinary 1-OHP (ng/mL)					24-hour urinary 1-OHP (ng/mL)				
	No.	GM (95% CI)	Percentiles			No.	GM (95% CI)	Percentiles		
			10^{th} (95% CI)	50^{th} (95% CI)	90^{th} (95% CI)			10^{th} (95% CI)	50^{th} (95% CI)	90^{th} (95% CI)
All	200	1.01 (0.78-1.30)	0.16 (0.07-0.20)	0.85 (0.78-1.30)	10.85 (7.07-14.09)	200	0.74 (0.57-0.97)	0.10 (0.05-0.10)	0.77 (0.57-0.97)	7.28 (5.47-11.09)
Non-coke oven workers	100	0.30 (0.24-0.39)	0.10 (0.05-0.10)	0.31 (0.24-0.39)	0.80 (0.62-1.98)	100	0.22 (0.16-0.30)	0.06 (0.02-0.09)	0.24 (0.16-0.30)	0.85 (0.77-2.73)
Coke workers	100	3.16 (2.35-4.24)	0.69 (0.32-0.70)	3.37 (2.35-4.24)	16.71 (11.39-31.49)	100	2.40 (1.92-2.99)	0.57 (0.43-0.78)	2.51 (1.92-2.99)	9.41 (7.40-13.47)
Winter	100	1.09 (0.77-1.54)	0.14 (0.07-0.18)	0.81 (0.77-1.54)	11.70 (6.65-16.92)	100	0.85 (0.61-1.17)	0.12 (0.06-0.16)	0.70 (0.61-1.17)	7.70 (4.61-11.09)
Non-coke oven workers	50	0.28 (0.21-0.36)	0.10 (0.06-0.12)	0.27 (0.21-0.36)	0.61 (0.48-1.28)	50	0.22 (0.17-0.28)	0.09 (0.05-0.10)	0.20 (0.17-0.28)	0.67 (0.50-0.98)
Coke workers	50	4.31 (3.06-6.08)	1.03 (0.54-1.37)	5.26 (3.06-6.08)	16.78 (13.56-34.46)	50	3.23 (2.43-4.27)	0.70 (0.58-1.26)	3.10 (2.43-4.27)	11.75 (8.27-17.80)
Summer	100	0.92 (0.63-1.35)	0.17 (0.05-0.20)	0.87 (0.63-1.35)	7.25 (5.87-16.73)	100	0.64 (0.42-0.98)	0.08 (0.03-0.19)	0.79 (0.42-0.98)	4.85 (4.97-15.78)
Non-coke oven workers	50	0.34 (0.21-0.54)	0.16 (0.03-0.18)	0.36 (0.21-0.54)	1.07 (0.74-4.56)	50	0.21 (0.11-0.42)	0.01 (0.01-0.03)	0.37 (0.11-0.42)	1.01 (0.79-10.29)
Coke workers	50	2.24 (1.39-3.64)	0.57 (0.13-0.87)	2.30 (1.39-3.64)	13.61 (10.31-38.42)	50	1.74 (1.25-2.42)	0.28 (0.22-0.61)	1.98 (1.25-2.42)	8.04 (4.92-12.07)

GM = Geometric Mean
CI = Confidence interval

Table 2. 3. Intra-person difference between the first morning and 24-hour urine voids in urinary 1-OHP (ng/mL) and creatinine-adjusted 1-OHP (μmol/mol).

	Number of paired samples	$[1\text{-}OHP_M] - [1\text{-}OHP_{24}]$ (ng/mL)	$[1\text{-}OHP_M] - [1\text{-}OHP_{24}]$ (μmol/mol)
All subjects	193	0.76 (51%)**	0.24 (42%)**
Work			
Non-coke oven workers	96	-0.04 (54%)*	-0.05 (46%)
Coke workers	97	1.56 (48%)**	0.53 (38%)*
Season			
Winter	99	0.92 (40%)**	0.17 (30%)
Summer/Fall	94	0.60 (62%)*	0.32 (55%)*

Intra-individual differences in parenthesis were shown as of relative standard deviation (RSD).

** $p<0.05$; ** $p<0.01$*

Table 2. 4. Linear Regression Model Results between [1-OHP_{24}] and [1-OHP_M].

	No.	Regression Coefficient (95% CI)	r^2
Creatinine unadjusted (ng/ml)			
Coke workers**	92	0.48 (0.28, 0.53)	0.22
Season**			
Winter	50	0.51 (0.35, 0.67)	0.46
Summer	42	0.31 (0.12, 0.50)	0.17
Sex			
Female	16	0.33 (-0.12, 0.79)	0.07
Male	76	0.41 (0.28, 0.54)	0.31
Non-coke oven workers*	88	0.36 (0.09, 0.62)	0.07
Season			
Winter	48	0.52 (0.27, 0.79)	0.13
Summer	40	0.32 (-0.12, 0.75)	0.05
Sex			
Female	47	0.32 (-0.26, 0.91)	0.02
Male	41	0.36 (0.11, 0.62)	0.15
Creatinine adjusted (µmol/mol)			
Coke workers**	92	0.44 (0.32, 0.56)	0.38
Season**			
Winter	50	0.56 (0.39, 0.72)	0.64
Summer	42	0.36 (0.20, 0.53)	0.38
Sex			
Female	16	0.42 (-0.18, 1.03)	0.12
Male	76	0.44 (0.32, 0.56)	0.38
Non-coke oven workers*	88	0.37 (0.12, 0.62)	0.08
Season			
Winter	48	0.74 (0.49, 0.99)	0.24
Summer	40	0.27 (-0.14, 0.68)	0.04
Sex			
Female	47	0.34 (-0.19, 0.87)	0.03
Male	41	0.38 (0.12, 0.64)	0.16

CI = confidence interval.
* $p<0.05$; **$p<0.01$.

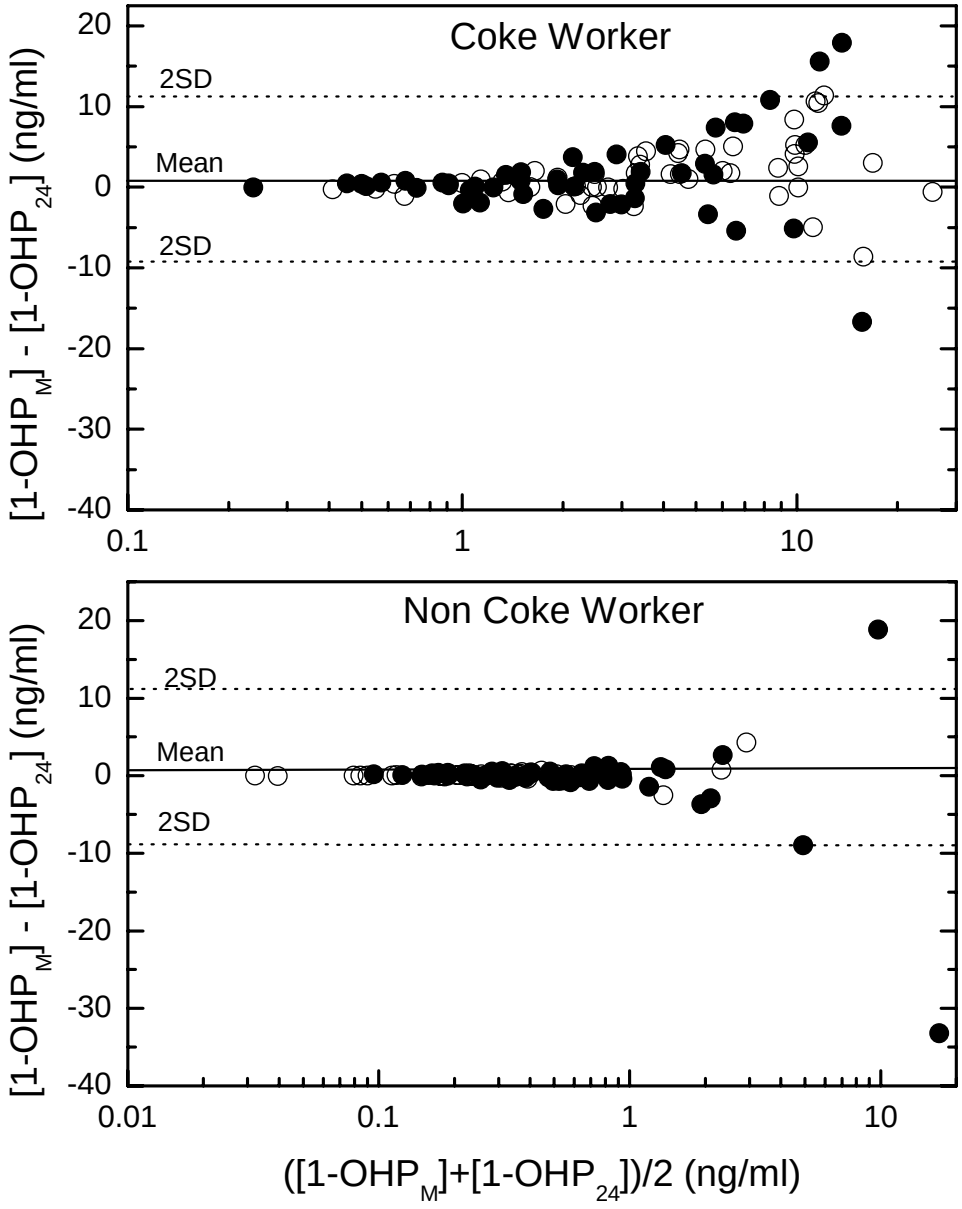

Figure 2. 1. Bland-Altman plot of difference (ng/ml) between the first morning urinary 1-OHP concentrations ([1-OHP_M]) and 24-hour urinary 1-OHP concentrations ([1-OHP_{24}]) by winter (•) and summer/fall (o) season (n=193).

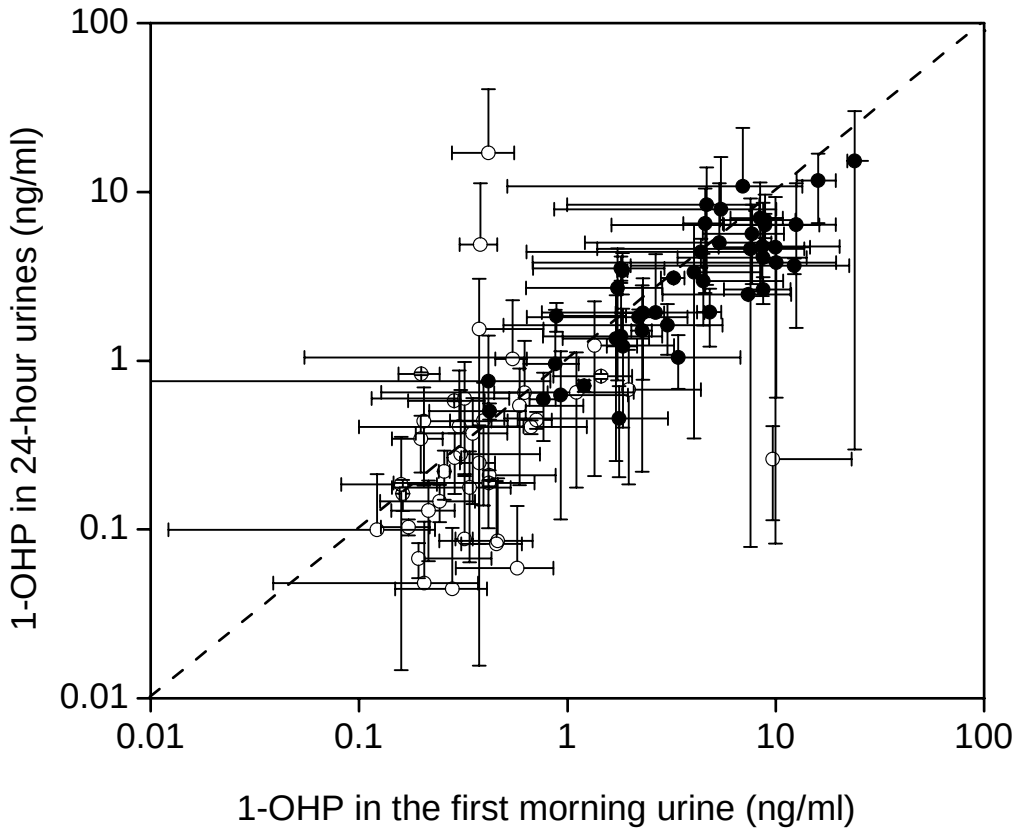

Figure 2. 2. Correlation between 1-OHP concentration in the first morning urines and in 24-hour urines, with estimated standard deviation, plotted on logarithmic scale for coke oven workers (•) and non-coke oven workers (o). The dotted line is 1:1 line (n=193).

Chapter 3

Usability of Urinary 1-Hydroxypyrene for Predicting Inhalation Exposure to Benzo[a]pyrene

3.1 Abstract

Urinary 1-hydroxypyrene (1-OHP), a metabolite of pyrene, is considered as a biomarker of exposure to pyrene in particular and PAH in general. Because benzo[*a*]pyrene (BaP) is generally measured for PAH health-risk assessment, it is necessary to find a biomarker for predicting BaP exposure. The association between urinary 1-OHP and pyrene/BaP inhalation exposure is well known in occupational settings with high BaP exposures but is less known under typical environmental conditions. It remains unknown whether there is a concentration range within which urinary 1-OHP can serve as an effective biomarker. The study focused on 100 non-smoking adults who had a wide range of PAH exposure, including 50 coke oven workers and 50 non-coke oven workers. 1-OHP concentrations of 24-hour composite urine samples were related to 24-hour average concentrations of pyrene and BaP, measured in the breathing zone, after controlling dietary intake of pyrene and BaP. Results suggest an exposure-response relationship when personal air pyrene and BaP concentrations were above 49 and 20 ng/m^3, respectively. With the same exposure to pyrene or BaP, urinary 1-OHP concentrations were significantly higher in male subjects than in female subjects. When urinary concentrations above 0.46 μmol/(mol creatinine), 1-OHP can reasonably predict 24-hour personal air concentration of BaP. An increase in 1-OHP concentration by 1 μmol/(mol creatinine) predicts an increase in personal air concentration of BaP by

0.12 μg/m^3 (95^{th} CI: -0.02, 0.26) and 0.13 μg/m^3 (95^{th} CI: 0.04, 0.23) in males and females, respectively.

3.2 Introduction

It is well known that 1-hydroxypyrene (1-OHP) concentration in urine is associated with exposure to polycyclic aromatic hydrocarbons (PAHs) in occupational settings (Dor et al. 2000; Jacob and Seidel 2002; Jongeneelen 2001; Jongeneelen et al. 1990; Mielynska et al. 1997; Ovrebo et al. 1995b; Wu et al. 2003). As a metabolite of pyrene, 1-OHP in urine typically represents 90% of the urinary excretion of pyrene in humans (Brzeznicki et al. 1997). Because pyrene is typically correlated with benzo[*a*]pyrene (BaP) in the air (Unwin et al. 2006; Wu et al. 2002), urinary 1-OHP measurement has been suggested as an alternative of personal air sampling for BaP exposure (Merlo et al. 1998; Siwinska et al. 1999). Given that the current framework for assessing cancer risk associated with PAH inhalation exposure is based on the use of airborne BaP concentrations (life-time averages), it will be useful if urinary 1-OHP concentrations can be directly "translated" to airborne BaP concentrations. However, the usability of urinary 1-OHP for quantitatively predicting BaP or PAH exposure has not been thoroughly examined.

In occupational settings, a number of studies have shown associations between 1-OHP concentrations in urine and BaP concentrations in personal air (i.e., air samples collected in subjects' breathing zone). According to these relationships, some studies have suggested urinary 1-OHP concentration values equivalent to the occupational exposure limit (OEL) of airborne BaP to protect workers' health (Jongeneelen 1992; Kuljukka et al. 1996; Mielynska et al. 1997; VanRooij et al. 1993; Wu et al. 1998a). However, it is still unknown as to whether there exist quantitative relationships between urinary 1-OHP and personal air BaP concentrations when BaP concentrations are lower

than the OEL (2 μg/m^3). (BaP concentrations are typically lower than OEL in the general environment and even in polluted urban environments.) Positive associations between urinary 1-OHP and air concentrations of pyrene/BaP have been observed in individuals without significant occupational PAH exposures (Castano-Vinyals et al. 2004); but the findings were inconsistent across different studies (Merlo etal., 1998; Sorensen et al., 2003). This perhaps results from the presence of a threshold level in 1-OHP excretion, corresponding to a low PAH exposure, below which there is no exposure-response relationship as observed at higher exposure levels in occupational settings.

The main objective of the present analysis is to examine the usability of urinary 1-OHP concentration in predicting personal air concentration (inhalation exposure) of BaP by analyzing the data collected in 100 male and female non-smoking adults who had a wide range of PAH inhalation exposure. Specially, we attempt to identify the concentration range within which a quantitative relationship between BaP/pyrene inhalation exposure and urinary 1-OHP concentrations can be established.

3.3 Methods

3.3.1 Data Collection

Data used for the present analysis were collected as part of an exposure biomarker study conducted in An-Shan, China, where a steel manufacturing factory was built in 1918. The factory employed > 5,000 workers and had 17 coke oven plants in operation at the time of data collection. During 2002-2003, 50 non-smoking coke oven workers at various job sites from this factory were recruited to participate in the exposure biomarker study. Another 50 non-smoking adults were recruited through advertisement in several

communities several kilometers away from the factory. These 50 subjects were urban residents who were not directly exposed to coke-oven emissions. Subjects at each group were not matched by age and sex but within the defined age range (21-55 years) for both coke-oven workers and non-workers. Detailed demographic information was summarized in Chapter 2. Institutional Review Board (IRB) approval was obtained before the commencement of the subject recruitment and renewed approval was maintained throughout the entire course of the study including the data analysis phase. Subjects participated in the study provided written and informed consent.

3.3.2 Sample collection and analysis

We used 24-hour urinary 1-OHP concentrations, personal air and dietary PAH exposure data in the present analysis. The urine samples were analyzed for 1-OHP and creatinine. Urine sample collection and analytical method were described in Chapter 2 in detail.

3.3.2.1 Personal air sample collection

Personal air sampling was conducted to measure 24-hour integrated PAH concentrations in the breathing-zone of each participant. Battery-powered BGI personal pumps (BGI, AFC 400S, Waltham, MA) were used for the personal air sampling of PAHs in particulate matter with an aerodynamic diameter less than 10 μm and gas-phase PAHs. The sampler consisted of a 37 mm quartz fiber filter (PALL FLEX, Pall Life Sciences) and a polyurethane foam plug (PUF, density 0.02 g/cm^3). Subjects were instructed to wear the samplers whenever they were awake except during showing, bathing, or swimming (in these circumstances, the samplers were placed away from getting wet but as close to the breathing zones as possible).

All samples were stored separately in glass jars in complete darkness at - 4°C, thus minimizing PAHs losses, until extraction and analysis were completed. Filter and PUF samples were individually placed in separate Soxhlet extraction apparatus and extracted for 16 hours with 120 mL of HPLC-grade dichloromethane. Sample extracts were concentrated to 4 mL via rotary evaporation and then cleaned using solid-phase extraction cartridges (i.e, Waters Sep-Pak silica cartridges by Waters Division, Millipore Corporation, Milford, MA). Prior to use, each cartridge was rinsed with 10 mL of hexane. The concentrated extract was then loaded onto the cartridges, and the flask containing the residue was rinsed twice with 1 to 2 mL of hexane. After discarding the hexane eluent, the cartridges were eluted with 5 to 6 mL of 15% dichloromethane in hexane, and the eluent was collected in a 10 mL test tube. This was evaporated slowly to near dryness under a stream of oxygen free nitrogen, and re-dissolved in 1 mL of acetonitrile. The sample was then subjected for PAH analysis.

3.3.2.2 Food sample collection

Dietary sampling was conducted using a "Duplicate Plate" method that required participants to set aside the same amount of their three meals and snacks during the 24-hour monitoring period. The participants were asked to keep a diary of the food consumed including approximate amounts and method of food preparation. These samples had been placed in an aluminum container, sealed, and refrigerated or frozen, by the subjects before sample collection. The field study personnel collected the food samples, along with the other sample, at the end of each 24-h monitoring period. A 24-h composite of the food samples were made in the laboratory by homogenizing all the food

samples using a food blender. Composite food samples were stored at -20°C in a dedicated freezer before chemical analysis.

The preparation of food samples were based on the work of Howard et al. (1986). Digestion and saponification of a 100 g aliquot of the aggregated food sample were carried out in a 7% potassium hydroxide in absolute ethanol (200 ml) solution with stirring and refluxing for 2 hours. The liquid portion of this mixture was decanted into a separated funnel. The remaining residual material was washed with three 50 ml portions of isooctane. This wash solution was used to extract the decanted ethanol solution. The ethanol solution was further extracted with an additional two portions (60 ml) of isooctane. The extract solutions were combined and washed repeatedly with warm (60 °C) water until the isooctane solution was clear. If an emulsion existed, 20-30 ml of saturated NaCl solution was added. Residual water was removed from the isooctane extract by adding 20 g Na_2SO_4 and then filtering. The isooctane extract was passed through a treated and tested fluorosil column followed by three 60 ml portions of benzene. The BaP containing benzene eluate was collected and rota-evaporated to 5 ml. After adding 10 ml cyclohexane, the solution was evaporated to 2 ml, and transferred to a vial where three 2 ml washes of the flask were added. This solution was blown down to 1 ml under nitrogen. The analytical method of PAHs in food was the same as the analytical method for air sample analysis.

3.3.2.3 Sample Analysis for PAHs

Identification and quantification of PAHs were performed on reversed-phase HPLC (Waters 600E) with programmable fluorescence detector (LDC Analytical Inc., Riviera Beach, FL, LDC FluoroMonitor 4100). The HPLC column was a Waters Radial-

Pak Cartridge (5.0×100 mm, 10 μm particle size C_{18} packing) with its guard column. A gradient elution program, using the mixture of acetonitrile (ACN) and distilled deionized water as the mobile phase, was as follows: 35% of ACN to 95% for the first 42 minutes, then 95% of ACN for 6 minutes, then 100% of ACN for 5minutes, and then 35% of ACN for the last 7 minutes. The fluorescence detector was set at an excitation wavelength of 250 nm and an emission wavelength of > 370 nm. This analytical technique was able to quantify the following 14 PAHs: acenaphthene, anthracene, benz(a)anthracene, chrysene, benzo(a)pyrene (BaP), benzo(b)fluoranthene, benzo(ghi)perylene, benzo(k)fluoranthene, dibenz(ah)anthracene, fluoranthene, fluorene, naphthalene, phenanthrene, and pyrene. Only pyrene and BaP data were used in this chapter.

3.3.3 Statistical Analysis

For descriptive analysis, percentiles of personal pyrene/BaP exposure and urinary 1-OHP concentrations were computed. For urinary 1-OHP values below the limit of detection (LOD), a value equal to one-half the LOD was used. PAHs and urinary 1-OHP concentrations were stratified by gender to investigate the potential for confounding. Personal pyrene/BaP exposure and urinary 1-OHP values were highly skewed. The relationship between PAH inhalation exposure and urinary 1-OHP was first examined by classifying the subjects into 10 groups with every 10^{th} percentile increase of personal pyrene or BaP exposure. Group means of urinary 1-OHP concentrations were computed across percentiles. In the second set of analysis, two regression analyses explored the relationship between personal PAH exposure and urinary 1-OHP levels. We used repeated-measures mixed-effects linear regression models for model 1 to explore personal PAH exposure-dose (urinary 1-OHP) relationships. These models included

urinary 1-OHP concentration as the response (dependent variable), the personal air pyrene or BaP concentration, dietary pyrene (or BaP) intake, and appropriate covariates as independent (fixed effects) variables. Gender was entered as covariate for the model. Age and BMI were not included in the final models because the both covariates were not significant predictors when included. A repeated measure correlation structure was introduced to model correlations between measurements taken across season for each individual subject. The form of the model is as follows:

$$Y_{ij} = \beta_0 + A_{ij} + F_{ij} + \tau_i + \beta_1 \text{Gender} + \gamma_j + \varepsilon_{ij}$$

such that Y_{ij} is the value of the urinary 1-OHP concentration for the j^{th} subject during the i^{th} season; A_{ij} represents the 24-hour average concentration of personal air pyrene (or BaP) level (continuous fixed-effects variable); F_{ij} represents the 24-hour average concentration of personal pyrene (or BaP) dietary intake (continuous fixed-effects variable); τ_i represents the effect of the i^{th} season (i=0 for winter and 1 for summer); γ_j represents the subject-specific random effect to adjust for the correlation within a subject between season; and ε_{ij} represents the random error, which is assumed to be normally distributed. The variance covariance structure for the ε_{ij} was found to be best represented using a compound symmetry via Akaike's Information Criteria (AIC) and Schwarz' Bayesian Criterion (SBC). In the model above, A and F represented personal air pyrene(or BaP) and dietary pyrene (or BaP) exposure. A test of these terms reveal whether there are significant differences between an effect of A (or F) in the changes in urinary 1-OHP. Using these models, we performed both non-stratified analyses (for all subjects combined) and stratified analyses by low and high exposure groups. We report

the regression coefficients *A* and *F* representing personal pyrene (or BaP) exposure level increase related to change of urinary 1-OHP levels. We report regression coefficients and standard error (SE) of regression coefficients, i.e., 1 μmol/mol increase in 1-OHP in urine, associated with a unit change of personal air or dietary PAH concentration.

A 2nd set of models was constructed to predict personal air pyrene (or BaP) level using urinary 1-OHP concentration. These models included the personal air pyrene or BaP concentration as the response (dependent variable), urinary 1-OHP concentration, and season as independent (fixed effects) variables. Age and BMI were not included in the final models because we found no significant effects when included. Same repeated measure correlation structure was introduced to model correlations between measurements taken across season for each individual subject, as done in the first set of models described above. The generic form of the models is as follows:

$$Y_{ij} = \beta_0 + C_{ij} + \tau_i + \gamma_j + \varepsilon_{ij}$$

such that Y_{ij} is the value of the personal air pyrene(or BaP) concentration for the j^{th} subject at the i^{th} time; C_{ij} represents the 24-hour average concentration of 1-OHP in urine (continuous fixed-effects variable); τ_i represents the effect of the i^{th} season (i=0 for winter and 1 for summer); γ_j represents the subject-specific random effect to adjust for the correlation within a subject between season; and ε_{ij} represents the random error, which is assumed to be normally distributed. The variance covariance structure for the ε_{ij} was compound symmetry as in 1st set of models. In the 2nd set of models, *C* represents urinary 1-OHP concentration. A test of this term reveals whether there are significant differences between urinary 1-OHP levels in the changes in personal air pyrene or BaP.

Gender-stratified analyses were also performed using the 1st set of models but only for the high exposure group. We report the predicting values of personal pyrene (or BaP) exposure level associated with the unit change of urinary 1-OHP levels with SE.

3.4 Results

3.4.1 Descriptive statistics

The 100 subjects had a mean (±SD) age and BMI of 36 ± 7.5 years and 23.2 ± 3.0, respectively. Detailed demographic information was summarized in Chapter 2. Distributions of personal PAH exposure concentrations and 24-hour composite urine 1-OHP concentrations are presented in Table 3.1. Pyrene and BaP were detected in 100% of air and composite food samples; and 1-OHP was detected in > 98% of the urine samples. Spearman correlation between personal air pyrene and BaP was 0.83 (95% CI: 0.78, 0.87).

3.4.2 Relationships between urinary 1-OHP and PAH inhalation exposure

The relationship between pyrene/BaP personal air concentrations and urinary 1-OHP concentrations was examined in 10 exposure groups classified according to subjects' personal air concentration. As shown in Figure 3.1, urinary 1-OHP concentrations increased with an increase in personal air concentrations of pyrene or BaP across the upper 5 exposure groups (i.e., when concentration above 50th percentile across all the subjects). When below the 50th percentile, 1-OHP concentrations did not show increases with increased personal air concentrations. The 50th percentiles of airborne

pyrene and BaP were 49 and 20 ng/m^3, respectively, corresponding to urinary 1-OHP levels of 0.46 μmol/mol for pyrene and 0.24 μmol/mol for BaP. A similar graphical method was used for the relationship between dietary intake of pyrene or BaP and urinary 1-OHP across the 10 exposure groups. However, we did not find any association between dietary intake of pyrene or BaP and urinary 1-OHP (Figure 3.2). Means for urinary 1-OHP concentrations were not statistically significant among the 10 groups. We determined the pattern of differences after observing the mean differences among groups. We made this determination by partitioning the exposure group. Detailed SAS programs are summarized in Appendix.

3.4.3 Regression of urinary 1-OHP against PAH inhalation exposure

The effect of personal air PAH and dietary PAH intake on 1-OHP in urine was investigated in this regression analysis. Regression coefficients from the mixed-effect linear regression models are presented in Table 3.2. Intercept values may reflect the baseline 1-OHP values, i.e., the values corresponding to "zero" personal inhalation exposure. These were 1.29 and 1.36 μmol/mol for pyrene and BaP, respectively. When all the subjects were included in the regression, each of air concentration and gender was statistically significant; and dietary intake or measurement season was not significant. A 1 μg/m^3 increase in pyrene was associated with 0.22 μmol/mol increase in urinary 1-OHP. A 1 μg/m^3 increase in BaP was associated with 0.36 μmol/mol increase in urinary -OHP. With the same inhalation exposure to pyrene or BaP, male subjects tended to have higher urinary 1-OHP excretions than female subjects. As noted in Table 3.2, regression

coefficients for all subjects in both pyene and BaP were higher than those for high exposure subjects (Figure 3.3.).

Based on results from the graphical analyses described above, relationships between urinary 1-OHP and personal air concentrations of pyrene and BaP should be different for the high and low exposure groups (separated at 50th percentile of air concentration). When only the subjects with personal air concentrations above the 50th percentile were included in the regression, the baseline 1-OHP levels corresponding to "zero" concentration of pyrene and BaP were 1.95 and 1.80 μmol/mol, respectively. The effect of personal air pyrene on urinary 1-OHP levels was significant (P=0.010) whereas the effect of personal air BaP was marginally significant (P=0.121). A 1 μg/m^3 increase in pyrene was associated with 0.11 μmol/mol increase in urinary 1-OHP. A 1 μg/m^3 increase in BaP was associated with 0.20 μmol/mol increase in urinary 1-OHP. With the same inhalation exposure to pyrene or BaP, male subjects excreted higher concentrations of urinary 1-OHP. Dietary PAH intake and seasonal variation did not affect urinary 1-OHP concentrations.

3.4.4 Prediction of personal air PAH level by urinary 1-OHP levels

Table 3.3 shows the regression results for the prediction of urinary 1-OHP for personal air pyrene or BaP concentrations. In male subjects, air pyrene concentrations were significantly associated with urinary 1-OHP concentrations (p=0.0105) whereas air BaP concentrations were marginally associated with urinary 1-OHP concentrations (p=0.0966). In female subjects, both personal air pyrene (p=0.0143) and BaP (p=0.0149) concentrations were significantly associated with urinary 1-OHP concentrations.

We computed the predicted concentrations of personal air pyrene and BaP in the exposure groups representing 50^{th}, 75^{th}, 90^{th}, 95^{th}, and 99^{th} percentiles of 1-OHP concentration (see Figure 3.4). Results indicated that predicted concentrations of air pyrene and BaP agreed well with actual measurements when urinary 1-OHP ranged between 75^{th} and 95^{th} percentiles. At urinary 1-OHP concentration of 3.8 μmol/mol (90^{th} percentile), corresponding measured and predicted air BaP concentrations were 0.79 and 0.85 (95% CI: -0.11, 1.83) $\mu g/m^3$, respectively. However, predicted concentrations of air pyrene and BaP were lower than actual measurements when urinary 1-OHP levels were higher than 95^{th} percentile, e.g., at urinary 1-OHP concentration of 10 μmol/mol, corresponding measured and predicted air BaP concentrations were 6.82 and 1.60 (95% CI: -0.23, 3.44) $\mu g/m^3$, respectively.

3.5 Discussion

Associations between urinary 1-OHP concentrations and air BaP concentrations have been reported by previous studies in occupational settings (Merlo et al. 1998; Ovrebo et al. 1995a). Although significant associations between urinary 1-OHP and air BaP concentrations were observed in highly exposed workers, whether such associations exist in the more general population with lower PAH exposure has not been examined thoroughly. To serve as a more widely usable biomarker, urinary 1-OHP and personal air BaP should have a quantitative relationship for the general population. In the present analysis, we found a good linear relationship between urinary 1-OHP and pyrene/ BaP personal air exposure when 24-hour composite urine concentrations of 1-OHP were above 0.46 μmol/(mol creatinine) (or 0.85 ng/ml). However, no significant associations

were found when urinary 1-OHP concentration was below 0.46 μmol/mol (0.85 ng/ml). Hence, this concentration may be suggested as a "threshold" value for the relationship between urinary 1-OHP and personal pyrene/BaP exposure. The corresponding concentrations of personal air pyrene and BaP were 49 and 20 ng/m^3, respectively

In the lower exposure groups, the lack of a significant association between urinary 1-OHP and personal air pyrene/BaP concentrations may be explained by several factors. First, low concentration of pyrene and BaP may not give rise to an appreciable increase of excretion in comparison with baseline urinary values (Cirillo et al. 2006). Fiala et al. (2001) reported that urinary 1-OHP might be a non-sensitive marker of the environmental inhalation exposure to pyrene/PAHs if the pollution of air is not excessive. It is possible that urinary 1-OHP excretion is little influenced by low-level PAH exposure such as that corresponding to personal air pyrene concentrations below 49 ng/m^3 (or BaP 20 ng/m^3). Dor et al. (1999) reported background level of urinary 1-OHP ranged from 0.03 to 0.79 μmol/mol for the population without occupational or significant environmental exposures. Jongeneelen et al (2001) also estimated the baseline level of urinary 1-OHP below 1 ng/ml or 0.76 μmol/(mol creatinine). The "threshold" values ranged within the baseline level of urinary 1-OHP suggested by previous studies.

Secondly, the relatively successful use of 1-OHP as a marker of PAH exposure is based on an underlying assumption that the fraction of pyrene in total PAH mixture (gas-phase and particle-phase) is constant (Ovrebo et al. 1995c). This assumption may be true for a specific PAH source under defined conditions like a coke oven factory but can not be applied to different sources and to a wide range of environmental conditions. Thus, the

lack of a significant association between urinary 1-OHP and personal air pyrene/BaP exposure may be partly explained by the discrepancies of particle/gas partition of pyrene.

Dietary intake of PAH may be an important contributor to 1-OHP excretion in urine. Previous research reported dietary exposure consists of 95% ~ 99% of daily total PAH exposure (Vanrooij et al. 1994; Vyskocil et al. 2000). Viau et al (2002) also estimated that dietary pyrene intake accounts for 87.5% ~ 99.8 % of the sum of dietary and inhalation intake. Average daily BaP intake of 530 ng/day in this study was comparable with 500-600 ng/day (Scherer et al. 2000) but higher than 123~206 ng/day (Buckley et al. 1995; Vyskocil et al. 1997; Waldman et al. 1991). The relative high dietary PAH intake may elevate baseline excretion of 1-OHP in urine.

In the present analysis, we did not find positive associations between dietary PAH intake and urinary 1-OHP concentrations. The lack of a significant association may be attributable to several factors. First, the metabolism of PAH in the body through diet is different from that via inhalation route. For example, pyrene absorbed from GI tract is metabolized in the liver and the majority eliminated in the bile as glucuronide (about 95%). Only 2% to 5% of metabolite enter kidney and are metabolized as 1-OHP in urine. However, pyrene absorbed through the lung can be metabolized and glucuronidated; these metabolites are distributed to liver and kidney (approximately 15% ~ 25%) (Viau et al. 1999). Second, large inter-individual variability may affect poor correlation between urinary 1-OHP and dietary PAH intake. According to Viau et al (1999), bioavailability and enzymatic polymorphism may contribute to the variability.

To my knowledge, other studies have not explored the usability of urinary 1-OHP levels to estimate personal air pyrene or BaP concentrations for a wide range of exposure

concentrations. Some studies estimated urinary 1-OHP concentrations that are equivalent to airborne occupational BaP exposure. A comparison of data available in the literature is shown in Table 3.4. Jongeneelen proposed an occupational limit for coke oven workers about 2.3 μmol/mol and its equivalent estimated BaP concentrations were 2 $\mu g/m^3$ (Jongeneelen 1992). At 2.0 μmol/mol of urinary 1-OHP, the predicted BaP concentrations were lower than those reported in three studies of coke oven workers (Mielzynska et al 1997, Pyy et al 1997, and Joneneelen 1992) but higher than that from various industry workers (Unwin 2006). The previous studies used during-work-shift average concentrations of BaP in the air (independent variable) and urinary 1-OHP levels (dependent variable) in cokeoven workers. No random effect for subject was investigated in their studies. In contrast, we used 24-hour average air concentrations and controlled subject-specific random effect adjusting for season and within-subject correlation.

In conclusion, we found that personal air BaP concentrations were well correlated with personal air pyrene concentrations. We observed an exposure-response relationship between airborne PAH concentrations and urinary 1-OHP concentrations when personal air pyrene and BaP concentrations were above 49 and 20 ng/m^3, respectively. These air concentrations corresponded to a urinary 1-OHP "threshold" concentration of 0.46 μmol/(mol creatinine) or 0.85 ng/ml. With the same exposure to pyrene or BaP, urinary 1-OHP concentrations were significantly higher in male subjects than in female subjects. When above the threshold concentration, 1-OHP can reasonably predict 24-hour personal air concentration of BaP. An increase in 1-OHP concentration by 1 μmol/(mol creatinine) predicts an increase in personal air concentration of BaP by

0.12 μg/m^3 (95^{th} CI: -0.02, 0.26) and 0.13 μg/m^3 (95^{th} CI: 0.04, 0.23) in males and females, respectively.

3.6 Reference

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Table 3.1. Summary statistics for pyrene, BaP and urinary 1-OHP concentrations**.**

Sample type	Analyte	No.	Median	Min	25th percentile	75th percentile	90th percentile	95th percentile	99th percentile	Max
Inhalation concentration[a]	Pyrene (ng/m^3)	200	48.7	0.44	20.8	249	1,750	3,749	15,799	24,744
	BaP[c] (ng/m^3)	200	19.7	0.13	6.04	193	790	1,324	6,822	10,370
Food Intake	Pyrene (µg/day)	161	3.75	0.01	1.72	5.46	9.72	14.0	48.4	49.8
	BaP[c] (µg/day)	161	0.53	<0.01	0.33	0.97	1.57	2.40	25.3	34.7
Urinary concentrition[b]	1-OHP[c] (µmol/mol)	200	0.46	< 0.01	0.14	1.55	3.79	5.90	9.99	14.7
	1-OHP (ng/mL)	200	0.85	< 0.02	0.30	3.07	8.59	10.3	28.0	47.8

[a] 24-hour average concentrations of particle-phase and gas-phase PAHs

[b] Concentrations in 24-hour composite urine samples (during the same 24-hour of air and food measurements)

[c]BaP: Benzo[a]pyrene; 1-OHP: 1-hydroxypyrene

Table 3.2. Regression coefficients of the independent variables from the multiple linear regression model of creatinine adjusted urinary 1-OHP levels (dependent variable).

Variable	All subjects (N=100)[a]				High exposure subjects (N=49)[a]			
	Pyrene		BaP		Pyrene		BaP	
	Coefficient ± SE	*p* value	Coefficient ± SE	*p* value	Coefficient ± SE	*p* value	Coefficient ± SE	*p* value
Intercept	1.29±0.30	<0.0001	1.36±0.32	<0.0001	1.95±0.37	<0.0001	1.80±0.51	0.0008
Personal air (μg/m^3)	0.22±0.05	0.0001	0.36±0.13	0.0076	0.11±0.03	0.0098	0.20±0.11	0.1213
Dietary intake (μg/day)	-0.02±0.02	0.3311	-0.02±0.04	0.5872	-0.05±0.04	0.2363	0.02±0.06	0.7013
Gender								
Female	-0.77±0.33	0.0224	-0.85±0.34	0.0151	-1.50±0.53	0.0066	-1.75±0.50	0.0007
Male	0.00±0.00	N/A	0.00±0.00	N/A	0.00±0.00	N/A	0.00±0.00	N/A
Season								
Winter	0.19±0.31	0.5409	0.12±0.30	0.6823	0.47±0.34	0.2056	0.37±0.46	0.4477
Summer	0.00±0.00	N/A[b]	0.00±0.00	N/A	0.00±0.00	N/A	0.00±0.00	N/A

[a] Number of subjects. Each subject was measured twice during the study period.

[b] N/A, not applicable. High exposure subjects were determined when personal air pyrene and BaP concentrations were above 49 ng/m^3 and 20 ng/m^3, respectively.

Table 3.3. Regression coefficients of the independent variables from the multiple linear regression model of personal air pyrene or BaP concentrations (dependent variable) in high exposure subjects.

Variable	Male(N=42)[a]				Female (N=7)[a]			
	Pyrene		BaP		Pyrene		BaP	
	Coefficient ± SE	*p* value	Coefficient ± SE	*p* value	Coefficient ± SE	*p* value	Coefficient ± SE	*p* value
Intercept	0.61±0.51	0.2385	0.40±0.22	0.0697	0.05±0.07	0.4394	0.06±0.03	0.0585
1-OHP (μmol/mol)	0.44±0.16	0.0105	0.12±0.07	0.0966	0.26±0.08	0.0143	0.13±0.04	0.0149

[a] Number of subjects. Each subject was measured twice during the study period.

Table 3.4. Comparison of personal air BaP concentrations with urinary 1-OHP levels of 2 μmol/mol.

BaP	Population	Reference[a]
0.32~0.66	Mostly coke oven workers (> 50th percentile)	The present study
2.1	Coke workers	Mielzynska et al 1997
10	Coke workers	Pyy et al 1997
2.0	Coke workers	Jongeneelen 1992
0.26[b]	Various industry workers	Unwin 2006

[a]Previous studies estimated urinary 1-OHP level of 2 μmol/mol with personal air BaP concentrations.

[b] Corresponding urinary 1-OHP level is 4 μmol/mol.

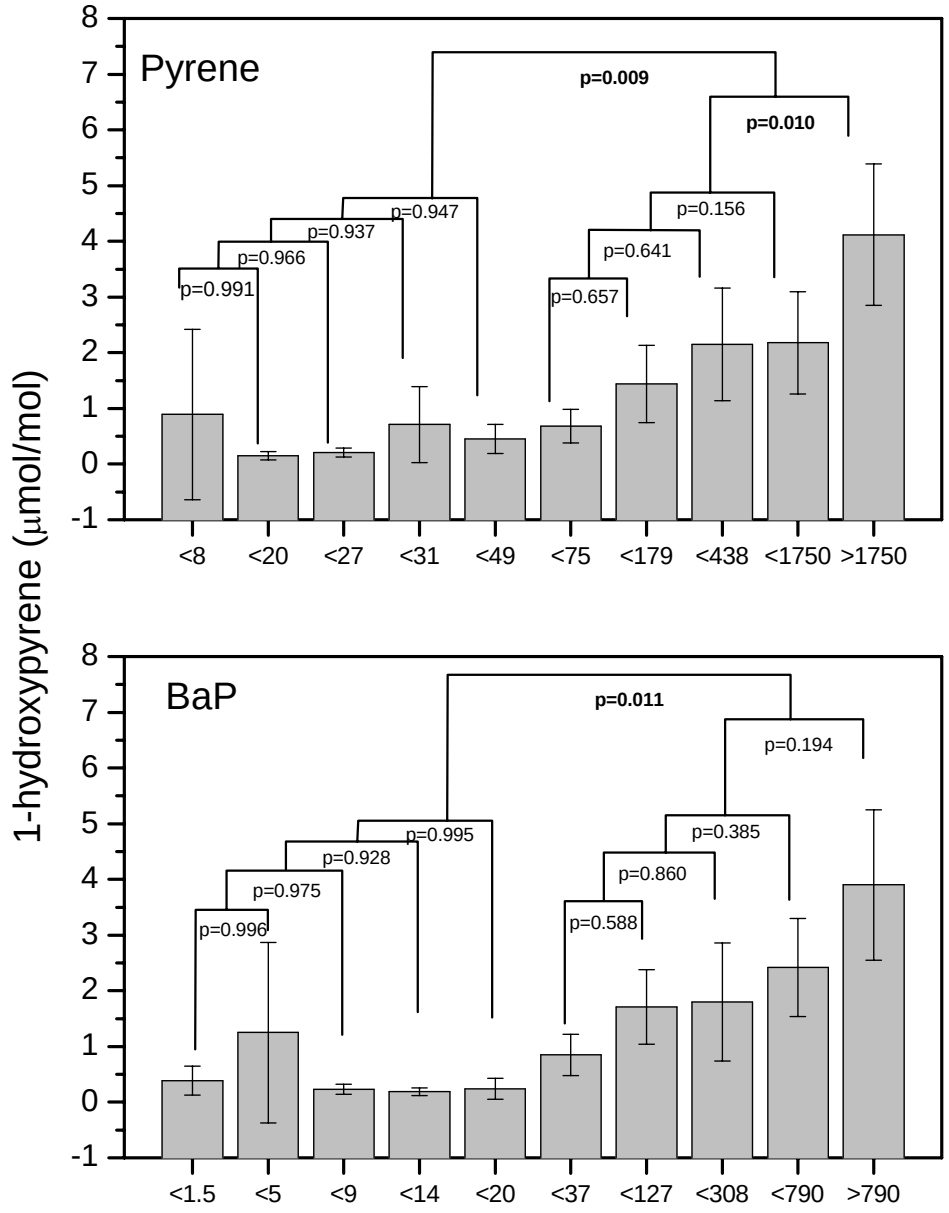

Figure 3.1. Relationship between urinary 1-OHP and personal air pyrene/BaP exposure according to every 10^{th} percentiles. Error bars represents 95% confidence intervals.

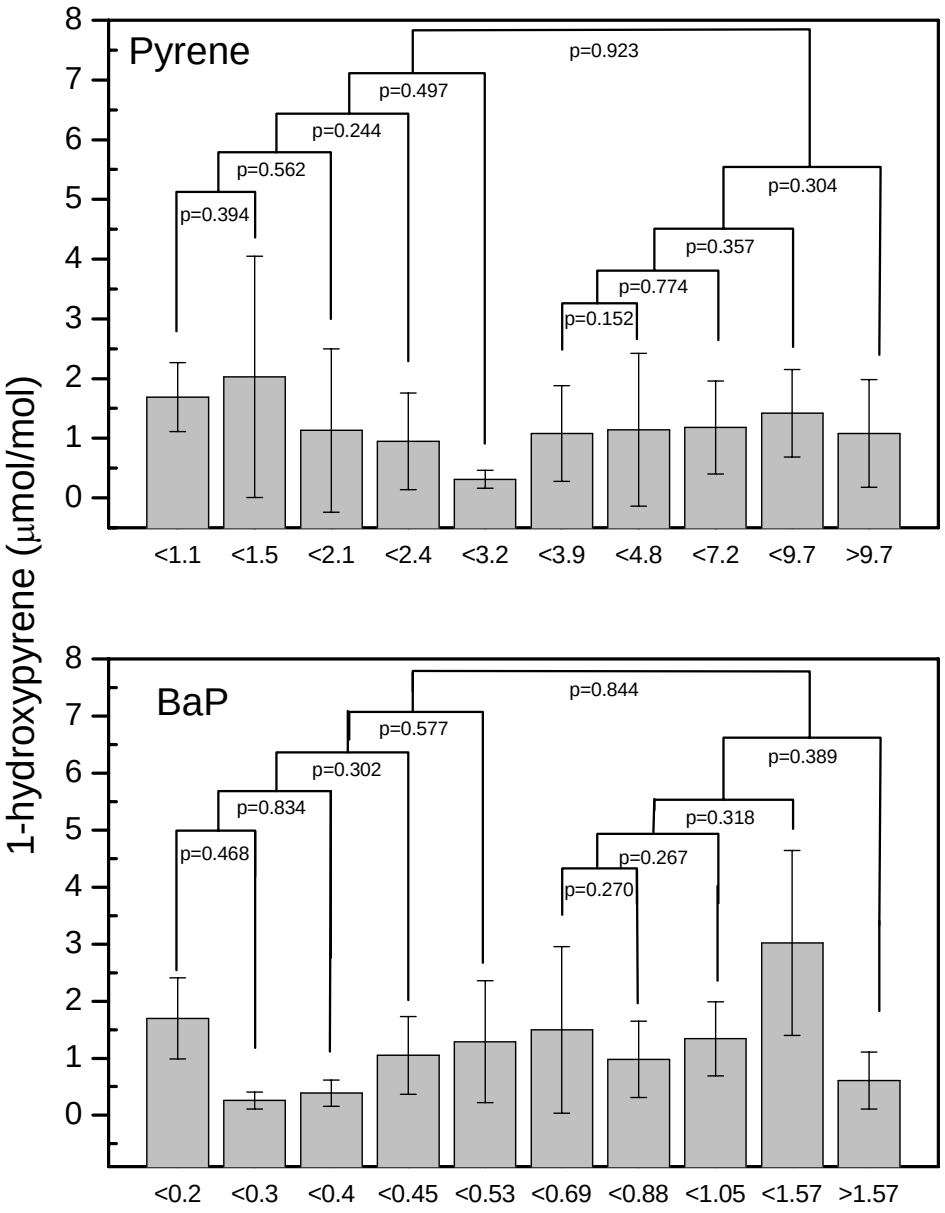

Figure 3.2. Relationship between urinary 1-OHP and dietary pyrene/BaP intake according to every 10th percentiles. Error bars represents 95% confidence intervals.

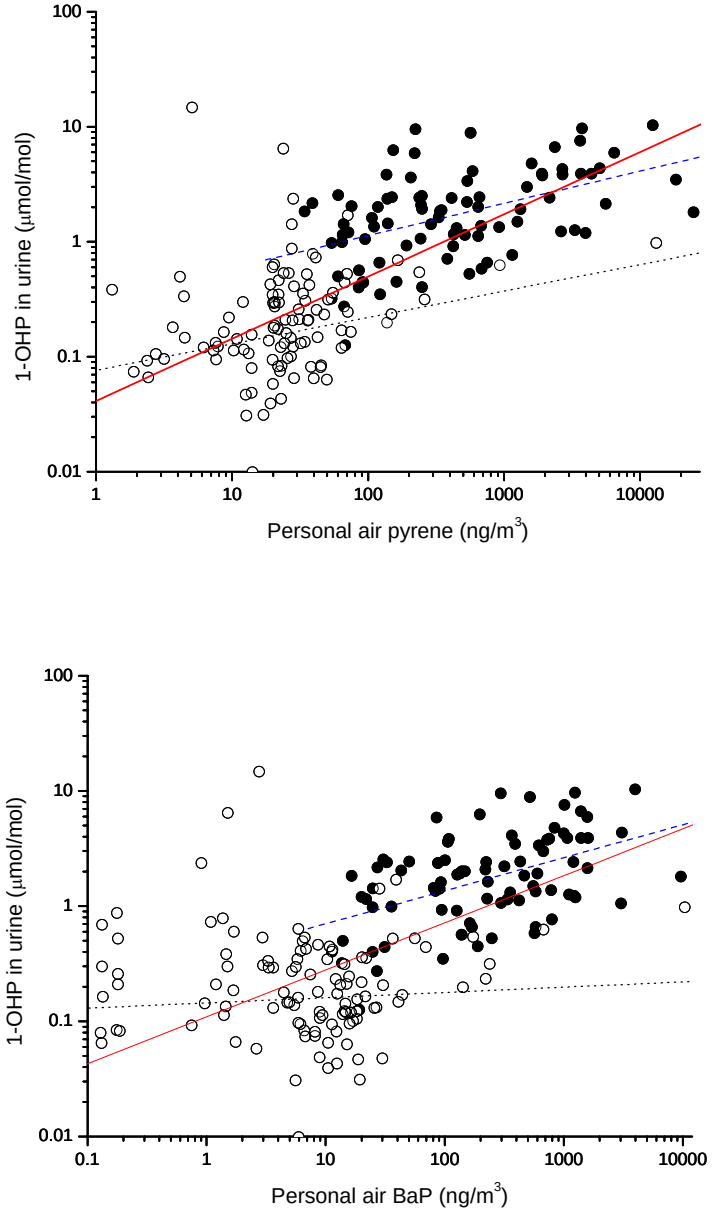

Figure 3. 3. Scatter plot between personal air pyrene/BaP exposure and urinary 1-OHP levels. Straight lines represent regression lines for all subject. Dashed lines represent regression lines for high exposed subjects. Dotted lines for low exposed subjects.

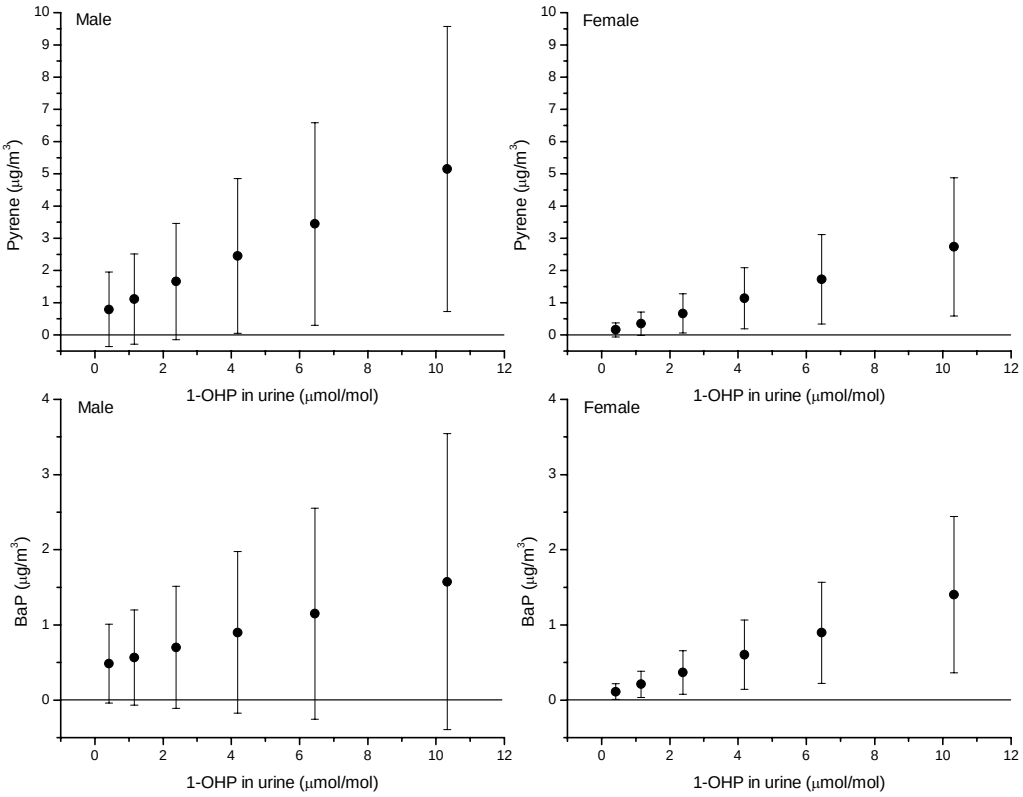

Figure 3. 4. Predicted values for personal air pyrene and BaP concentrations with selected urinary 1-OHP levels. Error bars indicate 95% confidence intervals.

Chapter 4

Seasonal Variability and Between-person Variability in Urinary 1-hydroxypyrene Concentration

4.1 Abstract

Urinary 1-hydroxypyrene (1-OHP) has been used as a biomarker of polycyclic aromatic hydrocarbons (PAH) exposure in occupational settings. To better understand the implication of using a single measurement of urinary 1-OHP to characterize PAH exposure, We investigated subject-specific between- and within- person variabilities in personal air and diet PAH concentrations and urinary 1-OHP concentrations from 100 persons with a wide range of PAH inhalation exposure. Each participant was sampled two times over 6 to 9 months. We fit a mixed-effects model to estimate mean levels and the variation of pyrene and urinary 1-OHP between work places and sampling times. Results show that personal air pyrene was moderately correlated both across coke oven workers (r=0.48) and across non-coke oven workers (r=0.40). No significant correlation of dietary PAH intake was observed across both coke oven workers and non-coke oven workers. Urinary 1-OHP was moderately correlated only across coke oven workers (r=0.55). Adjusting for season, the correlation of personal air pyrene concentrations within person over time was 0.49 for coke oven workers and 0.48 for non-coke oven workers. The correlation of dietary pyrene intake within person over time was 0.46 for coke oven workers and 0.45 for non-coke oven workers. The correlation of urinary 1-OHP within person over time was 0.46 for coke oven workers and 0.43 for non-coke oven workers. Results also show that between-person variance was smaller than within-

person variance for personal air pyrene, dietary pyrene intake, and urinary 1-OHP. The highest variance ratio (within- to between-person) was found in urinary 1-OHP. The results suggested the importance of repeated measurements on same individuals in obtaining a reliable measure of average daily PAH exposures.

4.2 Introduction

Urinary 1-hydroxypyrene (1-OHP), a metabolite of pyrene, has been suggested as a biomarker of polycyclic aromatic hydrocarbon (PAH) exposure. A single measurement over a short period of time (e.g., 1 day) has been used to estimate exposure in previous studies to assess the effects of exposure to PAH (Bouchard and Viau 1999; Hansen et al. 2005; Kuusimaki et al. 2004). However, the reliability of conclusions drawn from these studies depends largely on the representativeness of such single-sample measurements. In general, temporal variability in concentrations of environmental contaminants or their exposure biomarkers often complicates exposure assessment. Ideally, repeated measurements over many days, weeks, or months would provide more reliable assessment of longer-term or average exposure but may not be practical because of cost constraints and subject burden.

Some studies have estimated inter-individual variability or intra-individual variability (Van Rooij et al. 1994; Viau et al. 2002). Several studies have characterized temporal trends in 1-OHP concentration related to occupational exposure (Lin et al. 2006; Vyskocil et al. 1997; Wu et al. 1998b), and fewer examined that in relation to environmental exposure (Bouchard et al. 2001; Castano-Vinyals et al. 2004; Cocco et al. 2006). Although previous studies have found temporal changes in 1-OHP concentration, they did not quantify the variance related to temporal variability (Adonis et al. 2003; Cocco et al. 2006; Fiala et al. 2001; Vyskocil et al. 1997). To date, little is known about the temporal variability and reproducibility of repeated measures in urinary 1-OHP concentration within same individuals.

Exposure to air pollutants may vary not only within individuals over time but also across different individuals. Therefore understanding of within- and between-individual variance components is essential for accurately estimating individuals' and population exposures (Peretz et al. 2002). In this study, we hypothesized that within-individual variation in urinary 1-OHP concentration over a few months was smaller compared to between-person variation in non-smoking adults having stable daily PAH exposures.

To test this hypothesis, we analyzed seasonal variability of urinary 1-OHP concentration in order to understand the sources of 1-OHP variability, we also analyzed seasonal variability of pyrene inhalation and dietary exposure across 100 men and women.

4.3 Methods

4.3.1 Data collection

4.3.1.1 Subjects

Participants were 100 non-smoking Chinese residents of Anshan city. Among them, 50 participants were workers of a large coke oven factory and the rest of 50 were non-coke oven workers residing several kilometers away from the coke oven factory. The participants ranged in age from 23 to 51 years, with a mean age of 36.0 years (SD=7.7). All subjects provided informed consent prior to participating. Detailed demographic information is summarized in Table 2.1 of Chapter 2.

4.3.1.2 Sample collection and analysis

The data used in this analysis included personal air concentrations of pyrene, dietary intake of pyrene, and 1-OHP concentration in first morning urine void samples. We collected particle-phase and gas-phase pyrene during 24-hour close to a subject's

breathing zone. Food samples were collected using the "second plate" method, as described in more detail in Chapter 3. First morning urine sample voids were collected in a plastic container provided by a study staff and stored in a freezer until collected by study staff. Two samples were obtained from each subject during 2002-2003. The first sample was collected in December 2002 (winter season) and the second sample was collected in August-September 2003 (summer season). Detailed information about sample collection and analysis is described in Chapter 3.

4.3.2 Data analysis

4.3.2.1 Data description

We reported personal air concentrations of pyrene (ng/m^3), dietary pyrene intake (μg/day), and urinary 1-OHP concentrations (ng/ml and μmol/mol creatinine). We conducted statistical analysis on both unadjusted and creatinine-adjusted urinary 1-OHP levels. Because we was interested in reproducibility in population samples, we did not exclude outliers from any analyses.

4.3.2.2 Statistical analysis

Statistical analyses consisted of summary and descriptive statistics, Pearson and Spearman correlation analyses between the season, and random effects models to estimate between- and within-person variance components. Mixed-effects models were necessary to accommodate the between- and within-person variation in the unbalanced, longitudinal data (Littell et al. 1998). The mixed-effect model is described as follows:

$$Y_{ij} = \ln(X_{ij}) = A_y + \sum_{m=1}^{p} \tau_m C_{mij} + \beta_i + \varepsilon_{ij}$$

where i=1, 2, …, k individuals

j=the 1st and 2nd measurements of the i th individual, and

m=1, 2, …, p covariates

$\boldsymbol{X_{ij}}$ is a vector of the chemical measurement (i.e, personal air pyrene, dietary intake of pyrene, and urinary 1-OHP) for the i-th individual on the j th monitoring period; $\boldsymbol{A_y}$ representing the intercept; the product of the regression coefficients $\tau_1, \tau_1, \ldots, \tau_p$ (fixed effects; season, exposure group) and the observed values of their corresponding covariates $C_{1ij}, C_{2ij}, \ldots, C_{pij}$ (age, gender, and BMI); β_i representing the random effect for the ith individual; and ε_{ij} representing the residual error for jth observation on the ith individual. The random variables and residual error were assumed to be independently and normally distributed with means of 0 and variance of $\hat{\sigma}_B^2$ and $\hat{\sigma}_W^2$ (representing the between- and within-person components of variance, respectively). A compound symmetry covariance structure was used in all models. To compute the between- and within-person correlation coefficient between the response variable and fixed effects, estimated from exposure, while adjusting for the effect of a confounding factor Z, we considered subjects as a random effect in the mixed-effect model.

We calculated the "intra-class correlation coefficient " (ICC) for personal air PAH, dietary PAH intake, and urinary 1-OHP levels. ICC characterizes the reproducibility (stability) of repeated measurements over time by the following equation (Fleiss, 1985; Rosner 1995);

$$\rho_r = \hat{\sigma}_B^2 / (\hat{\sigma}_B^2 + \hat{\sigma}_W^2)$$

We designated the ratio of σ_W^2 to σ_B^2 as the variance ratio ($\lambda = \sigma_W^2 / \sigma_B^2$). This variance ratio can be used to evaluate attenuation bias when estimating an exposure-disease relationship, given that personal air, dietary intake, or urinary biomarker levels

were used as surrogates for actual exposure levels (Rappaport and Kupper 2004; Rappaport et al. 1995).

4.4 Results

4.4.1 Distribution of the data

Personal air concentrations of pyrene and dietary intakes of pyrene from all subjects were all above detection limits, while 98% urine samples were above detection limit for 1-OHP. Table 4.1 presents the average concentrations for personal air pyrene, dietary pyrene intake, and urinary 1-OHP. The data were stratified by coke and non-coke oven workers. Among the 50 coke oven workers, personal air pyrene ranged from 3.33 to 24,745 ng/m^3, with a median of 3,615 ng/m^3. Among the 50 non-coke oven workers, personal air pyrene ranged from 0.44 to 74.5 ng/m^3, with a median of 22.2 ng/m^3. Dietary pyrene intake ranges for both coke oven workers and noncoke oven workers were less than three orders of magnitude. Ranges of urinary 1-OHP levels for both coke oven workers and non-coke oven workers were up to five orders of magnitude.

4.4.2 Seasonal variation

The data did not show a distinct seasonal pattern in personal air pyrene, dietary pyrene intake, and urinary 1-OHP levels (Table 4.2). For the coke oven workers, a significant seasonal effect was found only on personal air pyrene concentrations, although personal air pyrene, dietary intake of pyrene and urinary 1-OHP in the winter measurements all appeared to be higher than in the summer measurements. For non-coke oven workers, only dietary pyrene intake statistically differed by season. Measured concentrations of personal air pyrene and urinary 1-OHP were similar in both seasons.

4.4.3 Correlations between Winter and Summer Measurements

Correlations of personal air pyrene and urinary 1-OHP between two seasons were moderate in coke oven workers. Correlation of urinary 1-OHP concentrations were slightly improved when they were adjusted for creatinine concentrations (Table 4.3). In non-coke oven workers, only personal air pyrene showed significant correlation coefficient between two seasons. Figure 4.1 presents scatter plots between the two repeated measurements for personal air pyrene, dietary pyrene intake, and urinary 1-OHP concentrations.

4.4.4 Reproducibility

We calculated the ICCs as a measure of reproducibility. For the coke oven workers, personal air pyrene had the highest estimate of reproducibility, with and ICC of 0.49, followed by dietary pyrene intake (ICC=0.46) and creatinine adjusted urinary 1-OHP (ICC=0.46), and then unadjusted urinary 1-OHP (ICC=0.45). For non-coke oven workers, the ICC values and trends for measured pollutants and biomarker were similar as those for coke oven workers (Table 4.4).

4.4.5 Bias in Estimating Exposure-Response Relationships

Potential bias in the estimation of exposure-response relationship was evaluated by examining the estimated variance ratio. In general the values of urinary 1-OHP for coke oven workers and non-coke oven workers were slightly higher than those for personal air pyrene and dietary pyrene intake. Based on this result, we found that using

urinary 1-OHP as a surrogate exposure measure would tend to provide almost same estimate as the use of personal air pyrene and dietary pyrene intake measurements. Figure 4.2 shows the variance ratios for personal air pyrene, dietary pyrene intake and urinary 1-OHP, stratified by exposure settings. All of them had variance ratio values higher than 1 (smaller than 1 means less potential bias). Variance ratios in coke oven workers were smaller than those in non-coke oven workers.

4.5 Discussion

In a biomarker validation study, the following two components of the relationship between the marker and exposure should be evaluated. The first component is at the group level, reflecting the degree of dependency between the two variables at the group (treatment) level. The second component is at the individual level, the correlation between individual measurements within the groups. The overall correlation coefficient is a weighted average of within- and between-group correlations, estimating the amount of variation between groups over the total variability. Therefore, the correlations include within- and between-individual variation in addition to measurement error that could be random or systematic. Investigating the errors and bias, from different exposure assessment methods and examining the components of the variability, would provide useful information to better understand the overall association between biomarker and exposure. Many authors have used variance components (between- and within- person variance) to examine assumptions about homogeneity of exposure within groups and the usefulness of exposure assessment strategies in epidemiological studies (Abraham et al. 2005; Egeghy et al. 2000). However, no studies have examined variance components for

PAH and PAH biomarkers. In this study, we used a similar approach to examine the reproducibility of PAH exposure and urinary biomarker measurement method for exposure assessment.

The data showed significant seasonal difference in personal air pyrene and urinary 1-OHP but not in dietary pyrene intake for coke oven workers. For non-coke oven workers, we observed significant seasonal variation in dietary pyrene intake but not in personal air pyrene and urinary 1-OHP. It is expected that urinary 1-OHP in winter should be higher than in summer because urinary 1-OHP was strongly correlated with personal air pyrene in coke oven workers (Jongeneelen 2001; McClean et al. 2004; Mielynska et al. 1997; Pan et al. 1998; Siwinska et al. 2004; Wu et al. 1998a). However, we could not draw the conclusion from the data. In Chapter 3, we have found a significant linear relationship between personal air concentration of pyrene and urinary 1-OHP. The linear regression model from table 2 of Chapter 3 confirmed that seasonal effect on urinary 1-OHP was not a significant predictor in the study.

We observed a moderate degree of reproducibility for personal air pyrene, dietary pyrene intake, and urinary 1-OHP. ICCs for creatinine-adjusted 1-OHP in urine were 0.46 for coke oven workers and 0.43 for non-coke oven workers. Given that 1-OHP has biologic half-lives of approximately 6-12 hours in humans and that exposures can vary from day to day and season to season, the observed ICCs were moderate as expected. A previous study reported low ICCs related with dietary nutrients which have substantial large within-person variation (Jahns et al. 2004). Although ICCs were not as high as those observed for other environmental exposures such as organochlorine pesticides (Gammon et al. 1997), these values were similar to or better than those observed for

metals in the environment (Egeghy et al. 2005). Based on the results from this study, two repeated measurements of personal air pyrene, dietary pyrene intake, and urinary 1-OHP may not provide a good estimation for average daily exposure.

The observed moderate ICCs for non-coke oven workers may be resulted from low PAH exposure in the environment resulting in large variation in exposure and internal dose relationship. An earlier study found that the relationship between urinary 1-OHP and environmental PAH exposure was not exposure/dose dependent (Viau et al. 2002). We also confirmed that there was no significant exposure-dose relationship between urinary 1-OHP and personal air concentrations of pyrene when pyrene concentrations were below 50 $\mu g/m^3$ (see Chapter 3). Viau et al. (2002) also found a large inter-individual variability in urinary 1-OHP excretion although identical dietary PAH ingestion dose was applied. The most likely explanation would be variability in metabolism of absorbed pyrene, which contributed to the non-reproducibility of urinary 1-OHP. The variability includes the difference in bioavailability, enzyme activity, and metabolism from each individual (Martin et al. 1996; Strickland and Groopman 1995).

We reported that all the ratio of within-person to between-person variance was larger than 1 for personal air pyrene, dietary pyrene intake, and urinary 1-OHP in both coke oven workers and non-coke oven workers. The usefulness of variance ratio provides measurement error effects that can bias the estimation of exposure assessment in epidemiological studies. Regarding the choice of covariance structure, We found that compound symmetry (CS) was appropriate for within- and between-variance in this study. However, CS assumes that two repeated measurements within a given individual have same correlation regardless of sample collection intervals. Thus, investigators should be

aware of potential problems arising from the sampling intervals of biomarkers and personal exposure measurements. CS should be more carefully used for urinary 1-OHP because urinary 1-OHP has short half-life of metabolism.

4.6 Conclusions

We identified great variability in personal concentration of air, dietary pyrene intake, and urinary 1-OHP for coke oven workers and non-coke oven workers. Seasonal effects on urinary 1-OHP were not found in both coke oven workers and non-coke oven workers. In the present analysis, we found that personal air pyrene was moderately correlated across 50 coke oven workers (r=0.48) and 50 non-coke oven workers (r=0.40). No significant correlation of dietary PAH intake was observed across both coke oven workers and non-coke oven workers. Urinary 1-OHP was moderately correlated only across coke oven workers (r=0.55). Adjusting for season, the correlation of personal air pyrene concentrations within person over time was 0.49 for coke oven workers and 0.48 for non-coke oven workers. The correlation of dietary pyrene intake within person over time was 0.46 for coke oven workers and 0.45 for non-coke oven workers. The correlation of urinary 1-OHP within person over time was 0.46 for coke oven workers and 0.43 for non-coke oven workers.

Between-person variance was smaller than within-person variance for personal air pyrene, dietary pyrene intake, and urinary 1-OHP, suggesting the importance of repeated measurements on same individuals in obtaining a reliable measure of average daily PAH exposures. We also present evidence that personal air concentration of pyrene and dietary pyrene intake have smaller variance ratios (within- to between-person) than urinary 1-OHP.

4.7 References

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Table 4.1. Weighted quantiles (95% CIs) of urinary 1-OHP (ng/mL) for the study participants.

		No[a]	Mean	STD	Min	25th percentile	50th percentile	75th percentile	Max
Coke workers									
	Air pyrene	50	1,507	3,615	3.33	93.6	250	1,041	24,745
	Dietary intake of pyrene	31	5.41	4.11	0.15	2.32	4.10	7.86	17.8
	1-OHP(μmol/mol)	50	3.43	7.53	0.002	0.72	1.71	3.41	69.22
	1-OHP (ng/ml)	50	8.04	19.89	0.003	1.28	3.48	8.51	184.3
Non-coke oven workers									
	Air PAH	50	22.2	15.3	0.44	11.4	20.8	27.9	74.5
	Dietary intake of PAH	50	4.72	7.85	0.02	1.59	2.71	4.59	49.7
	1-OHP(μmol/mol)	50	0.37	1.19	0.001	0.09	0.16	0.28	11.89
	1-OHP (ng/ml)	50	0.70	1.99	0.003	0.19	0.32	0.57	19.20

Based on two repeated measurements in personal air, food, and first morning void samples from 100 subjects, Anshan, China, 2002-2003. [a] Average of two measures per subject. If both measures were missing, the data were not included in here. Outliers were included.

Table 4.2. Seasonal variation for urinary 1-OHP levels –mixed-effect regression models.

	Summer[a]	Winter[a]	p-Value
Coke workers			
Air pyrene (ng/m^3)	166	1175	< 0.001
Dietary intake of pyrene (μg/day)	2.34	4.47	0.126
1-OHP(μmol/mol)	1.26	1.92	0.753
1-OHP (ng/ml)	2.41	4.31	0.209
Non-coke oven workers			
Air pyrene (ng/m^3)	18.6	23.9	0.298
Dietary intake of pyrene (μg/day)	2.09	3.39	0.013
1-OHP(μmol/mol)	0.21	0.13	0.753
1-OHP (ng/ml)	0.37	0.28	0.967

[a] Geometric means were compared between summer and winter.

Table 4.3. Correlation coefficients for personal air PAH, dietary PAH intake, and urinary 1-OHP measurements.

Dependent Variable	Pearson	Spearman
Coke workers	0.19	**0.48****
Air pyrene	-0.17	-0.21
Dietary intake of pyrene	0.05	**0.55****
1-OHP(μmol/mol)	0.05	**0.52****
1-OHP (ng/ml)		
Non-coke oven workers	0.20	**0.40****
Air pyrene	-0.07	0.03
Dietary intake of pyrene	-0.07	0.06
1-OHP(μmol/mol)	-0.07	-0.08
1-OHP (ng/ml)		

** $p < 0.01$

Table 4.4. Restricted maximum likelihood-estimates of between- (σ_b^2) and within-person covariance (σ_w^2) parameters and intraclass correlation coefficients (ρ_r) based on mixed-effects models.

Dependent Variable	Random effects only		Random + fixed effects		ICC (ρ_r)
	σ_b^2	σ_w^2	σ_b^2	σ_w^2	
Coke workers					
Air pyrene	0.94	1.68	1.12	1.18	0.49
Dietary intake of pyrene	0.97	1.21	1.00	1.18	0.46
1-OHP(μmol/mol)	1.05	1.29	1.06	1.27	0.46
1-OHP (ng/ml)	1.04	1.35	1.06	1.30	0.45
Non-coke oven workers					
Air pyrene	1.13	1.22	1.11	1.22	0.48
Dietary intake of pyrene	0.99	1.25	1.01	1.22	0.45
1-OHP(μmol/mol)	0.98	1.30	0.93	1.28	0.43
1-OHP (ng/ml)	0.96	1.34	0.82	1.33	0.42

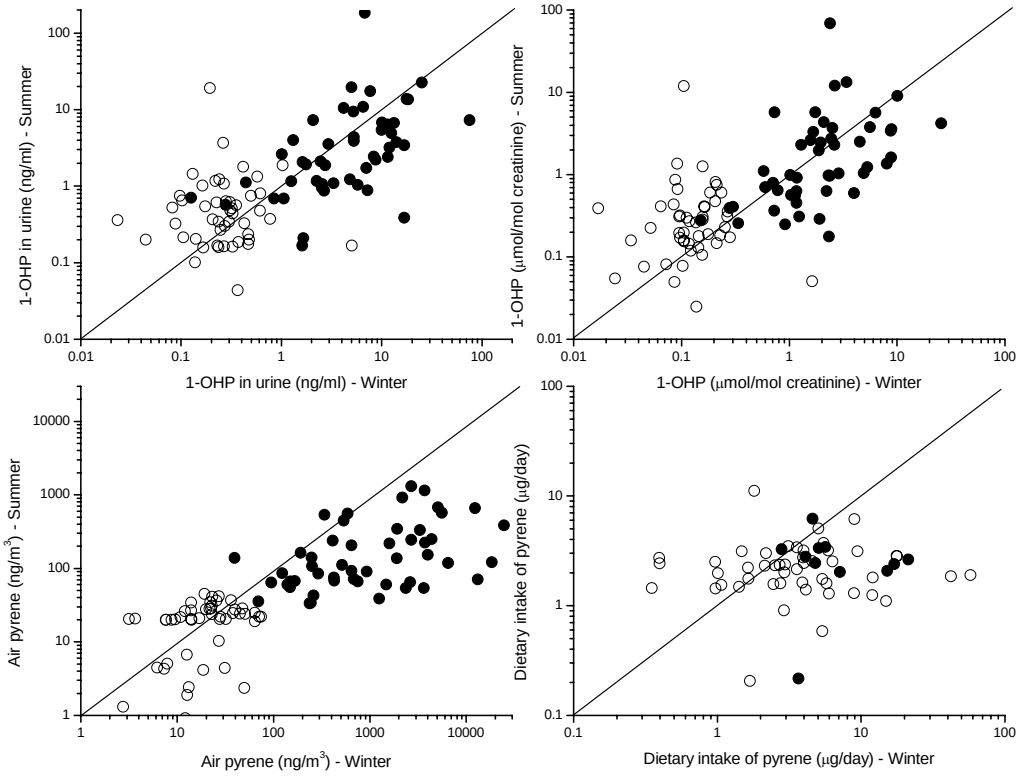

Figure 4.1. Scatter plots of urinary 1-OHP levels and personal air PAH concentrations during winter and summer. 1:1 line indicated on each plot. Closed circle represents coke oven workers and open circle represents non-coke oven workers.

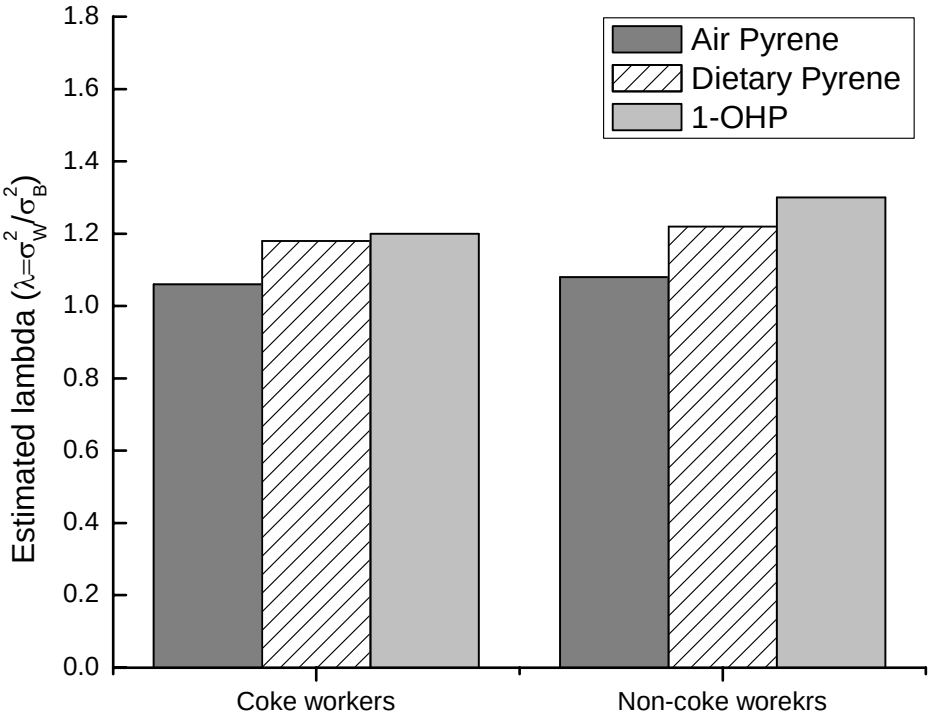

Figure 4.2. Estimated lambda (λ) values of personal air pyrene, dietary pyrene intake and urinary 1-OHP for coke oven workers and non-coke oven workers.

Chapter 5

Summary, Study Limitations and Future Research Directions

5.1 Conclusions

This dissertation research examined the validity of urinary 1-OHP as a biomarker of PAH exposure for coke oven workers and non-coke oven workers. Personal air pyrene/BaP, dietary intakes of pyrene/BaP, and urine samples were collected during winter and following summer. 1-OHP concentrations in frist morning urine were compared with those in 24-hour composite voids intra- and inter-individually. The usability of urinary 1-OHP was also examined in predicting personal air pyrene/BaP concentration. Seasonal variability and between- and within-person variances were investigated to evaluate the reproducibility of urinary 1-OHP measurement.

First morning urines samples were often used to assess PAH exposures in environmental settings. However, it still remains unknown as to how well the first morning urinary 1-OHP concentrations can reflect the daily average 1-OHP concentrations. The results in Chapter 2 showed that medians of first morning urinary 1-OHP concentrations were always higher than those of 24-hour urinary 1-OHP concentrations regadless of work environment and sampling periods. Significant intra-individual differences were found between 1-OHP concentrations in the first morning urine and that of 24-hour urine. The median difference concentrations were 0.76 ng/mL. 1-OHP concentrations in the first morning urine were 51% higher than those in 24-hour urine voids. Stratified analysis by work places showed that the concentration differences

between the paired samples were higher in coke oven workers than non-coke oven workers. Although significant concentration differences were found, two paired samples showed 96% of agreement. Correlation between two sample types was examined for inter-person comparison. An increase of 1 ng/mL of 1-OHP concentration in first morning urine resulted in 0.48 ng/mL increase in 24-hour urinary 1-OHP concentrations for coke oven workers and 0.36 ng/mL increase in 24-hour urinary 1-OHP concentrations for non-coke oven workers. We observed that the correlations and variance explanations between first morning urine and 24-hour urine were higher in winter than those in summer. Creatinine adjustment effects on urine samples were investigated. Although creatinine adjustement did not reduce intra-individual differences, it increased the variance explanation in regression analyses.

We used reverse dosimetry approach to use urinary 1-OHP concentrations to predict personal air concentrations of pyrene/BaP. The result in Chapter 3 showed that the relationships between personal air concentrations of pyrene/BaP and urinary 1-OHP were significant. However, no significant associations were observed between personal air concentrations of pyrene/BaP and urinary 1-OHP concentrations when pyrene concentrations in the air were below 49 ng/m^3 or BaP concentrations in the air were below 20 ng/m^3. No significant associations between dietary intakes of pyrene/BaP and urinary 1-OHP concentrations were observed. Given the results, 0.46 μmol/mol of urinary 1-OHP levels were suggested as threshold values. Regression analyses were performed with the data above the threshold value. The results showed that an increase of 1 μmol/mol of urinary 1-OHP was equivalent to 0.44 $\mu g/m^3$ of pyrene in the air and 0.26 $\mu g/m^3$ of pyrene in the air for male and female, respectively. An increase of 1μmol/mol

of urinary 1-OHP increased 0.12 μg/m^3 of BaP in the air and 0.13 μg/m^3 of BaP in the air for male and female, respectively. The comparison of personal air BaP concentrations with urinary 1-OHP levels of 2 μmol/mol in previous studies was conducted and the ranges of predicted BaP concentrations were comparable with those studies.

Seasonal variability and between- and within-person variability were examined in Chapter 4. Ranges of urinary 1-OHP levels were up to five orders of magnitude for both coke oven workers and non-coke oven workers. The results confirmed that personal air pyrene concentrations in winter were higher than those in summer for coke oven workers. Although urinar 1-OHP in winter were higher than those in summer, no significant differences were found between seasons. For non-coke oven workers, only dietary intakes of pyrene were higher in winter than those in summer. Intraclass correlation coefficients (ICCs) were calculated to estimate reproducibility of urinary 1-OHP measurements. For coke oven workers, personal air pyrene had the highest estimate of reproducibility, with an ICC of 0.49, followed by dietary pryene intakes (ICC=0.46), and urinary 1-OHP (ICC=0.46). For non-coke oven workers, the ICC values and trends for measured pollutants and urinary 1-OHP were similar as those for coke oven workers. Given the results, variance ratios for all measurements wer higher than 1 (smaller than 1 means less potential bias). The results suggested the importance of repeated measurements on same individuals to obtain a reliable measure of average daily PAH exposures.

5.2 Study limitations and Future Research Directions

Although intra-individual differences between first morning voids and 24-hour composite urines have been examined, concentrations in individual spot urine specimens

within a day were not measured because of the combination of 24-hour urine voids. Daily intra-individual differences among multiple spot voids and 24-hour composite urines were not answered in this dissertation research. Moreover, the variability between individual morning urine specimens was not determined because multiple spot urine specimens were not measured from the subjects during several days. Hence, whether a creatinine adjustment will improve consistency in urinary 1-OHP concentration among multiple spot urine specimens can not be drawn directly from this research. It is recommended that future work examine whether the variability in spot urine samples (or first morning urine) is decreased by creatinine adjustment in multiple spot urine specimens within a day or several days.

Relationships between personal air concentrations of PAHs and 1-OHP concentrations in urine were examined in Chapter 3. Results indicated that no significant association was found when urinary 1-OHP level was below 0.46 μmol/(mol creatinine). This may result from several confounding factors. One possible explanation is the contribution of ingested PAHs. Researchers demonstrated that diet is a major route of PAH exposure for non-occupationally exposed populations. However, previous studies did not quantify the contribution of dietary PAH intake on urinary 1-OHP concentrations. Results from this dissertation research were consistent with those from previous studies. Although the metabolism of ingested PAHs is different from inhaled PAHs, an ingested dose still appears as 1-OHP in urine.

Relatively large variabilities in personal air, dietary intake, and urinary 1-OHP were identified both between and within persons in Chapter 4. The smallest (within- to between-person) variance ratio should be the least biasing surrogate for PAH exposure.

The highest variance ratio in urinary 1-OHP concentration may relate with relatively short half-life of 6-12 hours. In addition, this dissertation research was constrained by the limited number of samples and by only two repeated measurements per subject during 6 to 9 months. The small sample size particularly limited the power to draw conclusions in stratified comparison by work environments. Because this sample included only coke oven workers and non-coke urban workers, the results may generalize only to highly polluted industrial areas and their vicinities. Due to the limited number of sample size, only two covariates were used to explain the variance structure. Measurement error effects were only considered in the context of individual based measurement. The results in this dissertation study based on individual measurements are somewhat different from those in a group based study, where the mean health outcome for each group is compared with the corresponding group mean of the exposure measure. It is recommended that future work consider repeated measurements to reduce measurement error rather than only one measurement within the time period of interest.

Appendix

SAS Program Used in this Dissertation

```
options nodate nonumber;
run;

* data import;
PROC IMPORT OUT= WORK.data
        DATAFILE= "G:\Backup\Inkyu\Mypaper\Ongoing\UMDNJ\data\verified_data.xls"
        DBMS=EXCEL REPLACE;
    SHEET="'test$'";
    GETNAMES=YES;
    MIXED=NO;
    SCANTEXT=YES;
    USEDATE=YES;
    SCANTIME=YES;
RUN;

* check variables on imported data;
proc contents data=work.data;
run;

proc univariate data=work.data;
        var um1ohpngml ut1ohpngml uohpngml;
run;

data work.data2;
        set work.data;
        logtpyr=log10(tpyr); logtbap=log10(tbap); logtpah=log10(tpah);
        fpyring=fpyr*food/1000; fbaping=fbap*food/1000; fpahing=fpah*food/1000;
   *    Male = 1.02x16hr(normal) + 0.62x8hr(sleep)     Female = 1.02x16hr(normal) + 0.51x8hr(sleep);
        tpyrinh=tpyr*20/1000; tbapinh=tbap*20/1000; tpahinh=tpah*20/1000;
        tpyrratio=tpyrinh/(tpyrinh+fpyring);                    tbapratio=tbapinh/(tbapinh+fbaping);
        tpahratio=tpahinh/(tpahinh+fpahing);
run;

proc print data=work.data2;
        var tpyrratio -- tpahratio;
run;

proc univariate data=work.data;
        class season;
        var tpyr tbap tpah;
run;

* PAH intake ratio by route;
proc univariate data=work.data2;
        where tpyr > 50;
        title "air pyrene >50";
        var tpyrratio -- tpahratio;
run;
proc univariate data=work.data2;
        where tpyr <= 50;
        title "air pyrene <=50";
        var tpyrratio -- tpahratio;
run;
proc univariate data=work.data2;
        where tbap > 20;
        title "air BaP >20";
        var tpyrratio -- tpahratio;
run;
proc univariate data=work.data2;
        where tbap <= 20;
        title "air BaP <=20";
        var tpyrratio -- tpahratio;
```

```
run;

* Wilcoxon singed rank sum test to compare personal PAH exposure by season;
PROC IMPORT OUT= WORK.paired
        DATAFILE= "G:\Backup\Inkyu\Mypaper\Ongoing\UMDNJ\data\verified_paired.xls"
        DBMS=EXCEL REPLACE;
    SHEET="'sheet1$'";
    GETNAMES=YES;
    MIXED=NO;
    SCANTEXT=YES;
    USEDATE=YES;
    SCANTIME=YES;
RUN;
data work.paired;
            set work.paired;
            if subject=' ' then delete;
            tpyrdiff=tpyr-tpyr1; tbapdiff=tbap-tbap1; tpahdiff=tpah-tpah1;
            fpyrdiff=(fpyr*food-fpyr1*food1)/1000;     fbapdiff=(fbap*food-fbap1*food1)/1000;     fpahdiff=(fpah*food-
            fpah1*food1)/1000;
            uohpngdiff=uohpngml-uohpngml1; um1ohpdiff=um1ohp-um1ohp1; ut1ohpdiff=ut1ohp-ut1ohp1;
            drop f63--f105;
run;
proc univariate data=work.paired;
            var tpyrdiff -- ut1ohpdiff;
run;

proc univariate data=work.data2;
            var logtpyr;
run;
proc univariate data=work.data2 noprint;
            var tpyr tbap tpah fpyring fbaping fpahing uohpngml ut1ohp;
            output out=work.percentiles pctlpts=0 to 100 by 5 pctlpre=tpyr_ tbap_ tpah_ fpyring_ fbaping_ fpahing_
            uohpngml_ ut1ohp_
            pctlname=p0 p05 p10 p15 p20 p25 p30 p35 p40 p45 p50 p55 p60 p65 p70 p75 p80 p85 p90 p95 p100;
run;
proc print data=work.percentiles;
run;
* Added july 17;
proc univariate data=work.data2 noprint;
            var tpyr tbap tpah uohpngml ut1ohp;
            output out=work.percentiles pctlpts=0 to 100 by 10 pctlpre=tpyr_ tbap_ tpah_ uohpngml_ ut1ohp_
            pctlname=p0 p10 p20 p30 p40 p50 p60 p70 p80 p90 p100;
run;
proc print data=work.percentiles;
run;


data work.data3;
            set work.data2;
            pyrbapratio=tpyr/tbap;
            if tpyr le 10 then rgr=1;
            else if 10<tpyr<50 then rgr=2;
            else rgr=3;
run;
/*
data work.data3;
            set work.data2;
            pyrbapratio=tpyr/tbap;
            if tpyr le 50 then rgr=1;
            else rgr=2;
run;
*/
```

```
proc sort data=work.data3; by rgr; run;
proc plot data=work.data3;
        by rgr;
        plot ut1ohp*tpyr;
run; quit;
proc univariate data=work.data3;
        var pyrbapratio ut1ohp;
run;
proc univariate data=work.data3;
        class rgr;
        var pyrbapratio ut1ohp;
run;

data work.data4;
        set work.data2;
        logut1ohp=log10(ut1ohp);
        if logtpyr le 1.32 then logrgr=1;
        else if 1.32<logtpyr<1.69 then logrgr=2;
        else if 1.69<logtpyr<2.40 then logrgr=3;
        else logrgr=4;
        if ut1ohp < 0.87 then bgl=1;
        else bgl=2;
run;
proc sort data=work.data4; by logrgr; run;
proc plot data=work.data4;
        by logrgr;
        plot ut1ohp*logtpyr;
run; quit;
proc sort data=work.data4; by bgl; run;
proc plot data=work.data4;
        by bgl;
        plot ut1ohp*tpyr;
        plot logut1ohp*logtpyr;
run;


/*****************************************************************
*
*                       Chapter 3 Dose-response relationship
*
*****************************************************************/

* personal air exposure;
proc univariate data=work.data;
        var tpyr tbap tpah;
run;

*dietary exposure;
proc univariate data=work.data;
        var fpyr fbap fpah;
run;

* making group based on percentiles;
data work.data2;
        set work.data;
        if tpyr le 3.9 then exgroup=1; *5%;
        else if 3.9 < tpyr le 7.8 then exgroup=2; *5-10%;
        else if 7.8 <tpyr le 20.8 then exgroup=3; *10-25%;
        else if 20.8 < tpyr le 48.7 then exgroup=4; *25-50%;
        else if 48.7 < tpyr le 249.6 then exgroup=5; *50-75%;
        else if 249.6 < tpyr le 1750 then exgroup=6; *75-90%;
        else if 1750 < tpyr le 3709 then exgroup=7; *90-95%;
```

```
        else exgroup=8;
run;

proc freq data=work.data2;
        table exgroup;
run;

proc means data=work.data2 n mean std median maxdec=3;
        var uohpngml ut1ohp;
run;
proc means data=work.data2 n mean std median maxdec=3;
        class exgroup;
        var uohpngml ut1ohp;
run;

* percentile increase by 10%;
proc univariate data=work.data all noprint ;
        var tpyr tbap tpah fpyr fbap fpah ut1ohp uohpngml;
        output out=work.percentiles pctlpts=0 to 100 by 5 pctlpre=tpyr_ tbap_ tpah_ fpyr_ fbap_ fpah_ ut1ohp_
        ohpngml_
        pctlname=p0 p05 p10 p15 p20 p25 p30 p35 p40 p45 p50 p55 p60 p65 p70 p75 p80 p85 p90 p95 p100;
run;
ods rtf file="c:\percentile.rtf";
proc print data=work.percentiles;
run;
ods rtf close;
data work.data4;
        set work.data;
        /* Air strata */
        if tpyr le 7.77 then tpgroup=1;
        else if 7.77 < tpyr le 19.9 then tpgroup=2;
        else if 19.9 < tpyr le 22.9 then tpgroup=3;
        else if 22.9 < tpyr le 31.1 then tpgroup=4;
        else if 31.1 < tpyr le 48.7 then tpgroup=5;
        else if 48.7 < tpyr le 75.1 then tpgroup=6;
        else if 75.1 < tpyr le 178.5 then tpgroup=7;
        else if 178.5 < tpyr le 437.1 then tpgroup=8;
        else if 437.1 < tpyr le 1750 then tpgroup=9;
        else tpgroup=10;
                if tbap le 1.46 then tbapgroup=1;
        else if 1.46 < tbap le 4.91 then tbapgroup=2;
        else if 4.91 < tbap le 8.37 then tbapgroup=3;
        else if 8.37 < tbap le 13.8 then tbapgroup=4;
        else if 13.8 < tbap le 19.7 then tbapgroup=5;
        else if 19.7 < tbap le 36.3 then tbapgroup=6;
        else if 36.3 < tbap le 127.1 then tbapgroup=7;
        else if 127.1 < tbap le 307.2 then tbapgroup=8;
        else if 307.2 < tbap le 789.9 then tbapgroup=9;
        else tbapgroup=10;
                        if tpah le 476 then tpahgroup=1;
        else if 476 < tpah le 593 then tpahgroup=2;
        else if 593 < tpah le 775 then tpahgroup=3;
        else if 775 < tpah le 1272 then tpahgroup=4;
        else if 1272 < tpah le 2060 then tpahgroup=5;
        else if 2060 < tpah le 3241 then tpahgroup=6;
        else if 3241 < tpah le 5062 then tpahgroup=7;
        else if 5062 < tpah le 7952 then tpahgroup=8;
        else if 7952 < tpah le 17412 then tpahgroup=9;
        else tpahgroup=10;
        /*food strata */
                if fpyring le 0.97 then fpyringgroup=1;
        else if 0.97 < fpyr le 1.55 then fpyrgroup=2;
```

```
            else if 1.55 < fpyr le 1.81 then fpyrgroup=3;
            else if 1.81 < fpyr le 2.33 then fpyrgroup=4;
            else if 2.33 < fpyr le 2.71 then fpyrgroup=5;
            else if 2.71 < fpyr le 3.27 then fpyrgroup=6;
            else if 3.27 < fpyr le 4.14 then fpyrgroup=7;
            else if 4.14 < fpyr le 5.75 then fpyrgroup=8;
            else if 5.75 < fpyr le 8.97 then fpyrgroup=9;
            else fpyrgroup=10;
                        if fbap le 0.21 then fbapgroup=1;
            else if 0.21 < fbap le 0.30 then fbapgroup=2;
            else if 0.30 < fbap le 0.36 then fbapgroup=3;
            else if 0.36 < fbap le 0.42 then fbapgroup=4;
            else if 0.42 < fbap le 0.48 then fbapgroup=5;
            else if 0.48 < fbap le 0.57 then fbapgroup=6;
            else if 0.57 < fbap le 0.75 then fbapgroup=7;
            else if 0.75 < fbap le 0.91 then fbapgroup=8;
            else if 0.91 < fbap le 1.37 then fbapgroup=9;
            else fbapgroup=10;
                                    if fpah le 22.2 then fpahgroup=1;
            else if 22.2 < fpah le 27.7 then fpahgroup=2;
            else if 27.7 < fpah le 34.6 then fpahgroup=3;
            else if 34.6 < fpah le 43.6 then fpahgroup=4;
            else if 43.6 < fpah le 53.2 then fpahgroup=5;
            else if 53.2 < fpah le 65.4 then fpahgroup=6;
            else if 65.4 < fpah le 83.9 then fpahgroup=7;
            else if 83.9 < fpah le 103.7 then fpahgroup=8;
            else if 103.7 < fpah le 150.8 then fpahgroup=9;
            else fpahgroup=10;
            loguohpng=log(uohpngml);
run;

proc sort data=work.data4; by season; run;
proc glm data=work.data4;
            by season;
            class tpgroup fpyrgroup season;
            model uohpngml=tpgroup/solution clparm;
run;quit;
proc glm data=work.data4;
            class tpgroup fpyrgroup season;
            model uohpngml=tpgroup fpyrgroup season/solution clparm;
run;quit;
proc mixed data=work.data4;
            class tpgroup subject season;
            model uohpngml=tpgroup/solution;
            random subject;
            repeated season;
run;

proc univariate data=work.data4;
            class tbapgroup;
            var uohpngml;
run;

/* making percetiles based on urine */

data work.data5;
            set work.data;
            if uohpngml < 0.3 then urinegroup=1;
            else if 0.3 <= uohpngml < 0.85 then urinegroup=2;
            else if 0.85 <= uohpngml < 3.07 then urinegroup=3;
            else if 3.07 <= uohpngml < 8.59 then urinegroup=4;
            else if 8.59 <= uohpngml < 10.26 then urinegroup=5;
```

```
            else urinegroup=6;
            logtpyr=log10(tpyr); loguohpngml=log10(uohpngml);
run;

proc sort data=work.data5; by urinegroup; run;
proc glm data=work.data5;
            class urinegroup;
            model uohpngml=urinegroup/solution;
run;quit;

proc plot data=work.data5;
            by urinegroup;
            plot uohpngml*tpyr;
run;quit;

proc print data=work.data5;
            var uohpngml urinegroup;
run;

proc glm data=work.data5;
            where 10> tpyr;
            class urinegroup;
            model loguohpngml=logtpyr/solution;
run;quit;

proc glm data=work.data5;
            where 10< tpyr<=75;
            class urinegroup;
            model loguohpngml=logtpyr/solution;
run;quit;

proc glm data=work.data5;
            where 50< tpyr;
            class urinegroup;
            model loguohpngml=logtpyr/solution;
run;quit;


proc plot data=work.data5;
            where 10< tpyr<=75;
            plot loguohpngml*logtpyr;
run;quit;

data work.data9;
            set work.data;
            /* Air strata */
            if tpyr le 3.9 then tpgroup=1;
            else if 3.9 < tpyr le 7.77 then tpgroup=2;
            else if 7.77 < tpyr le 13.6 then tpgroup=3;
            else if 13.6 < tpyr le 19.9 then tpgroup=4;
            else if 19.9 < tpyr le 20.8 then tpgroup=5;
            else if 20.8 < tpyr le 22.9 then tpgroup=6;
            else if 22.9 < tpyr le 27.0 then tpgroup=7;
            else if 27.0 < tpyr le 31.1 then tpgroup=8;
            else if 31.1 < tpyr le 37.5 then tpgroup=9;
            else if 37.5 < tpyr le 48.7 then tpgroup=10;
            else if 48.7 < tpyr le 64.8 then tpgroup=11;
            else if 64.8 < tpyr le 75.1 then tpgroup=12;
            else if 75.1 < tpyr le 122.2 then tpgroup=13;
            else if 122.2 <tpyr le 178.5 then tpgroup=14;
            else if 178.5 < tpyr le 250 then tpgroup=15;
            else if 250 < tpyr le 438 then tpgroup=16;
```

```
        else if 438 < tpyr le 669 then tpgroup=17;
        else if 669 < tpyr le 1750 then tpgroup=18;
        else if 1750 < tpyr le 3710 then tpgroup=19;
        else tpgroup=20;
run;
ods rtf file="c:\percent.rtf";
proc means data=work.data9 n mean median clm;
        class tpgroup;
        var uohpngml;
run;
proc univariate data=work.data9;
        class tpgroup;
        var uohpngml;
run;
ods rtf close;

proc print data=work.data9;
        where tpgroup=18;
run;

proc plot data=work.data9;
        plot uohpngml*tpgroup;
run;quit;

/*****************************************************************
*/
data work.data41;
        set work.data2;
        /* Air strata */
        if tpyr le 7.77 then tpgroup=1;
        else if 7.77 < tpyr le 19.9 then tpgroup=2;
        else if 19.9 < tpyr le 22.9 then tpgroup=3;
        else if 22.9 < tpyr le 31.1 then tpgroup=4;
        else if 31.1 < tpyr le 48.7 then tpgroup=5;
        else if 48.7 < tpyr le 75.1 then tpgroup=6;
        else if 75.1 < tpyr le 178.5 then tpgroup=7;
        else if 178.5 < tpyr le 437.1 then tpgroup=8;
        else if 437.1 < tpyr le 1750 then tpgroup=9;
        else tpgroup=10;
                if tbap le 1.46 then tbapgroup=1;
        else if 1.46 < tbap le 4.91 then tbapgroup=2;
        else if 4.91 < tbap le 8.37 then tbapgroup=3;
        else if 8.37 < tbap le 13.8 then tbapgroup=4;
        else if 13.8 < tbap le 19.7 then tbapgroup=5;
        else if 19.7 < tbap le 36.3 then tbapgroup=6;
        else if 36.3 < tbap le 127.1 then tbapgroup=7;
        else if 127.1 < tbap le 307.2 then tbapgroup=8;
        else if 307.2 < tbap le 789.9 then tbapgroup=9;
        else tbapgroup=10;
                        if tpah le 476 then tpahgroup=1;
        else if 476 < tpah le 593 then tpahgroup=2;
        else if 593 < tpah le 775 then tpahgroup=3;
        else if 775 < tpah le 1272 then tpahgroup=4;
        else if 1272 < tpah le 2060 then tpahgroup=5;
        else if 2060 < tpah le 3241 then tpahgroup=6;
        else if 3241 < tpah le 5062 then tpahgroup=7;
        else if 5062 < tpah le 7952 then tpahgroup=8;
        else if 7952 < tpah le 17412 then tpahgroup=9;
        else tpahgroup=10;
        /*food strata */
                if fpyring le 1.07 then fpyringgroup=1;
        else if 1.07 < fpyring le 1.52 then fpyringgroup=2;
```

```
            else if 1.52 < fpyring le 2.09 then fpyringgroup=3;
            else if 2.09 < fpyring le 2.43 then fpyringgroup=4;
            else if 2.43 < fpyring le 3.16 then fpyringgroup=5;
            else if 3.16 < fpyring le 3.86 then fpyringgroup=6;
            else if 3.86 < fpyring le 4.78 then fpyringgroup=7;
            else if 4.78 < fpyring le 7.17 then fpyringgroup=8;
            else if 7.17 < fpyring le 9.72 then fpyringgroup=9;
            else fpyringgroup=10;
                        if fbaping le 0.20 then fbapinggroup=1;
            else if 0.20 < fbaping le 0.31 then fbapinggroup=2;
            else if 0.31 < fbaping le 0.40 then fbapinggroup=3;
            else if 0.40 < fbaping le 0.46 then fbapinggroup=4;
            else if 0.46 < fbaping le 0.54 then fbapinggroup=5;
            else if 0.54 < fbaping le 0.69 then fbapinggroup=6;
            else if 0.69 < fbaping le 0.88 then fbapinggroup=7;
            else if 0.88 < fbaping le 1.05 then fbapinggroup=8;
            else if 1.05 < fbaping le 1.57 then fbapinggroup=9;
            else fbapinggroup=10;
                                    if fpahing le 21.0 then fpahinggroup=1;
            else if 21.0 < fpahing le 29.0 then fpahinggroup=2;
            else if 29.0 < fpahing le 40.0 then fpahinggroup=3;
            else if 40.0 < fpahing le 50.0 then fpahinggroup=4;
            else if 50.0 < fpahing le 58.0 then fpahinggroup=5;
            else if 58.0 < fpahing le 72.0 then fpahinggroup=6;
            else if 72.0 < fpahing le 101 then fpahinggroup=7;
            else if 101 < fpahing le 131 then fpahinggroup=8;
            else if 131 < fpahing le 176 then fpahinggroup=9;
            else fpahinggroup=10;
run;
%macro percentile(group);
proc univariate data=work.data41;
            class &group;
            var uohpngml;
run;
%mend percentile;
%percentile(fpyringgroup); %percentile(fbapinggroup); %percentile(fpahinggroup);

*** Regression model based on concentrations;

*Pooled data crude model;
%macro crude(var);
proc mixed data=work.data41;
            title "Crude model with single PAH compounds only";
            model loguohpng= &var /solution cl;
run;
%mend crude;
%crude(logtpyr); %crude(logtbap); %crude(logtpah);
%crude(logfpyr); %crude(logfbap); %crude(logfpah);

* Pyrene : 0-10, 10-50, >50 3 steps & BaP: 0-20, >20;
proc mixed data=work.data41;
            where tpyr < 10;
            title "0 < pyrene < 10";
            model loguohpng= logtpyr /solution cl;
run;
proc mixed data=work.data41;
            where 10 <= tpyr < 50;
            title "10 < pyrene < 50";
            model loguohpng= logtpyr /solution cl;
run;
proc mixed data=work.data41;
            where tpyr>50;
```

```
            title " pyrene > 50";
            model loguohpng= logtpyr /solution cl;
run;

* BaP: 0-20, >20 -- 2 steps;
proc mixed data=work.data41;
            where tbap < 20;
            title "0 < BaP < 20";
            model loguohpng= logtbap /solution cl;
run;
proc mixed data=work.data41;
            where tbap > 20;
            title "BaP > 20";
            model loguohpng= logtbap /solution cl;
run;

*Pooled data with adjusted single model;
%macro adj(var);
proc mixed data=work.data41;
            title "Crude model with single PAH compounds adjusted for age, gender, BMI";
            class subject gender season;
            model loguohpng= &var age gender BMI/solution cl;
            random subject;
            repeated season;
run;
%mend adj;
%adj(logtpyr); %adj(logtbap); %adj(logtpah);
%adj(logfpyr); %adj(logfbap); %adj(logfpah);

* Pyrene : 0-10, 10-50, >50 3 steps & BaP: 0-20, >20;
proc mixed data=work.data41;
            class subject gender season;
            where tpyr < 10;
            title "0 < pyrene < 10 adjusted for age, gender, BMI";
            model loguohpng= logtpyr age gender BMI /solution cl;
            random subject;
            repeated season;
run;
proc mixed data=work.data41;
            class subject gender season;
            where 10 <= tpyr < 50;
            title "10 < pyrene < 50 adjusted for age, gender, BMI";
            model loguohpng= logtpyr age gender BMI/solution cl;
            random subject;
            repeated season;
run;
proc mixed data=work.data41;
            class subject gender season;
            where tpyr>50;
            title " pyrene > 50 adjusted for age, gender, BMI";
            model loguohpng= logtpyr age gender BMI/solution cl;
            random subject;
            repeated season;
run;

* BaP: 0-20, >20 -- 2 steps;
proc mixed data=work.data41;
            class subject gender season;
            where tbap < 20;
            title "0 < BaP < 20 adjusted for age, gender, BMI";
            model loguohpng= logtbap age gender BMI/solution cl;
            random subject;
```

```
        repeated season;
run;
proc mixed data=work.data41;
        class subject gender season;
        where tbap > 20;
        title "BaP > 20 adjusted for age, gender, BMI";
        model loguohpng= logtbap age gender BMI/solution cl;
        random subject;
        repeated season;
run;

*Pooled data crude model adjusted for dietary PAH;
%macro total(var1, var2);
proc mixed data=work.data41;
        title "Inhalation and ingestion";
        model loguohpng= &var1  | &var2  /solution cl;
run;
%mend total;
%total(logtpyr, logfpyr); %total(logtbap, logfpyr); %total(logtpah, logfpah);

* Pyrene : 0-10, 10-50, >50 3 steps & BaP: 0-20, >20 adjusted for dietary PAH;
proc mixed data=work.data41;
        where tpyr < 10;
        title "0 < pyrene < 10 adjusted for dietary pyrene";
        model loguohpng= logtpyr|logfpyr /solution cl;
run;
proc mixed data=work.data41;
        where 10 <= tpyr < 50;
        title "10 < pyrene < 50 adjusted for dietary pyrene";
        model loguohpng= logtpyr|logfpyr /solution cl;
run;
proc mixed data=work.data41;
        where tpyr>50;
        title " pyrene > 50 adjusted for dietary pyrene";
        model loguohpng= logtpyr|logfpyr /solution cl;
run;

* BaP: 0-20, >20 -- 2 steps;
proc mixed data=work.data41;
        where tbap < 20;
        title "0 < BaP < 20 adjusted for dietary BaP";
        model loguohpng= logtbap|logfbap /solution cl;
run;
proc mixed data=work.data41;
        where tbap > 20;
        title "BaP > 20 adjsuted for dietary BaP";
        model loguohpng= logtbap |logfbap/solution cl;
run;

*Pooled data crude model adjusted for dietary PAH with covariates;
%macro total(var1, var2);
proc mixed data=work.data41;
        class subject gender season;
        title "Inhalation and ingestion";
        model loguohpng= &var1  | &var2 age gender BMI /solution cl;
        random subject;
        repeated season;
run;
%mend total;
%total(logtpyr, logfpyr); %total(logtbap, logfpyr); %total(logtpah, logfpah);

* Pyrene : 0-10, 10-50, >50 3 steps & BaP: 0-20, >20 adjusted for dietary PAH with covariates;
```

```
proc mixed data=work.data41;
	class subject gender season;
	where tpyr < 10;
	title "0 < pyrene < 10 adjusted for dietary pyrene";
	model loguohpng= logtpyr|logfpyr age gender BMI /solution cl;
	random subject;
	repeated season;
run;
proc mixed data=work.data41;
	class subject gender season;
	where 10 <= tpyr < 50;
	title "10 < pyrene < 50 adjusted for dietary pyrene";
	model loguohpng= logtpyr|logfpyr age gender BMI/solution cl;
	random subject;
	repeated season;
run;
proc mixed data=work.data41;
	class subject gender season;
	where tpyr>50;
	title " pyrene > 50 adjusted for dietary pyrene";
	model loguohpng= logtpyr|logfpyr age gender BMI/solution cl;
	random subject;
	repeated season;
run;

* BaP: 0-20, >20 -- 2 steps;
proc mixed data=work.data41;
	class subject gender season;
	where tbap < 20;
	title "0 < BaP < 20 adjusted for dietary BaP";
	model loguohpng= logtbap|logfbap age gender BMI/solution cl;
	random subject;
	repeated season;
run;
proc mixed data=work.data41;
	class subject gender season;
	where tbap > 20;
	title "BaP > 20 adjsuted for dietary BaP";
	model loguohpng= logtbap |logfbap age gender BMI/solution cl;
	random subject;
	repeated season;
run;

/*****************************************
*****************************************/
data work.data5;
	set work.data2;
	if uohpngml lt 0.147 then nggroup=1;
	else if 0.147 =< uohpngml < 0.231 then nggroup=2;
	else if 0.231 =< uohpngml lt 0.356 then nggroup=3;
	else if 0.356 =< uohpngml lt 0.560 then nggroup=4;
	else if 0.560 =< uohpngml lt 0.847 then nggroup=5;
	else if 0.847 =< uohpngml lt 1.551 then nggroup=6;
	else if 1.551 =< uohpngml lt 2.526 then nggroup=7;
	else if 2.526 =< uohpngml lt 3.941 then nggroup=8;
	else if 3.941 =< uohpngml lt 8.587 then nggroup=9;
	else nggroup=10;
run;

proc means data=work.data5 n mean median min clm std maxdec=3;
	class nggroup;
	var tpyr uohpngml ut1ohp;
```

```
run;

proc means data=work.data5 n mean median min clm std maxdec=3;
        class nggroup;
        var tbap uohpngml ut1ohp;
run;

proc glm data=work.data5;
        where nggroup le 6;
        title " low exposure group";
        class nggroup;
        model uohpngml=nggroup;
        contrast 'compare group 1 vs. 2' nggroup 1 -1 0 0 0 0;
        contrast 'compare group 1 & 2 vs. 3' nggroup 1 1 -2 0 0 0;
        contrast 'compare group 1, 2, & 3 vs. 4' nggroup 1 1 1 -3 0 0;
        contrast 'compare group 1, 2, 3, & 4 vs. 5' nggroup 1 1 1 1 -4 0;
        contrast 'compare group 1, 2, 3, ,4 & 5 vs. 6' nggroup 1 1 1 1 1 -5;
run;quit;

proc glm data=work.data5;
        where nggroup gt 5;
        title " high exposure group";
        class nggroup;
        model uohpngml=nggroup;
        contrast 'compare group 6 vs. 7' nggroup 1 -1 0 0 0;
        contrast 'compare group 6 & 7 vs. 8' nggroup 1 1 -2 0 0;
        contrast 'compare group 6, 7,  & 8 vs. 9' nggroup 1 1 1 -3 0;
        contrast 'compare group 6, 7, 8,  & 9 vs. 10' nggroup 1 1 1 1 -4;
run;quit;

proc glm data=work.data5;
        title " all exposure group";
        class nggroup;
        model uohpngml=nggroup;
        contrast 'compare group 1 vs. 2' nggroup 1 -1 0 0 0 0 0 0 0 0;
        contrast 'compare group 1 & 2 vs. 3' nggroup 1 1 -2 0 0 0 0 0 0 0;
        contrast 'compare group 1, 2, & 3 vs. 4' nggroup 1 1 1 -3 0 0 0 0 0 0;
        contrast 'compare group 1, 2, 3, & 4 vs. 5' nggroup 1 1 1 1 -4 0 0 0 0 0;
        contrast 'compare group 1, 2, 3, ,4 & 5 vs. 6' nggroup 1 1 1 1 1 -5 0 0 0 0;
        contrast 'comnpare group 1, 2, 3, 4, 5, & 6 vs. 7' nggroup 1 1 1 1 1 1 -6 0 0 0;
        contrast 'compare group 1, 2, 3, 4, 5, 6, & 7 vs. 8' nggroup 1 1 1 1 1 1 1 -7 0 0 ;
        contrast 'compare group 1, 2, 3, 4, 5, 6, 7, & 8 vs. 9' nggroup 1 1 1 1 1 1 1 1 -8 0;
        contrast 'compare group 1, 2, 3, 4, 5, 6, 7, 8, & 9 vs. 10' nggroup 1 1 1 1 1 1 1 1 1 -9;
run;quit;

* Pyrene;
proc glm data=work.data5;
        where nggroup le 6;
        title " low exposure group";
        class nggroup;
        model tpyr=nggroup /group;
        contrast 'compare group 1 vs. 2' nggroup 1 -1 0 0 0 0;
        contrast 'compare group 1 & 2 vs. 3' nggroup 1 1 -2 0 0 0;
        contrast 'compare group 1, 2, & 3 vs. 4' nggroup 1 1 1 -3 0 0;
        contrast 'compare group 1, 2, 3, & 4 vs. 5' nggroup 1 1 1 1 -4 0;
        contrast 'compare group 1, 2, 3, ,4 & 5 vs. 6' nggroup 1 1 1 1 1 -5;
run;quit;

proc mixed data=work.data5;
        where nggroup gt 5;
        title " high exposure group";
        class nggroup;
```

```
            model tpyr=nggroup;
            contrast 'compare group 6 vs. 7' nggroup 1 -1 0 0 0;
            contrast 'compare group 6 & 7 vs. 8' nggroup 1 1 -2 0 0;
            contrast 'compare group 6, 7,  & 8 vs. 9' nggroup 1 1 1 -3 0;
            contrast 'compare group 6, 7, 8,  & 9 vs. 10' nggroup 1 1 1 1 -4;
run;quit;

proc glm data=work.data5;
            title " all exposure group";
            class nggroup;
            model tpyr=nggroup;
            contrast 'compare group 1 vs. 2' nggroup 1 -1 0 0 0 0 0 0 0 0;
            contrast 'compare group 1 & 2 vs. 3' nggroup 1 1 -2 0 0 0 0 0 0 0;
            contrast 'compare group 1, 2, & 3 vs. 4' nggroup 1 1 1 -3 0 0 0 0 0 0;
            contrast 'compare group 1, 2, 3, & 4 vs. 5' nggroup 1 1 1 1 -4 0 0 0 0 0;
            contrast 'compare group 1, 2, 3, ,4 & 5 vs. 6' nggroup 1 1 1 1 1 -5 0 0 0 0;
            contrast 'comnpare group 1, 2, 3, 4, 5, & 6 vs. 7' nggroup 1 1 1 1 1 1 -6 0 0 0;
            contrast 'compare group 1, 2, 3, 4, 5, 6, & 7 vs. 8' nggroup 1 1 1 1 1 1 1 -7 0 0 ;
            contrast 'compare group 1, 2, 3, 4, 5, 6, 7, & 8 vs. 9' nggroup 1 1 1 1 1 1 1 1 -8 0;
            contrast 'compare group 1, 2, 3, 4, 5, 6, 7, 8, & 9 vs. 10' nggroup 1 1 1 1 1 1 1 1 1 -9;
run;quit;

symbol i=join r=10 l=1;
proc gplot data=work.data5;
            plot tpyr*season=subject;
            plot uohpngml*season=subject;
run;

proc mixed data=work.data5;
            where nggroup le 5;
            class season;
            model tpyr=uohpngml /solution;
run;

proc mixed data=work.data5;
            where nggroup gt 5;
            class subject gender season;
            model tpyr=uohpngml age gender /solution;
            repeated season;
run;

proc mixed data=work.data5;
            where nggroup gt 6;
            class subject gender season;
            model tpyr=uohpngml / solution noint;
            random subject;
run;

symbol1 i=join  l=1;
symbol2 i=join  l=2;
symbol3 i=join  l=3;
symbol4 i=join  l=4;
symbol5 i=join  l=5;
proc gplot data=work.data5;
            where nggroup le 6;
            plot tpyr*uohpngml=subject;
run;

proc print data=work.data5;
            where nggroup le 6;
            var tpyr uohpngml;
run;
```

```
proc freq data=work.data5;
	tables tpyr;
run;

data work.data6;
	set work.data5;
	if subject='0-051' then delete; * delete outlier when tpyr=13187.45;
run;

proc print data=work.data6;
	var tpyr;
run;
proc gplot data=work.data6;
	where nggroup gt 6;
	plot tpyr*uohpngml=subject;
run;
proc print data=work.data6;
	var uohpngml;
run;
data work.data6;
	set work.data6;
	if subject='0-081' then delete; * delete outlier when 1-ohp(ng/ml)=47;
run;

proc mixed data=work.data6;
	where nggroup gt 5;
	class subject gender season;
	model tpyr=uohpngml / solution;
	random subject;
run;

proc gplot data=work.data5;
	where group = 2;
	plot tpyr*uohpngml=subject;
run;quit;
proc mixed data=work.data6;
	where nggroup le 5;
	class subject gender season;
	model tpyr=uohpngml / solution;
	random subject;
run;

proc print data=work.data6;
	where subject='0-100';
run;

* May ;

proc sort data=work.data5; by subject; run;
proc print data=work.data5;
	by subject;
	var  season group nggroup;
run;


data work.high;
	set work.data5;
	if rgr='High' then output;
run;
ods html;
ods graphics on;
```

```
proc mixed data=work.high method=ml;
	where rgr='High';
	class subject gender season group;
	model tbap=uohpngml /solution influence(iter=10 estimates);
	repeated season / type=ar(1)  sub=subject(group) r rcorr;
	ods output influence=inf;
run;
ods graphics off;
ods html close;

data work.highxout;
	set work.high;
	if subject='0-060' then delete;
	else if subject='0-063' then delete;
	else if subject='0-073' then delete;
	else if subject='0-081' then delete;
run;
proc mixed data=work.highxout method=ml;
	where rgr='High';
	class subject gender season group;
	model tpyr=uohpngml /solution influence(iter=10 estimates);
	repeated season / type=ar(1)  sub=subject(group) r rcorr;
	ods output influence=inf;
run;
proc means data=work.highxout n mean median min max maxdec=3;
	class season;
	var tpyr uohpngml;
run;


data work.low;
	set work.data5;
	if rgr='Low' then output;
run;
proc mixed data=work.low;
	where rgr='Low';
	class subject gender season group;
	model tpyr=uohpngml /solution influence(iter=10 estimates);
	repeated season / type=ar(1)  sub=subject(group) r rcorr;
	ods output influence=inf;
run;
data work.lowxout;
	set work.low;
	if subject='0-029' then delete;
	else if subject='0-051' then delete;
run;
proc mixed data=work.lowxout;
	where rgr='Low';
	class subject gender season group;
	model tpyr=uohpngml /solution cl;
	repeated season / type=cs  sub=subject(group) r rcorr;
run;
proc mixed data=work.lowxout;
	where rgr='Low';
	class subject gender season group;
	model tbap=uohpngml /solution cl;
	repeated season / type=cs  sub=subject(group) r rcorr;
run;
/* Application of Heterogeneous Variance Models */
* Unstructured covariance;
proc mixed data=work.highxout;
	class subject gender season group;
```

```
            model tpyr=uohpngml/ solution;
            repeated season / type=un sub=subject(group) r rcorr;
run;
* Compound symmetry covariance;
proc mixed data=work.highxout;
            class subject gender season group;
            model tpyr=uohpngml/ solution cl;
            repeated season / type=cs sub=subject(group) r rcorr;
run;
proc mixed data=work.highxout;
            class subject gender season group;
            model tbap=uohpngml/ solution cl;
            repeated season / type=cs sub=subject(group) r rcorr;
run;
proc mixed data=work.highxout;
            class subject gender season group;
            model tpyr=ut1ohp/ solution cl;
            repeated season / type=cs sub=subject(group) r rcorr;
run;
proc mixed data=work.highxout;
            class subject gender season group;
            model tbap=ut1ohp/ solution cl;
            repeated season / type=cs sub=subject(group) r rcorr;
run;


* Autoregressive model;
proc mixed data=work.highxout;
            class subject gender season group;
            model tpyr=uohpngml/ solution;
            repeated season / type=ar(1) sub=subject(group) r rcorr;
run;
*Spherical contrast;
proc mixed data=work.highxout;
            class subject gender season group;
            model tpyr=uohpngml/ solution;
            repeated season / type=hf sub=subject(group) r rcorr;
run;
* Heterogeneous autoregressive;
proc mixed data=work.highxout;
            class subject gender season group;
            model tpyr=uohpngml/ solution;
            repeated season / type=arh(1) sub=subject(group) r rcorr;
run;
* Heterogeneous compound symmetry;
proc mixed data=work.highxout;
            class subject gender season group;
            model tpyr=uohpngml/ solution;
            repeated season / type=csh sub=subject(group) r rcorr;
run;
* random coefficient model;
proc mixed data=work.highxout;
            class subject gender season group;
            model tpyr=uohpngml/ solution;
            random int uohpngml/ type=un sub=subject;
run;
* power of the mean model;
proc mixed data=work.highxout;
            class subject gender season group;
            model tpyr=uohpngml/ solution;
            random int uohpngml/ type=un sub=subject;
            make 'solutionf' out=sf;
```

```
run;
proc mixed data=work.highxout;
        class subject gender season group;
        model tpyr=uohpngml/ solution;
        repeated season/ local=pom(sf);
        random int uohpngml/ type=un sub=subject;
        make 'solutionf' out=sf1;
run;

/************************************************
        Compound symmetry
************************************************/
* Compound symmetry covariance;
proc mixed data=work.data5;
        class subject gender season group nggroup;
        model tpyr=uohpngml | uohpngml |season/ solution cl;
        repeated / type=cs sub=subject   r rcorr;
run;
proc mixed data=work.data5;
        class subject gender season group nggroup;
        model tpyr=uohpngml | uohpngml |season/ solution cl;
        random subject(group);
run;
proc mixed data=work.data5;
        class subject gender season group nggroup;
        model tpyr=uohpngml uohpngml*uohpngml uohpngml*season/  solution;
        *random subject(group)/  group=group;
        repeated/ type=cs sub=subject group=group r rcorr;
run;
proc mixed data=work.data5;
        class subject gender season group nggroup;
        model tpyr=uohpngml uohpngml*uohpngml /  solution;
        repeated/ sub=subject group=group r rcorr;
run;
proc mixed data=work.data5;
        class subject gender season group;
        model tpyr=uohpngml uohpngml*uohpngml /  solution cl;
        repeated/ type=cs  sub=subject  r rcorr;
run;
proc mixed data=work.data5;
        class subject gender season group;
        model tbap=uohpngml uohpngml*uohpngml /  solution cl;
        repeated/ type=cs  sub=subject  r rcorr;
run;


symbol1 i=join  l=1;
symbol2 i=join  l=2;
symbol3 i=join  l=3;
symbol4 i=join  l=4;
symbol5 i=join  l=5;
proc gplot data=work.lowxout;
        plot tpyr*uohpngml=subject;
run;quit;

proc print data=work.lowxout;
        var subject season tpyr uohpngml;
run;

data work.lowxout2;
        set work.lowxout;
        if subject='0-087' then delete;
```

```
run;
proc gplot data=work.highxout;
        plot tpyr*uohpngml=subject;
run;quit;

proc mixed data=work.lowxout2;
        where rgr='Low';
        class subject gender season group;
        model tpyr=uohpngml /solution cl;
        repeated season / type=cs  sub=subject(group) r rcorr;
run;
proc mixed data=work.lowxout2;
        where rgr='Low';
        class subject gender season group;
        model tbap=uohpngml /solution cl;
        repeated season / type=cs  sub=subject(group) r rcorr;
run;
proc mixed data=work.lowxout2;
        where rgr='Low';
        class subject gender season group;
        model tpyr=ut1ohp /solution cl;
        repeated season / type=cs  sub=subject(group) r rcorr;
run;
proc mixed data=work.lowxout2;
        where rgr='Low';
        class subject gender season group;
        model tbap=ut1ohp /solution cl;
        repeated season / type=cs  sub=subject(group) r rcorr;
run;


/*************************************************************
*       Journal of Exposure Science and Environmental Epidemiology
*
*************************************************************/
data work.jesee;
        set work.data;
logut1ohpngml=log(ut1ohpngml);
logum1ohpngml=log(um1ohpngml);
logum1ohp=log(um1ohp);
logut1ohp=log(ut1ohp);
run;
proc sort data=work.jesee; by gender; run;

*creatinine unadjusted;
proc glm data=work.jesee;
        by gender;
        class gender;
        where group gt 2;
        model   logut1ohpngml=logum1ohpngml |gender/solution clparm;
run;quit;
proc glm data=work.jesee;
        by gender;
        class gender;
        where group le 2;
        model   logut1ohpngml=logum1ohpngml gender/solution clparm;
run;quit;

* creatinine adjusted;
proc glm data=work.jesee;
        by gender;
        class gender;
```

```
            where group gt 2;
            model     logut1ohp=logum1ohp gender/solution clparm;
run;quit;
proc glm data=work.jesee;
            by gender;
            class gender;
            where group le 2;
            model     logut1ohp=logum1ohp gender/solution clparm;
run;quit;

* Figure 1;
data work.jesee;
            set work.jesee;
            blandx=(um1ohpngml+ut1ohpngml)/2;
            blandy=(um1ohpngml-ut1ohpngml);
run;
proc plot data=work.jesee;
            plot blandy*blandx;
run;

* July 31 2007 for Chapter 3;
data work.ehp;
            set work.data41;
            fpyrintake=fpyr*food/1000; fbapintake=fbap*food/1000;
            logtpyr=log(tpyr); logtbap=log(tbap); logtpah=log(tpah);
            logfpyrintake=log(fpyrintake); logfbapintake=log(fbapintake);
            logut1ohp=log(ut1ohp); loguohpngml=log(uohpngml);
run;

* Table 1;
proc univariate data=work.ehp;
            var tpyr tbap fpyrintake fbapintake ut1ohp uohpngml;
run;

* Figrue 1 and 2;
proc means data=work.ehp n mean clm maxdec=2;
            class  tpgroup;
            var ut1ohp uohpngml;
run;

ods rtf file="c:\ehpfigure12-by pyr.rtf";
proc means data=work.ehp n mean clm maxdec=2;
            class tpgroup;
            var ut1ohp uohpngml;
run;
ods rtf close;

ods rtf file="c:\ehpfigure12-by bap.rtf";
proc means data=work.ehp n mean clm maxdec=2;
            class tbapgroup;
            var ut1ohp uohpngml;
run;
ods rtf close;

ods rtf file="c:\ehpfigure12-by fpyr.rtf";
proc means data=work.ehp n mean clm maxdec=2;
            class fpyringgroup;
            var ut1ohp uohpngml;
run;
ods rtf close;

ods rtf file="c:\ehpfigure12-by fbap.rtf";
```

```
proc means data=work.ehp n mean clm maxdec=2;
        class fbapinggroup;
        var ut1ohp uohpngml;
run;
ods rtf close;

* Regression model 1-OHP vs. air and food;
proc mixed data=work.ehp; *all;
        class gender season subject rgr;
        model ut1ohp=tpyr fpyrintake season  gender / solution cl;
        repeated season / type=cs  sub=subject(rgr) r rcorr;
run;
proc mixed data=work.ehp; *tpyr high group;
        where tpgroup ge 5;
        class gender season subject rgr;
        model ut1ohp=tpyr fpyrintake season  gender / solution cl;
        repeated season / type=cs  sub=subject(rgr) r rcorr;
run;

proc mixed data=work.ehp; * tbap all;
        class gender season subject rgr;
        model ut1ohp=tbap fbapintake season  gender / solution cl;
        repeated season / type=cs  sub=subject(rgr) r rcorr;
run;
proc mixed data=work.ehp; * tbap high groupl;
        where tbapgroup ge 5;
        class gender season subject rgr;
        model ut1ohp=tbap fbapintake season  gender / solution cl;
        repeated season / type=cs  sub=subject(rgr) r rcorr;
run;

***************
Prediction
***************;
data work.ehpmale;
        set work.ehp;
        if gender='M' then output;
run;
data work.ehpfemale;
        set work.ehp;
        if gender='F' then output;
run;
proc mixed data=work.ehpmale;
        where tpgroup ge 5;
        class gender season subject rgr;
        model tpyr=ut1ohp /solution cl;
        repeated season /type=cs sub=subject(rgr) r rcorr;
run;
proc mixed data=work.ehpmale;
        where tpgroup ge 5;
        class gender season subject rgr;
        model tbap=ut1ohp /solution cl;
        repeated season /type=cs sub=subject(rgr) r rcorr;
run;

proc mixed data=work.ehpfemale;
        where tpgroup ge 5;
        class gender season subject rgr;
        model tpyr=ut1ohp /solution cl;
        repeated season /type=cs sub=subject(rgr) r rcorr;
run;
proc mixed data=work.ehpfemale;
```

```
            where tbapgroup ge 5;
            class gender season subject rgr;
            model tbap=ut1ohp /solution cl;
            repeated season /type=cs sub=subject(rgr) r rcorr;
run;

* Correlation between pyrene and BaP;
proc corr data=work.data41 fisher spearman ;
            var tpyr tbap fpyring fbaping;
run;

proc univariate data=work.ehp;
            where tpgroup ge 5;
            var ut1ohp uohpngml;
run;
proc univariate data=work.ehp;
            where tbapgroup ge 5;
            var ut1ohp uohpngml;
run;

proc sort data=work.data5; by gender; run;
proc mixed data=work.data5;
            by gender;
            where ut1ohp ge 0.46;
            class gender season subject rgr;
            model tpyr=ut1ohp /solution cl;
            repeated season /type=cs sub=subject(rgr) r rcorr;
run;

proc univariate data=work.data5;
            where ut1ohp ge 0.46;
            var tpyr tbap ut1ohp;
run;

proc sort data=work.ehp; by gender; run;
proc mixed data=work.ehp;
            *by gender;
            *where ut1ohp ge 0.46;
            where tbapgroup ge 5;
            class gender season subject rgr;
            model tbap=ut1ohp  gender/solution cl;
            repeated season /type=cs sub=subject(rgr) r rcorr;
run;

proc mixed data=work.data41;
            where tbapgroup ge 5;
            class gender season subject rgr;
            model tbap=ut1ohp ut1ohp*ut1ohp/solution cl;
            repeated season /type=cs sub=subject(rgr) r rcorr;
run;
proc mixed data=work.data41;
            where tbapgroup ge 5;
            class gender season subject rgr;
            model uohpngml=tbap/solution cl;
            repeated season /type=cs sub=subject(rgr) r rcorr;
run;


proc plot data=work.data41;
            where tbapgroup ge 5;
            plot tbap*ut1ohp;
run;
```

```
proc univariate data=work.data41;
            class gender;
            var tpyr tbap;
run;

/*****************************************************
*           Chapter 4 Reproducibility of 1-OHP and PAH
*
*****************************************************/
libname thesis "g:\backup\inkyu\mypaper\ongoing\umdnj\manuscript\dissertation\ehp";
PROC IMPORT OUT= WORK.wb
        DATAFILE= "G:\Backup\Inkyu\Mypaper\Ongoing\UMDNJ\Data\paired
-thesischap4.xls"
        DBMS=EXCEL REPLACE;
    SHEET="Sheet1$";
    GETNAMES=YES;
    MIXED=NO;
    SCANTEXT=YES;
    USEDATE=YES;
    SCANTIME=YES;
RUN;
data thesis.chapter4;
            set work.wb;
            if group le 2 then exposure=0;
            else exposure=1;
run;
data thesis.master0926;
            set work.data41;
            if group le 2 then exposure=0;
            else exposure=1;
            lgum1ohpngml=log10(um1ohpngml);
            lgum1ohp=log10(um1ohp);
            lgfpahing=log10(fpahing);
            lgut1ohp=log10(ut1ohp);
            lgut1ohpngml=log10(ut1ohpngml);
            lgtpyr=log10(tpyr);
            lgfpyring=log10(fpyring);
run;
*table 1;
proc univariate data=thesis.master;
            class exposure;
            var um1ohp ut1ohp um1ohpngml ut1ohpngml tpah fpahing tpyr fpyring;
run;
* table 2 correlation (spearman, pearson);
proc sort data=thesis.chapter4; by exposure;run;
proc corr data=thesis.chapter4 spearman pearson;
            by exposure;
            var umngwin umngsum;
run;
proc corr data=thesis.chapter4 spearman pearson;
            by exposure;
            var um1ohpwin um1ohpsum;
run;
proc corr data=thesis.chapter4 spearman pearson;
            by exposure;
            var tpahwin tpahsum;
run;
proc corr data=thesis.chapter4 spearman pearson;
            by exposure;
            var fpahwin fpahsum;
run;
```

```
proc corr data=thesis.chapter4 spearman pearson;
        by exposure;
        var tpyrwin tpyrsum;
run;
proc corr data=thesis.chapter4 spearman pearson;
        by exposure;
        var fpyrwin fpyrsum;
run;

*table 3;
proc means data=thesis.master0926 n mean std maxdec=2;
        class season rgr;
        var lgtpyr lgfpyring;
run;

proc print data=thesis.master (obs=1);
        var subject fpahing fpah;
run;

proc sort data=thesis.master0926; by rgr;run;
%macro table2 (var);
proc glm data=thesis.master0926;
        by rgr;
        class season;
        model &var=season;
run;
%mend table2;
%table2(lgtpyr); %table2(lgfpyring);

* table 4;
proc sort data=thesis.master0926; by rgr;run;
%macro season(var);
proc mixed data=thesis.master0926; *random only;
        by rgr;
        class group season subject;
        model &var= /cl;
        repeated season /type=cs subject=subject(group) r rcorr;
run;
proc mixed data=thesis.master0926; *random +fixed;
        by rgr;
        class group season subject;
        model &var= season /cl;
        repeated season /type=cs subject=subject(group) r rcorr;
run;
%mend season;
%season(lgum1ohp);
%season(lgum1ohpngml);
%season(lgtpyr);
%season(lgfpyring);
%season(lgut1ohp);


**********;
proc mixed data=thesis.master;
        where tbap ge 20;
        class gender season subject rgr;
        model ut1ohp=tbap fbaping/solution cl ;
        repeated season /type=cs sub=subject(rgr) r rcorr;
run;
proc mixed data=thesis.master;
        where tbap ge 20;
        class gender season subject rgr;
```

```
        model tbap=ut1ohp fbaping/solution cl ;
        repeated season /type=cs sub=subject(rgr) r rcorr;
run;
data work.log;
        set thesis.chapter4;
        logumngwin=log(umngwin);        logumngsum=log(umngsum);        logum1ohpwin=log(um1ohpwin);
        logum1ohpsum=log(um1ohpsum);
        logtaphwin=log(tpahwin); logtpahsum=log(tpahsum); logfpahwin=log(fpahwin); logfpahsum=log(fpahsum);
proc means data=work.log;
        class exposure;
run;
```

Curriculum Vita

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February, 1997	**B.S.,** Environmental Science, Hankuk University of Foreign Studies, Seoul Korea
February, 1999	**M.P.H.,** Environmental Health (Option: Environmental Chemistry), Seoul National University School of Public Health, Seoul, Korea
January, 2008	**Ph.D.,** Environmental and Occupational Health, University of Medicine and Dentistry of New Jersey
March, 1999 - February, 2000	Teaching Assistant, Seoul National University School of Public Health, Seoul, Korea
March, 2000 - February 2001	Lecturer, (Subject : Environmental Health), Wonju National College, Wonju, Kangwon-do, Korea
July, 2000 - July, 2002	Assistant Researcher, Seoul Metropolitan Institute of Public Health and Environment, Seoul, Korea
August, 2002 - May 2007	Graduate Assistant, Department of Environmental and Occupational Health, University of Medicine and Dentistry of New Jersey School of Public Health

Han, I. K., Duan, X., Zhang, L., Yang, H., Rhoads, G., Wei, F., and Zhang, J. 2007. 1-Hydroxypyrene Concentrations in First Morning Voids and 24-hour Composite Urine: Intra- and Inter-person Comparisons. *Journal of Exposure Science and Environmental Epidemiology*. In press.

McCreanor, J., Cullinan, P., Nieuwenhuijsen, M., Stewart-Evans, J., Malliarou, E., Jarup, L., Harrington, R., **Han, I.K.**, Ohman-Strickland, P., Chung, K.F., Zhang, J. 2007. Respiratory Effects of Real-Life Diesel Traffic in Persons with Asthma. *New England Journal of Medicine.* 357(23): 2348-2358.

Fan, Z., Wescheler, C., **Han, I.K.,** and Zhang, J. 2005. Co-formation of second aerosol particles. *Atmospheric Environment*. 39(28). 5171-5182.