AN ABSTRACT OF THE DISSERTATION OF

Oleksii Motorykin for the degree of Doctor of Philosophy in Chemistry presented on September 26, 2014.

Title: Human Exposure to Polycyclic Aromatic Hydrocarbons: Global Cancer Risk and Metabolism

Abstract approved:

_____

Staci L. Massey Simonich

In recent decades, there has been increased attention on human exposure to, and subsequently toxicity of, Polycyclic Aromatic Hydrocarbons (PAHs). PAHs are widespread organic pollutants and some have been shown to be toxic, carcinogenic and mutagenic. This research was focused on understanding the link between global PAH emissions and lung cancer risk, as well as the metabolism of PAHs by humans. We investigated human exposure to PAHs via inhalation and ingestion, their metabolism to hydroxy-PAHs (OH-PAHs) and compared the results to the OH-PAH concentrations in the general US population.

The objective of the first part of this research was to investigate the relationship between lung cancer mortality rates, carcinogenic PAH emissions, and smoking on a global scale. We also investigated this relationship in different socioeconomic country groups. The data from the World Health Organization provided two lung cancer mortality rates, including estimated lung cancer deaths per 100,000 people (ED100000) and age

standardized lung cancer death rate per 100,000 people (ASDR100000) for 136 countries. Both mortality rates were regressed on PAH emissions in benzo[a]pyrene equivalence (BaPeq), smoking prevalence, cigarette price, gross domestic product per capita, percentage of people with diabetes, and average body mass index. Both simple linear regression and multiple linear regressions with stepwise procedure were used. The results showed a statistically significant positive linear relationship between $\log_e$(ED100000) and $\log_e$(BaPeq) emissions for high socioeconomic country group (p-value<0.01). Additionally, both $\log_e$(ED100000) and $\log_e$(ASDR100000) were significantly positively correlated with $\log_e$(BaPeq) emissions for the combination of upper middle and high (p-value<0.05) socioeconomic country groups.

The objective of the second part of this research was to investigate human inhalation exposure to PAHs. We developed a method for the measurement of 19 parent PAHs and 34 hydroxylated PAHs (OH-PAHs) in urine and particulate matter less than 2.5 um in diameter ($PM_{2.5}$) using GC-MS. We validated this method using NIST SRM 3672 (Organic Contaminants in Smoker's Urine) and SRM 3673 (Organic Contaminants in Nonsmoker's Urine). The method was used to measure PAHs and OH-PAHs in urine and personal $PM_{2.5}$ samples collected during fish smoking activities at the Confederated Tribes of Umatilla Indian Reservation (CTUIR). Two different fish smoking facilities (tipi and smoke shed) were used and two different wood types (alder and apple) were burned. Urine samples were hydrolyzed, concentrated using solid phase extraction, and fractionated using silica phase to separate PAHs and OH-PAHs. The 34 OH-PAHs were derivatized using N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA), and both OH-PAHs and PAHs were measured by GC-MS. The personal $PM_{2.5}$ samples were

extracted using pressurized liquid extraction, derivatized with MTBSTFA and analyzed by GC-MS for PAHs and OH-PAHs. Isotopically labeled surrogates of PAHs and OH-PAHs were added to accurately quantify analytes. The results showed an increase in OH-PAH concentrations in urine after 6 hours of fish smoking and an increase in PAH concentrations in air within each smoking facility. In general, the PAH exposure in the smoke shed was higher than in the tipi and the PAH exposure from burning apple wood was higher than burning alder.

The objective of the third part of this research was to investigate human oral exposure to PAHs. We estimated excretion rates and half-lives of 4 PAHs and 10 OH-PAHs after the consumption of Native American traditionally smoked fish. Nine members of the CTUIR consumed smoked fish for breakfast and urine samples were collected during the following 24 hours. The results showed significant increase in OH-PAH concentrations 3 to 6 hr post-consumption. The lowest half-life was for retene (1.4 hr) and the highest was for 3-hydroxyfluorene (7.0 hr).

Human Exposure to Polycyclic Aromatic Hydrocarbons: Global Cancer Risk and Metabolism

by

Oleksii Motorykin

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented September 26, 2014

Commencement June 2015

Doctor of Philosophy dissertation of Oleksii Motorykin presented on September 26, 2014

APPROVED :

Major Professor, representing Chemistry

Chair of the Department of Chemistry

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Oleksii Motorykin, Author

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to my major advisor, Dr. Staci Simonich for her advice, guidance, support, and patience over the years. I am grateful to her for this precious opportunity to work on this project. I would also like to thank my committee members, Dr. Jennifer Field, Dr. Claudia Maier and Dr. William Baird, Dr. Victor Hsu and Dr. David McIntyre for sharing your expertise and time on this research. I wish to thank Dr. Melissa Matzke and Dr. Katrina Waters for excellent help in building and interpreting statistical models. I would like to say a special thanks to Dr. Stephen Wise for providing standard reference materials from National Institute of Technology. Also, I say thanks to Dr. Barbara Harper, Stuart Harris, Dr. Anna Harding and Dr. Molly Kile for an interesting collaboration with Native Americans.

I wish to express my thanks to my current and past laboratory members (Jill, Yuling, Carlos, Leah, Christopher, Scott, Melissa, Pun, Kevin, Shelby, Anna, and Ivan) for fun times spent in the lab. Special thanks for my friends in Corvallis for interesting journeys on the mountain tops. I appreciate all the help from the staff in the Chemistry and Environmental and Molecular Toxicology departments at Oregon State University.

I am extremely thankful to my brother, my mom and friends in Ukraine for their invisible, but important help over the years.

CONTRIBUTION OF AUTHORS

Dr. Staci L. Massey Simonich from Oregon State University provided advice and support in all aspects of this dissertation.

Chapter 2. Dr. Melissa Matzke and Dr. Katrina Waters helped with modelling lung cancer and interpreting the results. They also helped revising the paper.

Chapter 3. Dr. Yuling Jia helped conducting the experiment and with Jill Schrlau they extracted and analyzed $PM_{2.5}$ samples. Jill Schrlau also helped editing the paper.

Chapter 3 and 4. Dr. Barbara Harper, Stuart Harris and Dr. Anna Harding helped with experimental design, conducting the experiment and editing paper. Dr. David Stone and Dr. Dan Sudakin helped with experimental design, calculating the cancer risks and editing the paper.

Chapter 4. Lisandra Santiago-Delgado helped with analysis of urine samples. Dr. Diana Rohlman helped with experimental design, conducting the experiment and editing the paper. Dr. Molly Kile helped with experimental design and editing the paper.

TABLE OF CONTENTS

Page

1. INTRODUCTION 1

1.1 Parent-PAHs 1

1.1.1 Toxicity and Cancer 1

1.1.2 Ingestion 3

1.1.3 Inhalation 4

1.2 Hydroxy-PAHs 4

1.3 Measurement of PAHs and OH-PAHs 6

1.4 Impact of this work 7

2. ASSOCIATION OF CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBON EMISSIONS AND SMOKING WITH LUNG CANCER MORTALITY RATES ON A GLOBAL SCALE 9

ABSTRACT 10

2.1 Introduction 10

2.2 Experimental Section 13

2.2.1 Variables and Data Used 13

2.2.2 Statistical Analysis and Models Tested 15

2.3 Results 17

2.4 Discussion 25

TABLE OF CONTENTS (Continued)

Page

2.5 Acknowledgements 28

3. DETERMINATION OF PARENT AND HYDROXY PAHs IN PERSONAL PM2.5 AND URINE SAMPLES COLLECTED DURING NATIVE AMERICAN FISH SMOKING ACTIVITIES 30

ABSTRACT 31

3.1 Introduction 32

3.2 Experimental Procedures 35

3.2.1 Reagents 35

3.2.2 Collection of urine and personal $PM_{2.5}$ air samples 37

3.2.3 Urine analytical method 39

3.2.4 $PM_{2.5}$ analytical method 41

3.2.5 PAH and OH-PAH analysis and detection limits 41

3.2.6 Creatinine measurements 42

3.3 Results and Discussion 43

3.3.1 Development and Validation of Analytical Method 43

3.3.2 Urine Extraction Recoveries 43

3.3.3 $PM_{2.5}$ Extraction Recoveries 45

3.3.4 Storage stability of spiked OH-PAHs in frozen urine and derivatized OH-PAHs in extracts 45

TABLE OF CONTENTS (Continued)

Page

3.3.5 Intra- and inter-day variability of analytical method 46

3.3.6 Estimated detection limits (EDLs) 47

3.3.7 NIST urine SRM analysis for OH-PAH and PAH 47

3.3.8 PAH and OH-PAH Concentrations in Operators' Urine from Fish Smoking Activities and Comparison to NHANES 48

3.3.9 PAH Concentrations in Personal $PM_{2.5}$ Air Samples Collected During Fish Smoking Activities 54

3.4 Acknowledgements 56

4. METABOLISM AND EXCRETION RATES OF PARENT AND HYDROXY-PAHs IN URINE COLLECTED AFTER CONSUMPTION OF TRADITIONALLY SMOKED SALMON IN NATIVE AMERICANS 57

ABSTRACT 58

4.1 Introduction 58

4.2 Experimental section 61

4.2.1 Reagents and materials 61

4.2.2 Selection of Study Participants 63

4.2.3 Preparation of Smoked Salmon 63

4.2.4 Fish Consumption and Collection of Urine Samples 63

4.2.5 Analytical Method 64

TABLE OF CONTENTS (Continued)

Page

4.2.6 Pharmacokinetic Model 65

4.3 Results and Discussion 66

4.3.1 Creatinine, PAH and OH-PAH Concentrations 66

4.3.2 The ratio of the OH-PAH to PAH 67

4.3.3 Pharmacokinetics and Half-Life Estimates 70

4.4 Acknowledgements 71

5. CONCLUSIONS 74

BIBLIOGRAPHY 80

APPENDICES 93

LIST OF FIGURES

Figure Page

Figure 1.1 Sixteen EPA priority pollutant PAHs. 2

Figure 1.2 Proposed metabolic scheme of polycyclic aromatic hydrocarbons using phenanthrene as an example 5

Figure 2 Scatter plot between lung cancer mortality rate (ED100000) and BaPeq. 18

Figure 3.1. Flow diagram of the entire analytical method for the measurement of 19 PAHs and 34 OH-PAH in urine and $PM_{2.5}$ samples. 40

Figure 3.2 Mean PAH and OH-PAH recoveries from urine over the entire method using Plexa+C_{18} SPE combined. 44

Figure 3.3 Mean OH- and PAH concentrations in urine for A) Apple × Smoke Shed, B) Apple × Tipi, C) Alder × Smoke Shed, D) Alder × Tipi. 49

Figure 3.4 The boxplots of urinary OH-PAH concentrations for the CTUIR fish smoking activities and for NHANES values from 2007-2008 survey years 53

Figure 3.5 Concentrations of low molecular weight (LMW) PAHs, high molecular weight (HMW) PAHs, and oxy-PAHs (OPAHs) in the tipi and the smoke shed personal $PM_{2.5}$ samples. 55

Figure 4.1. The mean creatinine adjusted PAH and OH-PAH concentrations over 24 hr time period. 68

Figure 4.2 The boxplots of urinary OH-PAH concentrations for this study, the CTUIR inhalation study[130] and for NHANES values from 2007-2008 survey years[163]. 69

Figure 4.3 Urinary PAH and OH-PAH first order elimination curves. 72

LIST OF TABLES

Table Page

Table 2.1 The associated change in median LCMR (%), given a 10% increase in mean independent variable, in the simple linear regression. The 95% confidence interval is given in parenthesis. 20

Table 2.2 The associated change in the median LCMR (%), given a 10% increase in mean independent variable, in the stepwise multiple linear regression. The 95% confidence interval is given in parenthesis. 23

Table 4. Measured and modeled parameters for the elimination of PAHs and OH-PAHs. 73

LIST OF APPENDIX FIGURES

Figure Page

Appendix A.3 Scatter plot matrix for untransformed variables. 101

Appendix A.4 Scatter plot matrix for Log-transformed variables. 102

Appendix A.8 Scatter plot between lung cancer mortality rate (ED100000) and smoking prevalence. 106

Appendix A.9 Scatter plot between lung cancer mortality rate (ASDR100000) and smoking prevalence 107

Appendix A.10 Scatter plot between lung cancer mortality rate (ED100000) and cigarette price. 108

Appendix A.11 Scatter plot between lung cancer mortality rate (ASDR100000) and cigarette price. 109

Appendix A.12 Scatter plot between lung cancer mortality rate (ED100000) and GDP per capita. 110

Appendix A.13 Scatter plot between lung cancer mortality rate (ASDR100000) and GDP per capita. 111

Appendix A.14 Scatter plot between lung cancer mortality rate (ED100000) and percent people with diabetes. 112

Appendix A.15 Scatter plot between lung cancer mortality rate (ASDR100000) and percent people with diabetes. 113

Appendix A.16 Scatter plot between lung cancer mortality rate (ED100000) and body mass index (BMI). 114

Appendix A.17 Scatter plot between lung cancer mortality rate (ASDR100000) and body mass index (BMI). 115

Appendix A.18 Scatter plot between lung cancer mortality rate (ASDR100000) and BaPeq. 116

LIST OF APPENDIX FIGURES (Continued)

Figure Page

Appendix A.19 Scatter plot between smoking prevalence and cigarette price..... 117

Appendix A.20 Box plot of BMI across four income levels. 118

Appendix A.21 Box plot of life expectancy across four income levels..... 119

Appendix B.4 Experimental timeline for the collection of urine and personal $PM_{2.5}$ samples. 131

Appendix B.5 Tipi (upper left) and smoke shed (upper right), and fish positioning inside (lower left and lower right, respectively) 132

Appendix B.6 Mean OH-PAH recoveries from spiked UTAK urine using different SPE. 133

Appendix B.7 Mean PAH recoveries for the entire analytical method using spiked UTAK urine and different SPE phases. 134

Appendix B.8 Mean PLE recoveries for the extraction of PAH and OH-PAH from $PM_{2.5}$. 135

Appendix B.9 Stability of spiked OH-PAHs in frozen UTAK urine. 136

Appendix B.10 Mean stability of the products of OH-PAH and MTBSTFA derivatization in the extract (frozen at -20ºC). 137

Appendix B.11 Mean intra-day variability of the Parent and OH-PAH method for urine. 138

Appendix B.12 Mean inter-day variability of the OH-PAH method for urine. 139

Appendix B.14 PAH profile from Personal Air Samples 141

Appendix B.15 Mean concentrations of selected OH-PAHs in urine extracts after addition of different volumes of β-glucuronidase/arylsulfatase. 142

Appendix B.16 OH-PAHs recoveries from standard solution using Focus SPE with different eluent compositions. 143

LIST OF APPENDIX FIGURES (Continued)

Figure Page

Appendix C.1 Creatinine concentration for all participants. 145

Appendix C.2 The mean creatinine adjusted PAH and OH-PAH concentrations over 24 hr time period..... 146

Appendix C.3 The mean creatinine adjusted PAH and OH-PAH concentrations over 24 hr time period..... 147

Appendix C.5 The ratio of the sum of four hydroxy-phenanthrenes to phenanthrene concentrations (first row) for 9 participants and the ratio of 1-hydroxypyrene to pyrene concentrations (second row) for 6 participants..... 149

LIST OF APPENDIX TABLES

Table Page

Appendix A.1 Independent and dependent variables used in the regression models. 95

Appendix A.5 Correlation matrix (R^2) for Log_e-transformed variables. 103

Appendix A.6. Regression coefficients for SLR. 104

Appendix A.7. Regression coefficients for MLR. 105

Appendix B.2 The list of OH-PAH, LMW PAH, HMW PAH, and oxy-PAHs and their labeled surrogates, internal standards, their abbreviations and estimated detection limits. 124

Appendix B.3 Selected Ion Monitoring (SIM) windows and m/z ions monitored. 128

Appendix B.13 Analysis of NIST SRM samples..... 140

Appendix C.4 Median (minimum and maximum) urinary concentrations of PAHs and OH-PAHs..... 148

1. INTRODUCTION

1.1 Parent-PAHs

Polycyclic aromatic hydrocarbons (PAHs) are widespread organic pollutants that consist of two or more fused aromatic rings (Figure 1.1). They are persistent contaminants and produced mainly by incomplete combustion of organic matter, such as coal and fossil fuels. Forest fires and volcanic eruption also contribute to environmental contamination[1]. PAHs undergo atmospheric transport and have been found in remote areas[2, 3], including high elevation areas[4].

1.1.1 Toxicity and Cancer

The toxicity of the mixture of parent PAHs (PPAHs) is commonly expressed as benzo(a)pyrene equivalents (BaP_{eq}). Benzo(a)pyrene (BaP) itself is one of the most toxic and studied PAHs. Because of this, the relative potency factor (RPF) of BaP was chosen to be 1. All other PAHs are assigned RPFs relative to that of BaP. The sum of the concentrations of toxic PAHs multiplied by their respective RPFs is the BaPeq concentration, and indicates how toxic the mixture is compared to pure benzo(a)pyrene (Eq. 1).

$$BaPeq = \sum C_{PAH} * RPF \qquad [1]$$

Equation 1. BaPeq calculation. C_{PAH} is the concentration of each PAH in air, ng/m^3, RPF – relative potency factor[5].

The toxicity and carcinogenicity of parent PAHs has been evaluated by many laboratories. Sixteen PAHs (naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flo), phenanthrene (Phen), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbFlt),

benzo[k]fluoranthene (BkFlt), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DBahA), indeno[1,2,3-c,d]pyrene (Ind), benzo[g,h,i]perylene (BghiP)) are listed as US EPA priority pollutants (Figure 1.1)[6]. The International Agency for Research on Cancer (IARC) concluded that some PAHs (BaP, BaA, BbFlt, BkFlt, Chr, DBahA, Ind, and Nap) are probable or possible human and animal carcinogens[7, 8] and the US Environmental Protection Agency (EPA) Integrated Risk Information System ranked them as probable human carcinogens[9].

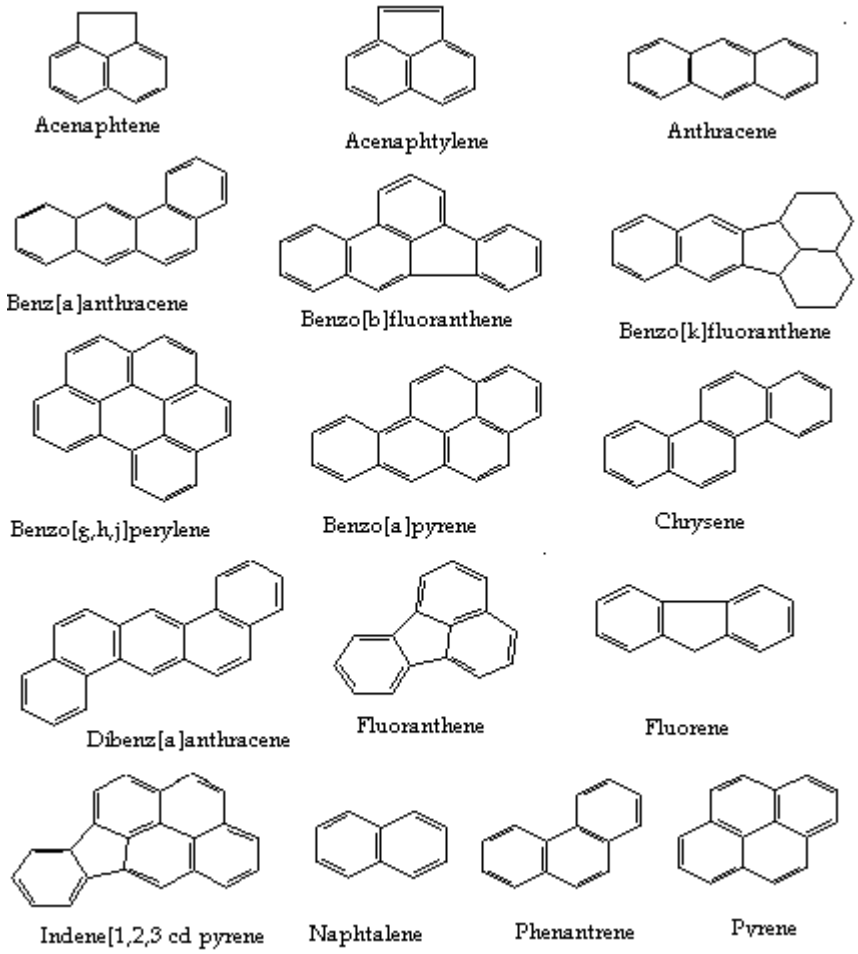


Figure 1.1 Sixteen EPA priority pollutant PAHs.

In recent decades, the association between exposure to carcinogenic PAHs and different types of cancer, including lung, skin, bladder, respiratory, and urinal tract cancers[10], has been investigated and showed positive correlation. Smoking has been attributed to lung cancer because PAHs are one of the main cancer-causing chemicals in the inhaled smoke (along with N-nitrosamines, aromatic amines, and aldehydes)[11, 12].

1.1.2 Ingestion

Cooking meat, such as grilling, charcoaling, barbequing and smoking, creates PAH contaminated food[13-16]. Smoking is particularly harmful because smoke particles are directly deposited on the surface of the food. In 2005, the European Commission established a limit of 5 µg/kg for BaP in any smoked fish or meat[17]. Research showed that smoked fish surfaces contain more PAHs that inner fish meat, but the skin has the highest concentration , exceeding the European limits by 9-fold[14]. It was shown that direct smoking, at high temperatures, leads to higher PAHs concentrations in fish than indirect smoking at low temperatures[14, 15]. Also, burning wood creates higher PAH concentrations in smoked meat than smoldering charcoal[13].

Some foods contain PAHs even without smoking. Veyrand et al. found that mollusks and oil-based products (i.e. margarine) are most contaminated with PAHs, and that chrysene (CHR), benzo[b]phenanthrene (BbPh) and benzo[a]anthracene (BaA) had highest concentrations[18]. Benzo[a]pyrene has been shown to be a good indicator of PAH contamination in food[16], as well as PAH4 (CHR+BbPh+BaA+BaP)[18].

Ingestion plays a significant role in PAH exposure not only through consumption of food, but also through occupational exposure. In addition, children playing outside can be exposed to dust from coal-tar sealed pavements, which have high PAH concentrations[19].

1.1.3 Inhalation

In air, PAHs undergo photodegradation, forming oxy-, hydroxy-, nitro-, methyl-PAHs, or any combination of these functional groups [20, 21]. PAHs and their photodegradation products were shown to be stable enough to undergo long range atmospheric transport[22, 23].

It has been shown that human exposure to PAHs from inhalation is lower than from ingestion[24]. Still, lung cancer was attributed to occupational PAH exposure in some regions in the World[25]. Svecova et al. showed that outdoor concentrations, environmental tobacco smoke, the type of home heating fuel used, the frequency of exhaust fan use, as well as cooking and commuting by car, are the factors that can increase personal exposure to PAHs[26].

China is the largest emitter of PAHs in the world[27] and research has been focused on human exposure to these carcinogens in China. E-waste recycling and residential heating during winter season have been shown to significantly increase human exposure to PAHs in China [28, 29]. In addition, Zhou et al. showed that up to 3% of the lung cancer deaths in Beijing could be avoided if emission of PAHs was absent[30].

1.2 Hydroxy-PAHs

Hydroxylated PAHs (OH-PAHs) are produced in the human body when parent PAHs enter the body through ingestion, inhalation or dermal exposure. They are formed via CYP P450 family of enzymes, the most active being CYP1A1 and CYP1B1[11], which transform PAHs to phenolic and epoxide forms (Figure 1.2). The epoxides undergo transformation to diol-epoxides, which are highly reactive and can bind to DNA, causing mutation and initiate carcinogenesis[31-36]. It was shown that the expression of CYP1A1

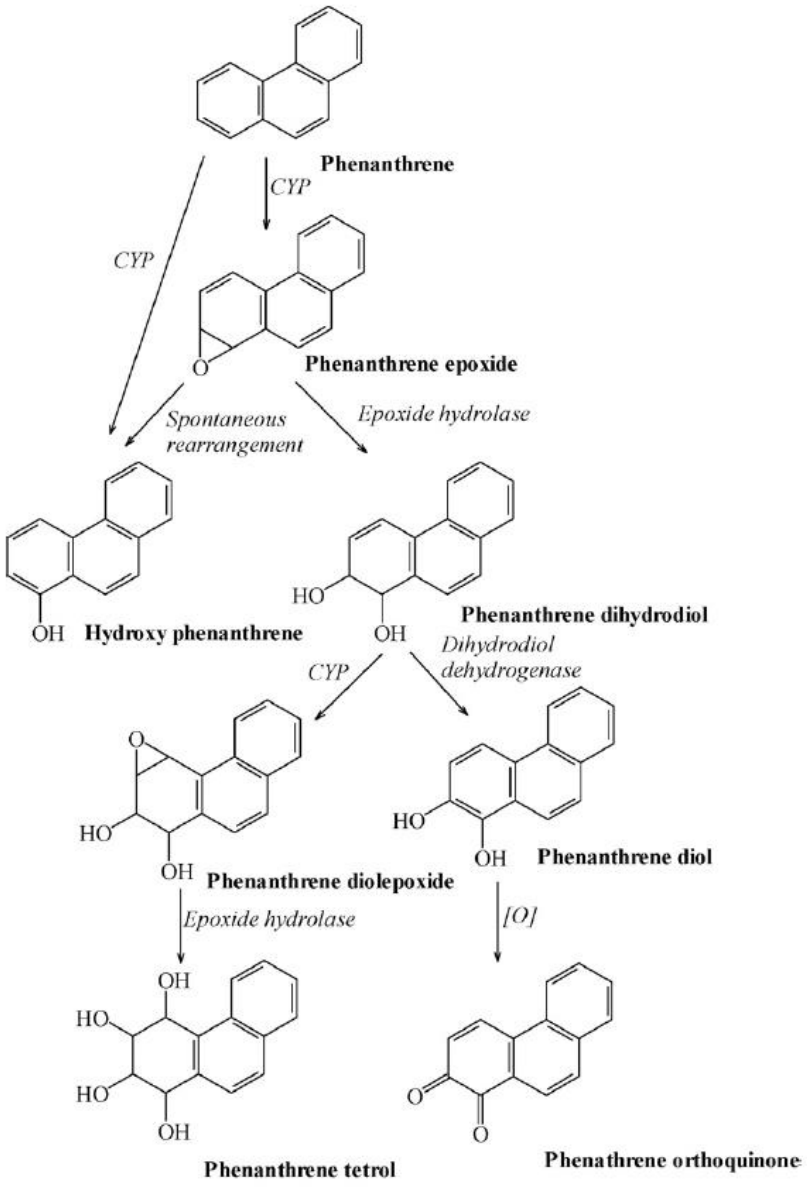


Figure 1.2 Proposed metabolic scheme of polycyclic aromatic hydrocarbons using phenanthrene as an example[37].

significantly correlated with PAH-DNA adducts in lung tissues[32]. Kuang et al. showed that the concentration of urinary 8-hydroxydeoxyguanosine (8-OHdG), which is the marker for oxidative damage to DNA, increases with increase of exposure to PAHs. Also, concentration of 8-iso-prostaglandin-F2α (8-iso-PGF2α), a biomarker of lipid peroxidation, and 8-OHdG were significantly correlated with urinary OH-PAH

concentration[38]. Yim et al. found that lung cancer risk increases with a decrease of urinary total antioxidant capacity and increase of 8-OHdG concentration[39]. In addition, several PAH metabolites, such as 1-OH-Pyr and 3-OH-BaP, have been shown to be good markers for overall PAH exposure[40-42].

1.3 Measurement of PAHs and OH-PAHs

A lot of methods were developed to measure parent PAHs in air, water, soil or sediments, urine and blood. The most popular technique is gas chromatography[43, 44], because PAHs are volatile or semi-volatile, but HPLC with fluorescent[45] or UV-VIS detectors[46] are also used. Solid phase extraction (SPE) is used to extract PAHs from aqueous solutions[45] and Pressurized Liquid Extraction (PLE) is used to extract PAHs from soil and particulate matter[20, 47].

Many methods were developed to measure OH-PAHs in water, urine, air and soil. The most common method for measuring OH-PAHs and PAHs in aqueous solutions, such as urine, is extraction using solid phase extraction (SPE), followed by either liquid chromatography coupled with mass spectrometry (LC-MS)[37, 48, 49] or derivatization of OH-PAHs, followed by gas chromatography coupled with mass spectrometry (GC-MS)[23, 43, 50-55]. Other methods include HPLC with fluorescence detection[56-58] and derivatization with pentafluorobenzyl bromide, followed by LC-MS/MS[59]. In urine hydroxy PAHs are bound to glucuronides and sulfates residues, and some methods measure either glucuronide form[60] or free OH-PAHs after deglucuronidation[48]. Derivatization converts OH-PAHs into more volatile compounds and increases the selectivity and sensitivity of the GC/MS analysis. Several derivatization reagents that have been used for OH-PAHs include *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA)[53, 55], *N*,*O*-

bis(trimethylsilyl)trifluoroacetamide (BSTFA)[51, 52, 54], N,O-bis(trimethylsilyl)acetamide (BSA) with 5% trimethylchlorosilane (TMCS)[50], N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA)[43, 54], and pentafluorobenzyl bromide[59].

1.4 Impact of this work

In Chapter 2 of this work, the correlation between PAH emissions in the world and lung cancer was investigated. Although a lot of work has been done before to establish correlation, this is the first work to show the correlation on a global scale. The statistical model was built in order to determine which variables significantly correlate with lung cancer mortality. Besides PAH emission, other variables, such as smoking prevalence, price of the pack of cigarettes, gross domestic product (GDP) per capita, body mass index (BMI), and per cent of people with diabetes in the country were taken into account. It was shown that the number of lung cancer deaths in the country was correlated with PAH emissions for the high and upper middle socioeconomic country groups.

Chapter 3 describes the development, validation and use of an analytical method to measure PAH and OH-PAH in urine and atmospheric particulate matter. The method was optimized and validated using urine Standard Reference Materials (SRM) from the National Institute of Science and Technology (NIST). The method was used to measure PAHs and OH-PAHs in urine and atmospheric particulate matter during traditional Native American fish smoking. This was the first time the inhalation exposure to PAHs during traditional smoking was assessed. The results showed increase urinary OH-PAH concentrations compare to general US population.

Chapter 4 describes the use of previously developed method to assess human oral exposure to PAHs. In this work human subjects ate Native American traditionally

smoked fish, potentially containing PAHs, and their urine was analyzed on the presence of PAHst and OH-PAHs. The results show increased levels of PAHs and OH-PAHs in urine after 3-6 hr of ingestion of smoked fish. Also, the kinetics of elimination was modelled for each particular PAH and OH-PAH and the rates of elimination were estimated. It was shown that all compounds have short half-lives (less than 8 hr.), and that PAHs have shorter half-lives than OH-PAHs.

2. ASSOCIATION OF CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBON EMISSIONS AND SMOKING WITH LUNG CANCER MORTALITY RATES ON A GLOBAL SCALE

OLEKSII MOTORYKIN[1], MELISSA M. MATZKE[2], KATRINA M. WATERS[2], STACI L. MASSEY SIMONICH[1,3 *]

[1]Department of Chemistry, Oregon State University, Corvallis, Oregon USA 97331;

[2]Computational Biology and Bioinformatics, Pacific Northwest National Laboratory, Richland, Washington USA 99352;

[3]Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon USA, 97331

Environ. Sci. Technol. 2013, 47, 3410−3416

DOI: 10.1021/es305295d

*Corresponding author, E-mail: staci.simonich@oregonstate.edu, fax: 541-737-0497.

ABSTRACT

The objective of this research was to investigate the relationship between lung cancer mortality rates, carcinogenic polycyclic aromatic hydrocarbons (PAHs) emissions, and smoking on a global scale, as well as for different socioeconomic country groups. The estimated lung cancer deaths per 100,000 people (ED100000) and age standardized lung cancer death rate per 100,000 people (ASDR100000) in 2004 were regressed on PAH emissions in benzo[a]pyrene equivalence (BaPeq), smoking prevalence, cigarette price, gross domestic product per capita, percentage of people with diabetes, and average body mass index using simple and multiple linear regression for 136 countries. Using stepwise multiple linear regression, a statistically significant positive linear relationship was found between $log_e(ED100000)$ and $log_e(BaPeq)$ emissions for high (p-value<0.01) and for the combination of upper middle and high (p-value<0.05) socioeconomic country groups. A similar relationship was found between $log_e(ASDR100000)$ and $log_e(BaPeq)$ emissions for the combination of upper middle and high (p-value<0.01) socioeconomic country groups. Conversely, for $log_e(ED100000)$ and $log_e(ASDR100000)$, smoking prevalence was the only significant independent variable in the low socioeconomic country group (p-value<0.001). These results suggest that reducing BaPeq emissions in the U.S., Canada, Australia, France, Germany, Brazil, South Africa, Poland, Mexico, and Malaysia could reduce ED100000, while reducing smoking prevalence in Democratic People's Republic of Korea, Nepal, Mongolia, Cambodia, and Bangladesh could significantly reduce the ED100000 and ASDR100000.

2.1 Introduction

Cancer is the second leading cause of death worldwide [61]. While one in four people die from cancer in industrialized countries, one in eight die worldwide [61, 62]. Worldwide, twelve percent of all cancer cases are lung cancer and lung cancer is the first leading cause of cancer deaths in men and the second leading cause of cancer deaths in women [61, 63]. In addition, lung cancer incidence and mortality rates vary among different geographic regions of the world and ethnicities [62, 64]. For most populations, approximately 80% of all lung cancer cases are associated with tobacco smoking and the remaining 20% have been attributed to exposure to secondhand smoke, radon (and its decay products), asbestos, some metals (including arsenic, beryllium, cadmium), organic compounds (including carcinogenic polycyclic aromatic hydrocarbons (PAHs)), radiation, and genetic susceptibility [61, 62]. In addition, ambient air pollution has been estimated to account for 5% of male cancer deaths and 3% of female cancer deaths in the U.S. [65].

The primary anthropogenic sources of PAHs to the atmosphere include incomplete combustion of coal, wood, and other liquid and solid fuels [66]. Natural processes, including forest fires and volcanic eruptions, also contribute to global PAH emissions [1, 67]. Some PAHs are known to be mutagens [68, 69] and animal carcinogens [66, 70] and are ranked as probable human carcinogens by the U.S. Environmental Protection Agency Integrated Risk Information System [9] and by the International Agency for Research on Cancer (IARC) (1983). In 2010, IARC concluded that indoor emissions from household combustion of coal are carcinogenic to humans and classified biomass burning emissions as probable carcinogens [8].

Cigarette smoking contributes the most to lung cancer occurrence and mortality [61]. Farrelly et al [71] found that increasing the cigarette price led to a decrease in the number of cigarettes smoked daily. Other studies have shown that obesity [72, 73] and diabetes [74-79] may affect lung cancer development and mortality. Gross Domestic Product (GDP) and GDP per capita were found to positively correlate with per capita health care expenditure and life expectancy [80, 81]. In addition, Ultraviolet B (UVB) irradiance has been shown to play a protective role in the development of some cancers, including lung cancer [82, 83].

Zhang et al [84] found that lung cancer mortality was highly correlated with ambient carcinogenic PAH exposure in China, while Grant [65] found a similar relationship for the U.S. The emission of particulate matter less than 2.5 μm in diameter, on which most carcinogenic PAH are sorbed, can also lead to an increased risk of lung cancer [65, 85]. Carcinogenic PAH concentrations are often reported in benzo[a]pyrene equivalents (BaPeq) because benzo[a]pyrene (BaP) is a well-studied PAH that is known to be carcinogenic. BaPeq is a measure of the concentration of carcinogenic PAHs in a mixture relative to pure BaP [5].

The objective of this research was to investigate the relationship between lung cancer mortality rates, carcinogenic PAH emissions, and smoking prevalence on a global scale, as well as for different socioeconomic country groups. Multiple linear regression (MLR) models were built to determine the influence of BaPeq and smoking prevalence on lung cancer mortality rates after taking into account other factors that may contribute to lung cancer mortality rates. The estimated lung cancer deaths per 100,000 people in 2004 (ED100000) and age standardized lung cancer mortality rate per 100,000 people in 2004 (ASDR100000) were regressed on smoking prevalence, cigarette price, gross

domestic product (GDP) per capita, the percentage of people with diabetes, average body mass index (BMI) and carcinogenic PAH emissions (in BaP_{eq}) using MLR and data for 136 different countries. The contribution of carcinogenic PAH emissions and smoking prevalence on lung cancer mortality rates was assessed for countries in different socioeconomic country groups. This is the first study to link carcinogenic PAH emissions, as a measure of air pollution, with lung cancer mortality rates on a global scale.

2.2 Experimental Section

2.2.1 Variables and Data Used

The most recent lung cancer mortality rate (LCMR) data available, including ED100000 and ASDR100000, were taken from the World Health Organization for 2004 [86]. The ASDR100000 is a weighted average of the age-specific lung cancer mortality rates per 100,000 persons, where the weights are the proportion of persons in the corresponding age groups of the WHO standard population [87]. In 2004, 1.28 million people died of lung cancer and, for the 136 countries we had entire datasets for, the highest ASDR100000 were in Maldives (67 deaths per 100,000 people), Hungary (52 deaths per 100,000 people) and Poland (42 deaths per 100,000 people) (Appendix A.1). The U.S. ranked fifth with 39 deaths per 100,000 people (Appendix A.1). The lowest rates were in Fiji (1 death per 100,000 people), Federated States of Micronesia (2.4 deaths per 100,000 people), and Mozambique (3.1 deaths per 100,000 people) (Appendix A.1).

Population and GDP data for 2004 were obtained from Zhang and Tao [27] (Appendix A.1). The total population for the 136 countries modeled was 5.93 billion people, with the highest populations in China (1.33 billion), India (1.08 billion), and the United States (0.29 billion). To account for the socioeconomic level of the country, GDP per capita was used to divide the countries into four groups recommended by the World Bank in 2004 [88]: 1) low income: ≤ $825 USD; 2) low middle income: $826 to $3,255 USD; 3) upper middle income: $3256 to $10,065 USD; and 4) high income: more than $10,065 USD (Appendix A.1). We also tested the combinations of low and low middle income countries, as well as upper middle and high income countries.

The most recent smoking prevalence and the cigarette price per pack data available, and closest to 2004, were compiled for all 136 countries [89]. The available smoking prevalence data was from 1997 to 2008, while the closest available cigarette price data to 2004 was from 2008 [89]. Cigarette price per pack does not accurately represent the price per cigarette because the number of cigarettes per pack (from 10 to 50) varies for different regions of the world [90, 91]. The highest smoking prevalence was in Nauru (47%), Russia (44%), and Austria (41%), while the lowest smoking prevalence was in Suriname (1%), Ethiopia (3%), and Ghana (4%) (Appendix A.1). The highest cigarette prices were in Iceland ($11.3 USD per pack), Norway ($10.1 USD per pack), and Singapore ($8.1 USD per pack), while the lowest cigarette prices were in Democratic People's Republic of Korea ($0.14 USD per pack), Paraguay ($0.20 USD per pack), and Pakistan ($0.23 USD per pack) (Appendix A.1).

Data on the percentage of people with diabetes (for 2000 and 2006) and average BMI (for 2000 and estimated for 2030), were taken from WHO [92, 93]. These data were

interpolated to 2004 for each country (see Appendix A.2). BMI is a relative measure of human body fat based on an individual's weight and height, and is equal to body mass (kg) divided by body height squared (m^2). The highest percentages of people with diabetes in 2004 were in Nauru (17%), Seychelles (11%), and Malta (10%), while the lowest percentages were in Uganda (0.5%), Nigeria (0.1%), and the Democratic Republic of the Congo (0.03%) (Appendix A.1). The highest BMIs in 2004 were in Nauru (33.6), Cook Island (32.3), and Tonga (31.9), while the lowest BMIs were in the Democratic Republic of the Congo (20.5), Ethiopia (20.18), and Bangladesh (20.13) (Appendix A.1).

The carcinogenic PAH emissions (in BaPeq) data for the 136 countries in 2004 were taken from Zhang and Tao [27] and were calculated using the BaPeq emission factors for different fuel types, in different countries. The highest BaPeq emission rates were in China (110,000 tons/year), India (90,000 tons/year), and United States (32,000 tons/year), while the lowest emission rates were in Dominica (0.74 tons/year), St. Vincent and the Grenadines (1.1 tons/year), and Nauru (1.3 tons/year) (Appendix A.1).

Although there is a 20 year lag time for developing measurable cancer [94], we did not account for this lag time in the independent variable datasets because the dependent variables (ED100000 and ASDR100000) are rates. By doing this, we assumed that the ED100000 and ASDR100000 rates would not change significantly over this 20 year period. In addition, the independent variables used in the models were not available from 20 years ago for the 136 countries.

2.2.2 Statistical Analysis and Models Tested

The ED100000 and ASDR100000 were independently modeled using a stepwise multiplicative multiple linear regression model to determine the statistically significant association between lung cancer mortality rates, carcinogenic PAH emissions (BaPeq), and smoking prevalence after accounting for other potentially influential factors:

[1] $$LCMR = BaPeq^{\beta 1} * SP^{\beta 2} * Price^{\beta 3} * GDP.CAP^{\beta 4} * MBI^{\beta 5} * Diabetes^{\beta 6}$$

where LCMR was either ED100000 or ASDR100000, *BaPeq* was the carcinogenic PAH emissions in BaPeq (Mt/year), *SP* was the smoking prevalence (%), *Price* was the cigarette price ($USD per pack), *GDP.CAP* was GDP per capita ($US'000), *BMI* was the average body mass index (kg/m^2), *Diabetes* was the percentage of people with diabetes, and $\beta_0 \ldots \beta_6$ were coefficients in the model. MLR models were independently generated for each of the four socioeconomic groups, as well as the combination of low and low middle country groups and upper middle and high country groups (Appendix A.7).

To investigate the percent change in LCMR as a function of a percent change in a given independent variable, equation [2] was used:

[2] $$\text{\% change in LCMR} = \left(\left(1+\frac{Z}{100}\right)^{\beta_x} - 1\right) * 100\%$$

where β_x is the variable's coefficient estimate and Z is the percent increase in the variable.

Standard regression model checking and refinement techniques, including residual plots, Q-Q plot, Cook's distance and stepwise variable selection using Akaike Information Criteria (AIC) [95], were used. Effects were deemed statistically significant for

a p-value < 0.05. The statistical package R v. 2.12.0 (Free Software Foundation, Inc., Boston, MA) was used for all modeling.

2.3 Results

An initial graphical review of the data, including LCMRs and independent variables, revealed the need for a logarithmic transformation (see Appendix A.3 and Appendix A.4). Specifically, these data tend to be highly skewed. Data from the LCMRs and independent variables were transformed to the $\log_e$ scale to resolve this issue and allowed the data to be modeled linearly, rather than multiplicatively. The resulting MLR model is written as:

$$[3]\ \log_e(LCMR) = \beta_0 + \beta_1 * \log_e(BaPeq) + \beta_2 * \log_e(SP) + \beta_3 * \log_e(Price) + \beta_4 * \log_e(GDP.CAP) + \beta_5 * \log_e(BMI) + \beta_6 * \log_e(Diabetes)$$

where *LCMR* is either ED100000 or ASDR100000 and the independent variables are consistent with previous descriptions.

Co-linearity among the $\log_e$ independent variables, for the entire dataset, was explored (Appendix A.4 and Appendix A.5). There were statistically significant linear relationships (p-value<0.05) between $\log_e$(SP), $\log_e$(Diabetes), $\log_e$(Price), $\log_e$(BMI), and $\log_e$(GDP.CAP). The PAH emission variable, $\log_e$(BaPeq), had a statistically significant negative linear relationship with $\log_e$(Diabetes) (r^2 = 0.09), $\log_e$(BMI) (r^2 = 0.22), and $\log_e$(Price) (r^2 = 0.05) (Appendix A.4 and Appendix A.5).

The linear relationships among the $\log_e$ LCMR and $\log_e$ independent variables for the entire dataset were explored using simple linear regression (SLR) (Appendix A.4 and

Appendix A.6). Linear relationships were further explored by individual socioeconomic country group (low, low-middle, upper-middle, and high), as well as for the combination of low and low middle country groups and upper middle and high country groups using SLR (Figure 2, Appendix A.6, Appendices A.8 - A.19). Appendix A.6 shows the regression coefficients, standard error, and percent of the total regression sum-of-squares due to β_n for the SLRs. Table 2.1 shows the percent change in the median LCMR, given a 10% increase in the mean of the independent variable from the SLRs, for the entire dataset, as well as the different socioeconomic country groups and groupings.

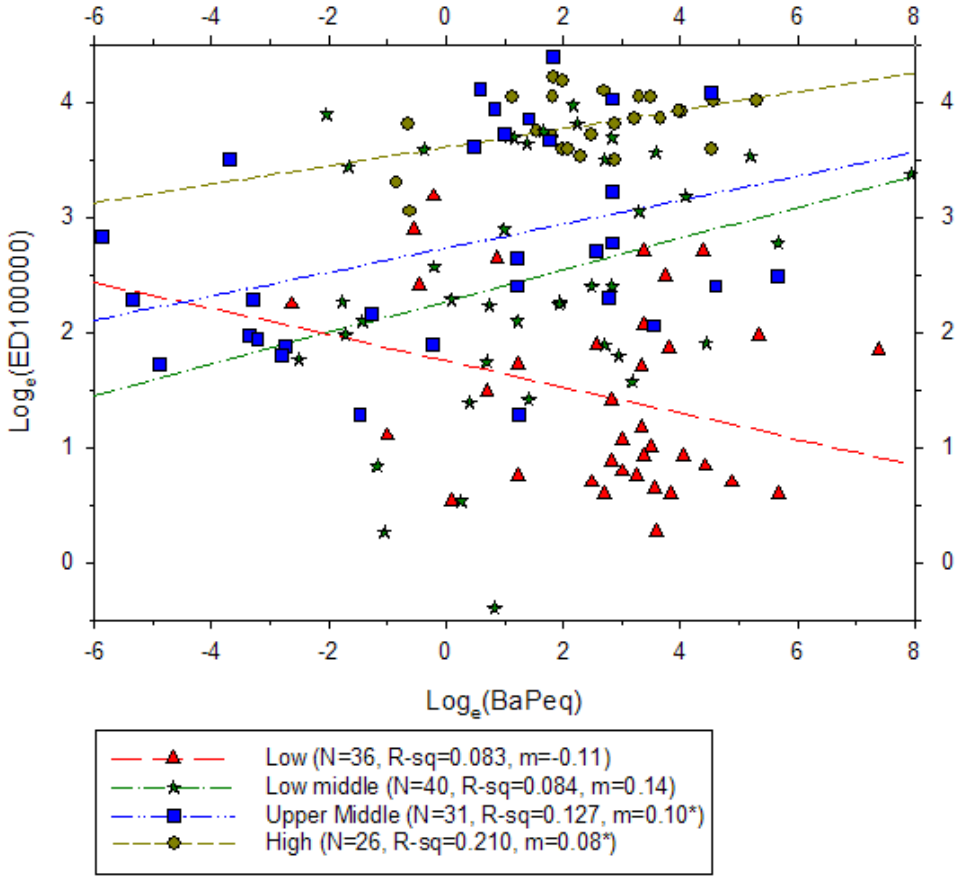


Figure 2 Scatter plot between lung cancer mortality rate (ED100000) and BaPeq. Significance of estimates: * p-value<0.05; **p-value<0.01, *** p-value <0.001.

The SLR analysis for the socioeconomic country group subsets showed that smoking prevalence (*log_e(SP)*) was significantly positively related to the LCMRs in the low, low-middle and upper-middle country groups, as well as for the combination of low and low middle country groups, upper middle and high country groups, and the entire dataset (Table 2.1, Appendices A.8 and A.9). The price of a pack of cigarettes (*log_e(Price)*) was significantly negatively related to the LCMRs for the low (both ED100000 and ASDR100000) and low-middle (ED100000 only) socioeconomic country groups (Appendices A.10 and A.11). However, the price of a pack of cigarettes (*log_e(Price)*) was significantly positively related to both ED100000 and ASDR100000 for the combination of upper middle and high socioeconomic country groups and the entire dataset (Appendix A.6). The GDP per capita (*log_e(GDP.CAP)*) and percentage of people with diabetes (*log_e(Diabetes)*) were significantly positively related to the LCMRs for only the low socioeconomic country group (Appendices A.12 – A.15). The average BMI (*log_e(BMI)*) was significantly negatively and positively related to *log_e(ED100000)* and *log_e(ASDR100000*) for different socioeconomic country groups and groupings (Appendices A.16 and A.17). The BaP equivalents emission (*log_e(BaPeq)*) was significantly positively related to *log_e(ED100000)* for the upper-middle and high socioeconomic country groups, as well as the combination of upper middle and high socioeconomic country groups (Table 2.1 and Figure 2). However, *log_e(BaPeq)* was significantly positively related to *log_e(ASDR100000)* only for the upper-middle socioeconomic country group and the combination of upper middle and high socioeconomic country groups (Table 2.1 and Appendix A.18).

Table 2.1 The associated change in median LCMR (%), given a 10% increase in mean independent variable, in the simple linear regression. The 95% confidence interval is given in parenthesis.
* p-value<0.05, **p-value<0.01, *** p-value<0.001

LCMR	Socioeconomic Group	BaPeq	SP	Price	GDP.CAP	BMI	Diabetes
ED100000	Low	-1.1 (-2.3, 0.17)	9.8*** (5.9, 14)	-6.4*** (-9.8, -2.8)	8.0** (2.7, 14)	27 (-8.9, 78)	4.1** (1.5, 6.7)
	Low Middle	1.3 (-0.11, 2.7)	6.1** (1.9, 10)	-4.4* (-8.4, -0.28)	0.16 (-9.1, 10)	-30.2 (-54, 4.7)	7.1 (-0.71, 15)
	Upper Middle	1.0* (0.01, 2.0)	9.6*** (5.4, 14)	-0.22 (-7.1, 7.2)	-1.9 (-12, 9.6)	-38* (-60, -1.8)	-1.7 (-8.1, 5.1)
	High	0.77* (0.14, 1.4)	4.2 (-0.13, 8.8)	-0.99 (-4.6, 2.8)	0.58 (-2.2, 3.4)	-4.0 (-22, 18)	-0.69 (-3.4, 2.1)
	Low + Low middle	-0.31 (-1.4, 0.75)	9.0*** (6.1, 12.0)	-2.0 (-5.1, 1.2)	6.3*** (3.8, 8.8)	30* (3.2, 63)	6.5*** (4.0, 9.0)
	Upper Middle + High	1.6*** (0.73, 2.4)	12*** (7.9, 16)	8.0*** (4.2, 12)	4.7** (2.0, 7.6)	-50*** (-64, -31)	-3.6 (-8.6, 1.6)
	World	0.27 (-0.50, 1.0)	11*** (8.7, 14)	4.8*** (2.8, 6.9)	4.7*** (3.7, 5.7)	31** (7.8, 58)	6.9*** (4.7. 9.2)

Table 2.1 (Continued)

LCMR	Socioeconomic Group	BaPeq	SP	Price	GDP.CAP	BMI	Diabetes
ASDR100000	Low	-0.98 (-2.0, 0.01)	7.3*** (4.1, 11)	-4.8** (-7.7, -1.9)	6.0** (1.6, 10)	21 (-8.0, 58)	3.0** (0.94, 5.1)
	Low Middle	0.76 (-0.33, 1.9)	4.0* (0.74, 7.3)	-2.7 (-5.9, 0.60)	0.85 (-6.3, 8.5)	-27* (-46, -1.0)	2.4 (-3.6, 8.6)
	Upper Middle	0.76* (0.18, 1.4)	5.3*** (2.6, 8.0)	-1.4 (-5.7, 2.9)	-3.2 (-9.4, 3.5)	-20 (-40, 6.5)	-1.4 (-5.3, 2.8)
	High	0.25 (-0.32, 0.82)	1.7 (-2.0, 5.6)	0.63 (-2.4, 3.8)	0.44 (-1.8, 2.8)	5.5 (-11, 25)	-0.54 (-2.8, 1.7)
	Low + Low middle	-0.29 (-1.1, 0.48)	5.8*** (3.6, 8.0)	-1.7 (-4.0, 0.62)	3.6*** (1.7, 5.5)	12 (-5.3, 33.0)	3.7*** (1.8, 5.6)
	Upper Middle + High	0.91*** (0.48, 1.3)	5.4*** (3.2, 7.6)	2.9** (0.82, 5.1)	2.1** (0.62, 3.6)	-24** (-37, -9.0)	-1.7 (-4.5, 1.1)
	World	0.14 (-0.35, 0.62)	6.3*** (4.7, 8.0)	2.0** (0.66, 3.3)	2.4*** (1.7, 3.1)	15* (1.9, 30)	3.8*** (2.4, 5.2)

The relationships among the LCMRs and the independent variables were modeled using equation [3] and stepwise multiple linear regression (MLR) for the entire dataset, as well as the different socioeconomic country groups and groupings. Table 2.2 shows the percent change in the median LCMR, given a 10% increase in the mean of the independent variable in the MLR models, for the entire dataset, as well as the different socioeconomic country groups and groupings. Appendix A.7 shows the regression coefficients, standard error, and percent of the total regression sum-of-squares due to β_n for the MLR models. The stepwise procedure primarily selected the smoking prevalence (*log_e(SP)*, positive relationship) and body mass index (*log_e(BMI)*, negative relationship) variables as the most predictive of the LCMRs for the entire dataset and the various socioeconomic country groups and groupings. However, *log_e(BMI)* was not selected by the stepwise procedure for the low and high socioeconomic country group MLR models. The BaP equivalents emission (*log_e(BaPeq)*) was selected by the stepwise procedure for ED100000 for the high socioeconomic country group and the combination of the upper middle and high socioeconomic country groups in the MLR (positive relationship). However, the BaP equivalents emission (*log_e(BaPeq)*) was selected by the stepwise procedure for ASDR100000 only for the combination of the upper middle and high socioeconomic country groups in the MLR (positive relationship). The entire dataset MLR models also included the average price of a pack of cigarettes (for ASDR100000), and GDP per capita (for ED100000 and ASDR100000). Price of cigarettes (*log_e(Price)*) was significantly positively correlated with smoking prevalence (*log_e(SP)*) only for the low socioeconomic country group (Appendix A.19). The variance inflation factors test was used to evaluate cross-correlation between *log_e(Price)* and *log_e(SP)* in the models

Table 2.2 The associated change in the median LCMR (%), given a 10% increase in mean independent variable, in the stepwise multiple linear regression. The 95% confidence interval is given in parenthesis.
D= stepwise procedure dropped variable from model.* p-value<0.05, ** p-value<0.01, *** p-value<0.001

LCMR	Socioeconomic Group	BaPeq	SP	Price	GDP.CAP	BMI	R^2
ED100000	Low	D	9.8*** (5.9, 14)	D	D	D	0.45***
	Low Middle	D	6.6** (2.6, 11)	D	D	-35* (-54, -6.1)	0.30**
	Upper Middle	D	11*** (7.2, 14)	D	D	-46*** (-60, -27)	0.66***
	High	0.81** (0.23, 8.5)	4.5* (0.68, 8.5)	D	D	D	0.37**
	Low + Low middle	D	6.0*** (3.2, 8.9)	-3.2* (-5.7, -0.70)	5.4*** (2.9, 7.8)	D	0.49***
	Upper Middle + High	0.70* (0.13, 1.3)	8.8*** (5.7, 12.1)	3.8** (1.2, 6.5)	D	-38*** (-51, -22)	0.68***
	World	D	8.8*** (6.7, 11)	D	4.5*** (3.5, 5.5)	-24*** (-34, -12)	0.63***

Table 2.2 (Continued)

LCMR	Socioeconomic Group	BaPeq	SP	Price	GDP.CAP	BMI	R^2
ASDR100000	Low	D	7.3*** (4.2, 11)	D	D	D	0.40***
	Low Middle	D	4.4** (1.4, 7.5)	D	D	-30* (-47, -7.5)	0.27**
	Upper Middle	D	5.8*** (3.3, 8.3)	D	D	-26** (-40, -8.0)	0.51***
	High	D	D	D	D	D	D
	Low + Low middle	D	4.3*** (2.1, 6.6)	-2.1* (-4.0, -0.04)	4.6*** (2.2, 7.0)	-18* (-32, -1.5)	0.38***
	Upper Middle + High	0.58** (0.20, 0.96)	4.5*** (2.6, 6.5)	D	D	-16* (-28, -2.4)	0.47***
	World	D	4.9*** (3.4, 6.4)	-1.8* (-3.2, -0.33)	3.1*** (2.1, 4.1)	-14** (-23, -4.0)	0.50***

where both were statistically significant. The results showed that there was no case of cross-correlation between these independent variables in any of the models. Appendix A.7 shows the percent of total sum-of-squares due to each of the variables in the models.

2.4 Discussion

Smoking prevalence and body mass index have been shown to be contributing factors to lung cancer mortality rates [61, 72, 73]. In this study, smoking prevalence was shown to be a significant predictor of the lung cancer mortality rates, with the exception of high socioeconomic countries status for the age standardized lung cancer death rate per 100,000 people. The lack of correlation for these countries may be explained by smoking habits in those individuals with higher incomes. Townsend et al. [96] found that smokers with high incomes tend to smoke less than smokers with lower incomes. Additionally, over the past few decades, smoking prevalence among people with high incomes has decreased significantly [97-100] and the quit rates are higher within the highest socioeconomic group [101]. The smoking prevalence data used in this study does not account for the frequency of smoking. It would be interesting to evaluate the association of carcinogenic PAH emissions and smoking with male and female lung cancer mortality rates separately. However, this could not be done because the lung cancer mortality rates, and independent variables, are not reported separately for males and females.

The negative relationship between body mass index and lung cancer mortality rates, after accounting for the other independent factors, is consistent with previous studies that found reduced lung cancer mortality with increasing BMI (especially when BMI>28) [72, 73, 102, 103]. Appendix A.20 shows $log_e(BMI)$ by socioeconomic country group.

The highest median BMI was for upper-middle socioeconomic country group, followed by the high and low-middle socioeconomic country groups. Only the low-middle and upper-middle socioeconomic country groups had individual countries with average BMI's greater than 28. This may explain the significant negative correlation of $log_e(ED100000)$ and $log_e(ASDR100000)$ with $log_e(BMI)$ for these groups. Another possible explanation for this negative relationship is that smokers tend to have lower BMI than non-smokers [104]. Additionally, decreased levels of vitamin A and carotene (which may be protective against lung cancer) were observed in lean men compared to obese men [105]. Other confounding factors, not accounted for in this study, may also play a role in this negative relationship. Further medical research is necessary to understand the relationship between BMI and lung cancer.

In addition to these prominent factors, PAH emissions, as measured by BaP equivalents, were significantly positively correlated with the estimated lung cancer deaths per 100,000 people in high socioeconomic countries, as well as with both ED100000 and ASDR100000 for the combination of upper-middle and high socioeconomic country groups. When inhaled, PAHs diffuse into lung cells and bind to the aryl hydrocarbon receptor [32]. After metabolic reactions with CYP-enzymes (P450, CYP1A1, CYP1B1, and CYP3A4) and microsomal epoxide hydrolase (EPHX1), PAHs form DNA-adducts [32, 106]. DNA-adducts have been shown to correlate with exposure to PAHs via smoking [32, 107] or exposure to environmental pollution [31, 34, 106]. DNA-PAH adducts may initiate carcinogenesis [35, 108] and their levels may be predictive of cancer risk [34, 35].

This suggests that reducing PAH emissions in high socioeconomic countries and with high PAH emissions, including the U.S., Canada, Australia, France and Germany,

could reduce the estimated lung cancer deaths per 100,000 people. In addition, there is strong evidence of the same trend in upper-middle socioeconomic countries with high PAH emissions, including Brazil, South Africa, Poland, Mexico, and Malaysia (Figure 2.1). Conversely, smoking prevalence was the only significant independent variable in the low socioeconomic country group for the estimated lung cancer deaths per 100,000 people and age standardized lung cancer mortality rate per 100,000 people. This suggests that reducing smoking prevalence in countries in low socioeconomic countries and with high smoking prevalence, including Democratic People's Republic of Korea, Nepal, Mongolia, Cambodia, and Bangladesh, could significantly reduce the estimated lung cancer deaths per 100,000 people, as well as the age standardized lung cancer mortality rate per 100,000 people.

The lack of a correlation between estimated lung cancer deaths per 100,000 people and age standardized lung cancer mortality rate per 100,000 people with PAH emissions for the low and low-middle socioeconomic country groups may be due to the different life expectancy for these two socioeconomic country groups, compared to the upper-middle and high socioeconomic country groups. In 2004, the mean life expectancy for the low, low-middle, upper-middle and high socioeconomic country groups was 54, 69, 72, and 79 years, respectively (Appendix A.21) [109]. Lung cancer incidence and mortality increase with age [110]. For a cohort of 22,874 people with lung cancer, 42.8% were 65 years old, or older, at diagnosis [110]. In addition, the development of clinically detectable cancer takes more than 20 years [94]. Therefore, people with a relatively shorter life expectancy may not develop detectable lung cancer in their lifetimes. If the population in all of the socioeconomic country groups had high life expectancy, there

might be a correlation between estimated lung cancer deaths per 100,000 people and age standardized lung cancer mortality rate per 100,000 people with PAH emissions for all of the different socioeconomic country groups. In the MLR, after accounting for smoking prevalence, $log_e(BaPeq)$ remained significantly correlated with $log_e(ED100000)$ for the high socioeconomic country group and with both $log_e(ED100000)$ and $log_e(ASDR100000)$ for the combination of upper-middle and high socioeconomic country groups (Table 2 and Appendix A.7). These results suggest BaPeq emissions influence lung cancer mortality rates in upper-middle and high socioeconomic country groups more than in the other socioeconomic country groups. Although this study found an association between PAH emissions and lung cancer mortality rates, no causal relationship can be proven and other factors (including other air pollutants) cannot be ruled out. Finally, future studies should test for the potential association between average UVB irradiance for a given country and lung cancer mortality rates as this data becomes available [82, 83].

2.5 Acknowledgements

This publication was made possible in part by grant number P30ES00210 from the National Institute of Environmental Health Sciences (NIEHS), NIH and NIEHS Grant P42 ES016465. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of the NIEHS, NIH. We thank John Molitor of Oregon State University for helpful suggestions.

Supporting Information

Supporting information is available via http://pubs.acs.com and contains the table with all independent and dependent variables, interpolation method, and figures that were not included in main article.

3. DETERMINATION OF PARENT AND HYDROXY PAHs IN PERSONAL PM2.5 AND URINE SAMPLES COLLECTED DURING NATIVE AMERICAN FISH SMOKING ACTIVITIES

OLEKSII MOTORYKIN[1], JILL SCHRLAU[2], YULING JIA[2], BARBARA HARPER[3], STUART HARRIS[3], ANNA HARDING[4], DAVID STONE[2], MOLLY L. KILE[4], DANIEL SUDAKIN[2], STACI L. MASSEY SIMONICH[1,2 *]

[1]Department of Chemistry, Oregon State University, Corvallis, Oregon 97331, USA

[2]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon 97331, USA

[3] Department of Science and Engineering, Confederated Tribes of the Umatilla Indian Reservation, Pendleton, Oregon 97801, USA

[4] School of Biological and Population Health Sciences, Oregon State University, Corvallis, Oregon 97331, USA

Submitted to Science of the Total Environment

*Corresponding author, E-mail: staci.simonich@oregonstate.edu, fax: 541-737-0497.

ABSTRACT

A method was developed for the measurement of 19 parent PAHs (PAHs) and 34 hydroxylated PAHs (OH-PAHs) in urine and personal air samples of particulate matter less than 2.5 um in diameter ($PM_{2.5}$) using GC-MS and validated using NIST SRM 3672 (Organic Contaminants in Smoker's Urine) and SRM 3673 (Organic Contaminants in Nonsmoker's Urine). The method was used to measure PAHs and OH-PAHs in urine and personal $PM_{2.5}$ samples collected from the operators of two different fish smoking facilities (tipi and smoke shed) burning two different wood types (alder and apple) on the Confederated Tribes of Umatilla Indian Reservation (CTUIR) while they smoked salmon. Urine samples were spiked with β-glucuronidase/arylsulfatase to hydrolyze the conjugates of OH-PAHs and the PAHs and OH-PAHs were extracted using Plexa and C18 solid phases, in series. The 34 OH-PAHs were derivatized using MTBSTFA, and the mixture was measured by GC-MS. The personal $PM_{2.5}$ samples were extracted using pressurized liquid extraction, derivatized with MTBSTFA and analyzed by GC-MS for PAHs and OH-PAHs. Fourteen isotopically labeled surrogates were added to accurately quantify PAHs and OH-PAHs in the urine and $PM_{2.5}$ samples and three isotopically labeled internal standards were used to calculate the recovery of the surrogates. Estimated detection limits in urine ranged from 6.0 to 181 pg/ml for OH-PAHs and from 3.0 to 90 pg/ml for PAHs, and, in $PM_{2.5}$, they ranged from 5.2 to 155 pg/m^3 for OH-PAHs and from 2.5 to 77 pg/m^3 for PAHs. The results showed an increase in OH-PAH concentrations in urine after 6 hours of fish smoking and an increase in PAH concentrations in air within each smoking facility. In general, the PAH exposure in the smoke shed was higher than in the tipi and the PAH exposure from burning apple wood was higher than burning alder.

3.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants, found even in remote ecosystems[22, 23, 111]. PAHs are emitted to the atmosphere during incomplete combustion of organic material (e.g. solid fuels, meat)[66], including natural processes (e.g. forest fires, volcano eruptions)[1, 67]. Human exposure to PAHs occurs mainly through ingestion of food with high PAH concentrations, such as mollusks, crustaceans, oil, margarine[18], but also barbequed[112], smoked[15, 113], or grilled meat[114]. Additional sources of PAH exposure include inhalation of vehicular exhaust gases[115], smoke from burning solid fuels[116], and cigarette smoke[117]. Chronic exposure to PAHs has been linked to lung cancer[118] and peripheral arterial disease[119]. The U.S. Environmental Protection Agency Integrated Risk Information System[9] and the International Agency for Research on Cancer (IARC)[7] identify some PAHs as probable human carcinogens. Additionally, some PAHs are known mutagens[68, 69] and animal carcinogens[66, 70].

PAHs are metabolized in the human body via CYP-450 enzymes[120]. The most abundant PAH metabolites in the human body are hydroxylated PAHs (OH-PAHs)[37] and some OH-PAHs are more potent carcinogens than parent PAHs because they can bind to DNA[36, 121] and cause cell mutations[69]. 2-hydroxyphenanthrene has been shown to be linked to cardiovascular diseases[122] and 1-hydroxynaphthalene has a slight endocrine effect in adult males[123]. Diols, diol epoxides and tetraols are also formed by metabolism[117]. Hydroxy and dihydroxy-PAHs are excreted from the body with urine in the form of glucuronides and sulfates[124]. Parent PAHs are not completely metabolized to OH-PAHs in the human body, therefore, urine contains a mixture of both PAHs and OH-PAHs[45, 125-127].

The most frequently measured OH-PAH biomarker for human exposure to PAHs is 1-hydroxypyrene (1-OH-Pyr)[41, 42, 128, 129]. However, 1-OH-Pyr does not provide a complete assessment of human exposure to PAH mixtures because of the different molecular size, shape, and rates of metabolism of different PAHs[40, 130, 131]. Recently, several other OH-PAHs have been used as biomarkers of human exposure to PAHs, including 4-hydroxyphenanthrene (4-OH-Phe)[58] and 3-hydroxybenzo(a)pyrene (3-OH-BaP)[40, 129] for total PAH exposure, 1,2-dihydroxynaphthalene (1,2-OH-Nap) for naphthalene exposure[50], and 2-hydroxynaphthalene and the sum of hydroxyfluorenes for tobacco smoke exposure[132]. Ideally, multiple PAH metabolites, including parent PAHs, should be used as biomarkers to better understand the extent of human exposure to PAHs[60, 124, 133].

The quantitative determination of multiple OH-PAH and PAH biomarkers in human urine is challenging due to difference in the polarity of these compounds and the potential for matrix interferrants[127, 134]. The most common method for measuring OH-PAHs and PAHs in aqueous solutions, such as urine, is extraction using solid phase extraction (SPE), followed by either liquid chromatography coupled with mass spectrometry (LC-MS)[37, 48, 49] or derivatization of OH-PAHs, followed by gas chromatography coupled with mass spectrometry (GC-MS)[43, 50-55]. Other methods include HPLC with fluorescence detection[56-58] and derivatization with pentafluorobenzyl bromide, followed by LC-MS/MS[59]. Derivatization converts OH-PAHs into more volatile compounds and increases the selectivity and sensitivity of the GC/MS analysis. Several derivatization reagents that have been used for OH-PAHs include *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA)[53, 55], *N*,*O*-

bis(trimethylsilyl)trifluoroacetamide (BSTFA)[51, 52, 54], N,O-bis(trimethylsilyl)acetamide (BSA) with 5% trimethylchlorosilane (TMCS)[50], N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA)[43, 54], and pentafluorobenzyl bromide[59]. The choice of derivatization reagent depends on the structure of the OH-PAH. While all of these derivatization reagents react with monohydroxylated PAHs, not all of them react with dihydroxy-PAHs, especially dihydroxy-PAHs with hindered OH groups[52]. Schummer et al[54] compared BSTFA and MTBSTFA and concluded that MTBSTFA was the preferred derivatization reagent for OH-PAHs without sterically hindered OH groups because it gave higher analytical response and better chromatographic resolution of OH-PAH isomers. Another advantage of using MTBSTFA for the derivatization of OH-PAHs is that the products were more stable to hydrolysis in the presence of trace water[135].

Limited amount of studies have been published on tribal exposure, including trace metals [136], polychlorinated biphenyls (PCBs)[137] and methyl mercury from fish[138]. This study was conducted in collaboration with the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) in May 2011[113]. Native Americans traditionally smoke fish for sustenance. During one of the fish smoking events, the fish was analyzed for PAHs before and after smoking[113]. In addition, personal air samples of particulate matter with diameter less than 2.5 um ($PM_{2.5}$) and urine samples were collected from the participants conducting the fish smoking activities in order to measure their exposure to PAHs due to inhalation resulting from their fish smoking activities. The objectives were to develop an analytical method for the determination of 19 PAHs and 34 OH-PAHs in urine and $PM_{2.5}$ and to use this method to better understand human exposure to OH-PAHs and PAHs via

inhalation during traditional Native American fish smoking. Secondary objective was to identify new biomarkers of human exposure to PAHs.

3.2 Experimental Procedures

3.2.1 Reagents

All parent and hydroxy-PAHs standards, including 1-Hydroxynaphthalene (1-OH-Nap), 2-Hydroxynaphthalene (2-OH-Nap), 2,3-Dihydroxynaphthalene (2,3-OH-Nap), 1,3-Dihydroxynaphthalene (1,3-OH-Nap), 1,5-Dihydroxynaphthalene (1,5-OH-Nap), 1,6-Dihydroxynaphthalene (1,6-OH-Nap), 2,7-Dihydroxynaphthalene (2,7-OH-Nap), 2,6-Dihydroxynaphthalene (2,6-OH-Nap), 9-Hydroxyfluorene (9-OH-Flo), 3-Hydroxyfluorene (3-OH-Flo), 2-Hydroxyfluorene (2-OH-Flo), 1-Hydroxy-9-fluorenone (1-OH-Flon), 2-Hydroxy-9-fluorenone (2-OH-Flon), 2-Hydroxyanthraquinone (2-OH-AntQn), 4-Hydroxyphenanthrene (4-OH-Phen), 3-Hydroxyphenanthrene (3-OH-Phen), 2-Hydroxyphenanthrene (2-OH-Phen), 1-Hydroxyphenanthrene (1-OH-Phen), 3-Hydroxyfluoranthene (3-OH-Flt), 1-Hydroxypyrene (1-OH-Pyr), 2-Hydroxyenzo(a)anthracene (2-OH-BaA), 3-hydroxybenzo(c)pnenanthrene (3-OH-BcPhen), 10-Hydroxybenzo(a)pyrene (10-OH-BaP), 12-Hydroxybenzo(a)pyrene (12-OH-BaP), 7-Hydroxybenzo(a)pyrene (7-OH-BaP), 9-Hydroxybenzo(a)pyrene (9-OH-BaP), 3-Hydroxybenzo(a)pyrene (3-OH-BaP), 4-Hydroxychrysene (4-OH-Chr), 6-Hydroxychrysene (6-OH-Chr), 3-Hydroxychrysene (3-OH-Chr), 2-Hydroxychrysene (2-OH-Chr), 1-Hydroxychrysene (1-OH-Chr), 2,6-Hydroxyanthraquinone (2.6-OH-AntQn), 11-Hydroxybenzo(b)fluoranthene (11-OH-BbFlt), Naphthalene (Nap), Acenaphtylene (Acy), Acenaphthene (Ace), Fluorene (Flo), Phenanthrene (Phen), Anthracene (Ant), Fluoranthene (Flt), Pyrene (Pyr), Retene (Ret), Benzo(a)anthracene (BaA), Chrysene

(Chr), Triphenylene (TriPh), Benzo(b)fluoranthene (BbFlt), Benzo(k)fluoranthene (BkFlt), Benzo(e)pyrene (BeP), Benzo(a)pyrene (BaP), Indeno(1,2,3-cd)pyrene (I(1,2,3-cd)Pyr), Dibenz(a,h)anthracene (BahA), Benzo(ghi)perylene (BghiPer) (Appendix B.2) were purchased from AccuStandards, Inc. (New Haven, CT), Sigma-Aldrich (Milwaukee, WI), MRI Global (Kansas City, MO), VWR international, Inc (Radnor, PA), or TCI America (Portland, OR). The isotopically labeled standards, including 1-Hydroxy[$^{2}H_{7}$]naphthalene, 2-Hydroxy[$^{2}H_{9}$]fluorene, 3-Hydroxy[$^{13}C_{6}$]phenanthrene, 1-Hydroxy[$^{13}C_{6}$]pyrene, 1-Hydroxy[$^{13}C_{6}$]benzo(a)anthracene, 3-Hydroxy[$^{13}C_{6}$]benzo(c)phenanthrene, 3-Hydroxy[$^{13}C_{6}$]chrysene, [$^{2}H_{10}$]-Fluorene, [$^{2}H_{10}$]-Phenanthrene, [$^{2}H_{10}$]-Pyrene, [$^{2}H_{12}$]-Triphenylene, [$^{2}H_{12}$]-Benzo(a)pyrene, [$^{2}H_{12}$]-Benzo(ghi)perylene (Appendix B.2) were purchased from Cambridge Isotope Laboratories (Andover, MA), Santa Cruz Biotechnology Inc. (Santa Cruz, CA), MRI Global (Kansas City, MO), or C/D/N isotopes Inc. (Pointe-Claire, Quebec, Canada). The Bond Elute Plexa (30 mg and 60 mg, 3 mL), Bond Elute C18 (100 mg, 3 mL), and Bond Elute Si (500 mg, 3mL) cartridges were purchased from Agilent Technologies (New Castle, DE). The Focus (60mg, 3mL) cartridges were purchased from Varian, Inc (Lake Forest, CA), and the Isolute 101 (100mg, 3 mL) cartridges were purchased from Biotage LLC (Charlotte, NC). Optima grade solvents (methanol, hexane (Hex), ethyl acetate (EA), acetonitrile (ACN), and dichlromethane (DCM)) were purchased from Fisher Scientific (Santa Clara, CA), toluene (>=99.9%), MTBSTFA (>97%), BSTFA+TMCS (99:1) and BSA:TMC:TMSI (3:2:3) were purchased from Sigma-Aldrich (Milwaukee, WI). β-glucuronidase/arylsulfatase was purchased from Roche Diagnostics Corporation (Indianapolis, IN). Acetate buffer (pH=5.5, APHA) was purchased from Ricca Chemical

Company (Arlington, TX). Certified drug free urine was purchased from UTAK laboratories, Inc (Valencia, CA). GC amber vials (1.5 mL) and inserts (300 µL) were purchased from VWR International (San Francisco, CA). Personal $PM_{2.5}$ samples were collected using a Leland Legacy Sample pump, Personal Environmental Monitor, and the quartz fiber filters were purchased from SKC Inc. (Eighty Four, PA). Standard Reference Materials (SRMs) were provided by National Institute for Standards and Technology (NIST) (Gaithersburg, MD).

3.2.2 Collection of urine and personal $PM_{2.5}$ air samples

Urine and personal $PM_{2.5}$ samples were collected from two male members of the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) in May 2011 who smoked salmon (see Appendix B.4 for the sample collection timeline). The participants were asked not to eat foods containing PAHs, including fried, broiled, charcoaled, roasted or toasted foods, three days prior to the observation period when they smoked salmon. Both participants were non-smokers. Each participant operated one smoking structure – either a smoke shed (SS) and or a tipi (TP), shown in Appendix B.5. The participants smoked two batches of salmon in each structure. The first batch was smoked using apple wood and the second batch was smoked using alder wood producing a cross-over study design.

Freshly caught Spring Chinook Salmon from the Columbia River was purchased from a commercial Native American fisherman near Celilo, Oregon[113]. In the smoke shed, fish fillets were placed on a shelf approximately 1.5 m feet above the fire, while in the tipi the fish fillets were hung on a string approximately 2 m above the fire. The smoke shed had a more confined space, with less ventilation, while the tipi had the open top,

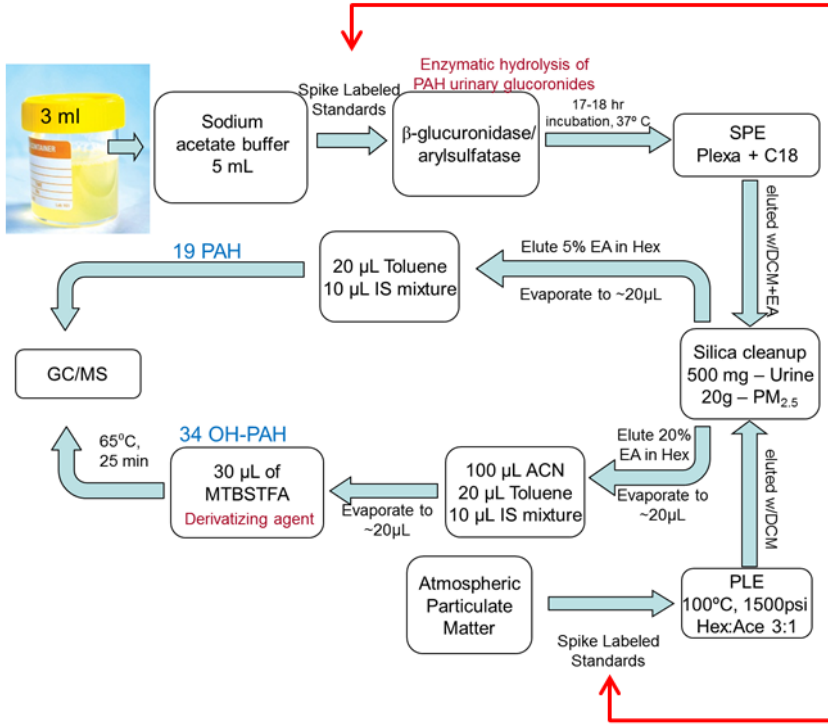
creating an updraft that carried the smoke over the fish. As needed, the operators periodically walked into each structure to add more wood to the fire. Depending on the structure and wood type, it took 22 to 35 hr to fully smoke the fish (Appendix B.4). Both operators wore a Leland Legacy Personal Environmental Monitor $PM_{2.5}$ air sampler (PAS) to collect $PM_{2.5}$ near their breathing zone. In order to capture total $PM_{2.5}$ PAH exposure that only resulted from being inside the smoke structures, operators turned on their PAS before entering the smoking structures and turned off the PAS upon leaving the smoking structure. For each batch, the operators entered and left the smoking structures approximately 10-16 times during the 22 to 35 hr smoking period. The $PM_{2.5}$ was collected on quartz filters (34 mm in diameter) at a flow rate of 10 L/min. The filters were stored at -20^{o}C until analysis.

Urine samples were collected in plastic urine collection cups using clean catch collection technique[139]. When the apple wood was used for fish smoking, four urine samples were collected per participant, per structure: the first sample was collected just before starting to operate the smoking structure and then three samples were collected after 4-5 hr, 12-13 hr, and 22-23 hr of initiating the fish smoking activity (Appendix B.4). When alder wood was used for fish smoking, three urine samples were collected per participant, per structure: after 10 hr, 22 hr and 33-34 hr of initiating activities to smoke the second batch of salmon (Appendix B.4). The sample that was collected prior to operating any smoke structure was also used as time zero for the second batch when alder wood was used in the smoking structures. The time between that elapsed between the end of smoking with apple wood and start of smoking with alder wood was 12.5 hr for the smoke shed operator and 10.9 hr for the tipi operator. Urine samples were stored at -25ºC

and analyzed within one week of collection. The study was approved by the Institutional Review Board of Oregon State University, the CTUIR Health Commission, and Portland Area Indian Health Board.

3.2.3 Urine analytical method

The analytical method developed for the determination of PAH and OH-PAH in urine is shown in Figure 3.1. For all steps of method development, the blank UTAK urine was used. The urine was analyzed for PAHs and OH-PAHs and all reported concentrations are background subtracted. A 3 ml aliquot of urine was mixed with 5 mL of acetate buffer (pH=5.5). Six labeled PAH surrogates (10 µl, 1000 pg/µl) and 9 labeled OH-PAH surrogates (20 µl, 500 pg/µl) were spiked into the mixture for quantitation, followed by the addition of β-glucuronidase/arylsulfatase (10 µl) and incubation overnight (37 °C, 15 hours) to hydrolyze OH-PAHs. Two SPE phases in series, Bond Elut Plexa (for OH-PAHs, top cartridge) and Bond Elut C18 (for PAHs, bottom cartridge), were used to extract the analytes from the urine matrix. The SPE cartridges were preconditioned with 3 mL of methanol, followed by 3 mL of deionized water. The urine was passed through the SPE cartridges and the analytes were retained on the two stationary phases. The analytes were then eluted with 3 ml of DCM, followed by 3 ml of EA and the two extracts were combined. The extract was then loaded onto a 500 mg silica column, previously conditioned with 3 mL of EA, 3 mL of DCM and 6 mL of Hex. The first silica fraction was eluted with 5 ml of 5% EA in Hex and contained parent PAHs. The second silica fraction was eluted with 5 ml of 20% of EA in Hex and contained OH-PAHs. The two fractions were concentrated separately to 300 µl using a



Hydroxy-PAHs surrogates	Parent PAHs surrogates
1-[$^{2}H_{7}$]hydroxynaphthalene	[$^{2}H_{10}$]-Fluorene
2-[$^{2}H_{7}$]hydroxynaphthalene	[$^{2}H_{10}$]-Phenanthrene
2-[$^{2}H_{9}$]hydroxyfluorene	[$^{2}H_{10}$]-Pyrene
4-[$^{13}C_{6}$]hydroxyphenanthrene	[$^{2}H_{12}$]-Triphenylene
3-[$^{13}C_{6}$]hydroxyfluoranthene	[$^{2}H_{12}$]-Benzo(a)pyrene
1-[$^{13}C_{6}$]hydroxypyrene	[$^{2}H_{12}$]-Benzo(ghi)perylene
1-[$^{13}C_{6}$]hydroxybenzo(a)anthracene	
3-[$^{13}C_{6}$]hydroxybenzo(c)phenanthrene	
3-[$^{13}C_{6}$]hydroxychrysene	

Figure 3.1. Flow diagram of the entire analytical method for the measurement of 19 PAHs and 34 OH-PAH in urine and $PM_{2.5}$ samples.

TurboVap® evaporator (nitrogen gas, 30 °C water bath), transferred into amber GC vials with inserts and evaporated to ~20µl under a gentle stream of nitrogen. Internal standards were spiked into the extracts (10 µL, 1 mg/L) to track surrogate recovery and 20 µl of toluene were added to both fractions. One hundred µl of acetonitrile (ACN) was added to OH-PAH fraction, the fraction was evaporated to 20 µl a second time, and 30 µl of MTBSTFA was added for derivitization and determination of 34 OH-PAHs. The OH-PAH fraction was incubated at 65 °C for 25 minutes and the PAH and OH-PAH fractions were analyzed separately by GC-MS (Appendix B.2, OH-PAHs and LMW PAHs). The benefits of the current method include the extensive number of analytes and the ability to quickly extract and analyze both PAHs and OH-PAHs. Additionally, silica cleanup significantly decrease the noise on the chromatogram, thus lowering estimated detection limits.

3.2.4 $PM_{2.5}$ analytical method

The analytical method for the determination of PAH and OH-PAH in $PM_{2.5}$ is also shown in Figure 3.1. The $PM_{2.5}$ filters were spiked with PAH surrogates (10 µl, 1000 pg/µl) and OH-PAH surrogates (20 µl, 500 pg/µl) and extracted with Hex:Ace (3:1, v/v) using pressurized liquid extraction (PLE, 100ºC, 1500 psi). The extract was loaded onto a silica column (20g), preconditioned with 50 ml of EA, 100 ml of DCM and 50 ml of Hex. The extract was eluted from the silica column using 100 ml of DCM. The eluent then was concentrated to 300 µL and a 40 µL aliquot was removed for PAH and OH-PAH analysis. The PM extract was then derivatized with MTBSTFA and analyzed for LMW PAHs, HMW PAHs, Oxy-PAHs, and OH-PAHs (Appendix B.2).

3.2.5 PAH and OH-PAH analysis and detection limits

An Agilent 6890 GC chromatograph, equipped with 7683 series autosampler and 5973N mass selective detector with electron impact ionization was used for the analysis of 19 PAHs and 34 OH-PAHs. A DB-5 GC column (Agilent, 30 m x 25 m, 0.25 μm) was used and the carrier gas was helium with a flow rate of 1 ml/min. The GC oven temperature program for the analysis of PAH and OH-PAH was: 70 °C, hold for 1 min, then ramp 5 °C/min to 200 °C, hold for 5 min, ramp 5 °C/min to 250 °C, ramp 2 °C/min to 286 °C, and ramp 34 °C/min to 320 °C, hold for 5 min. The inlet and interface temperatures were 260 °C and 280 °C, respectively. All analyses were done in selected ion monitoring (SIM) mode and Appendix B.3 lists the m/z ions monitored. The total run time for the determination of 19 PAHs and 34 OH-PAHs was 66 minutes. The method detection limits in urine and air were calculated based on response factors according to the US EPA method 8280A[140]. The personal $PM_{2.5}$ samples were also analyzed for high molecular weight PAHs (302 MW) and oxy-PAHs using previously published methods[20, 47, 141].

3.2.6 Creatinine measurements

Creatinine was measured using the method described in Chiaia et al.[142] Briefly, urine samples were brought to room temperature and 2 ml aliquot was centrifuged at 14 000 rpm. The supernatant was then transferred to HPLC vial and spiked with creatinine-D_3 (US Biological, Swampscott, MA) standard. Creatinine analyses were performed on the Agilent 1100 HPLC system equipped with Waters Quattro Micro tandem mass spectrometer (Milford, MA) operated in positive mode with an electrospray ionization interface. An injection volume of 100 μL was used. The 100 μL sample volume was directly injected into a 2.0 × 4.0 mm C18 security guard cartridge that was attached to a

150 × 4.6 mm 5 μm particle size Luna C18 column (Phenomenex, Torrance, CA). Isocratic conditions with a mobile phase of 10 mM ammonium acetate in 5% methanol were used at a column temperature of 35 °C and a flow rate of 500 μL/min.

3.3 Results and Discussion

3.3.1 Development and Validation of Analytical Method

Experiments were conducted to optimize and validate the analytical method (Figure 3.1) including: urine extraction recoveries, $PM_{2.5}$ extraction recoveries, the storage stability of underivatized OH-PAHs in frozen urine, the storage stability of the derivatized OH-PAHs, the intra- and inter-day variability of analytical method, and the estimated detection limits (EDLs). In addition, we tested different SPE stationary phases for the urine extraction, the SPE elution solvent composition, the volume of β-glucuronidase/arylsulfatase needed for the enzymatic hydrolysis of OH-PAHs and the choice of derivatizing agent (Appendix B.1). As a part of the validation of the analytical method, two NIST Standard Reference Materials (SRMs), SRM 3672 (Organic Contaminants in Smoker's Urine) and SRM 3673 (Organic Contaminants in Non-Smoker's Urine), were analyzed and the results compared to NIST reported concentrations.

3.3.2 Urine Extraction Recoveries

The testing of different SPE stationary phases for the urine extraction, including Bond Elute Plexa (30 mg and 60 mg), Bond Elute C18 (100 mg), Focus cartridges (60mg), and the Isolute 101 cartridges (100 mg), is described in the Appendix B.6. Based on the results of the PAH and OH-PAH recoveries from urine for the different SPE stationary phases, we chose to use the Bond Elute Plexa (60 mg) and Bond Elute C18 (50

mg), in series. A final recovery experiment was conducted for a PAH and OH-PAH mixture spiked in UTAK urine using the Bond Elute Plexa and Bond Elute C18, in series, with DCM and EA used as the elution solvents and the fractions combined (Figure 3.1). The recoveries for both PAHs and OH-PAHs for the entire analytical method are shown in Figure 3.2, and ranged from 14% to 125% (with a mean recovery of 64%). The OH-PAH recoveries were somewhat lower for the combination of SPE phases (61% ± 28%) (compared to Plexa alone, Figure 3.2 and Appendix B.6), but were higher for PAHs (69%±21%) (Appendix B.7). Mono- and dihydroxynaphthalenes had the lowest recoveries of all OH-PAHs (28%±10%), this may be due to their increased polarity and

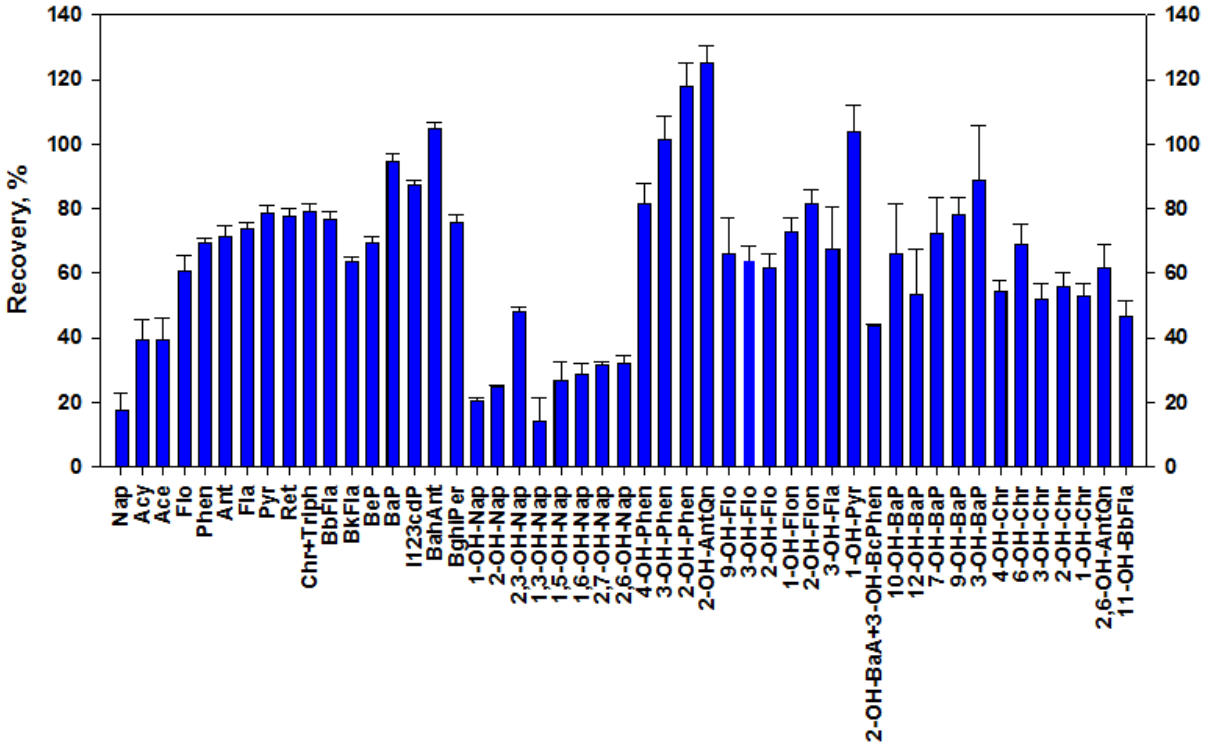


Figure 3.2 Mean PAH and OH-PAH recoveries from urine over the entire method using Plexa+C_{18} SPE combined.
Three independent extractions, error bars represent standard deviation

volatility. The combination of stationary phases was chosen to effectively extract both PAHs and OH-PAHs from urine matrix.

3.3.3 $PM_{2.5}$ Extraction Recoveries

The PAH and OH-PAH extraction recoveries from quartz filters using pressurized liquid extraction (PLE) was determined. Blank and baked quartz filters were spiked with a mixture of 25 µl PAH (1 ng/µl), 25 µl of OH-PAH (1 ng/µl), 10 µl of PAH surrogates (1 ng/µl), and 20 µl of OH-PAH surrogates (500 ng/µl), and extracted with PLE using DCM and Hexane:Acetone (3:1, v/v) solution (100ºC, 1500psi). Both fractions were evaporated to ~30 µl, followed by derivatization and GC-MS analysis. Appendix B.8 shows the PAH and OH-PAH PLE recoveries using these solvents. The mean recovery for PAHs was 68% using DCM (from 21% for Nap to 92% for B(ah)Ant) and 105% (from 47% for Acy to 190% for Phen) using Ace:Hex mixture. For OH-PAHs, the mean recoveries were 65% (range from 0% for 2,3-OH-Nap to 202% for 2-OH-AntQn) for DCM, and 92% (from 1.0% for 3-OH-BaP to 221% for 2-OH-Nap) for Hex:Ace. Overall, the mixture of Hexane and Acetone (3:1 v/v) had higher recoveries for both parent and hydroxy-PAHs and was chosen as the extraction solvent.

3.3.4 Storage stability of spiked OH-PAHs in frozen urine and derivatized OH-PAHs in extracts

The storage stability of OH-PAHs in frozen urine was investigated using six aliquots of UTAK urine (10 ml) that were spiked with 30 µl (1 ng/µl) of OH-PAHs from a standard mixture and frozen (-20 ºC). Over a two week period (on days 1, 3, 6, and 13), one sample at a time was thawed, extracted, and analyzed using the method shown in Figure 3.1. Appendix B.9 shows the percent change in OH-PAH concentrations over 13

days. Most of the OH-PAHs (24 out of 34) were stable (% change from 0.80% to 18%) for up to 6 days, with the exception of dihydroxy-PAHs, 2-OH-AntQn, 9-OH-Flo, and 1-OH-Pyr which were stable for up to 3 days In contrast, Hagedorn et al.[56] found that OH-PAHs were stable for at least seven months in frozen urine (-20 °C). However, in our study to determine stability of OH-PAHs in frozen urine we spiked UTAK urine with OH-PAHs that were unbound to sulfates and glucuronides (worst case scenario), while the Hagedorn et al. study used urine samples that contained OH-PAHs which were bound to sulfates and glucuronides, making them more stable.

The stability of derivatized OH-PAH products in acetonitrile was also investigated. One hundred microliters of acetonitrile was spiked with OH-PAHs (50 µl, 1 ng/µl), the derivatization reaction was performed, the extracts were stored at (-20 °C) and the OH-PAH concentrations were measured in the acetonitrile extracts over a two week period (on days 1, 2, 7, and 14). Appendix B.10 shows the percent change in concentration of derivatized OH-PAH products in acetonitrile over a two week time period. The experiment showed that all of the derivatized OH-PAH products, except 9-OH-Flo and 1,3-OH-Nap, were stable at -20 ºC for up to one week (% change from 0.09% to 10%), and up to two weeks at -20 ºC, with the exception of 9-OH-Flo, 1,3-OH-Nap, 2-OH-AntQn, and 2,6-AntQn.

3.3.5 Intra- and inter-day variability of analytical method

Intra- and inter-day variability of the entire analytical method was determined by spiking three different concentrations of PAH and OH-PAH standards (1000, 100, and 10 pg/µl) in UTAK urine, and analyzing each sample either three times during one day, or on three consecutive days, using the method shown in Figure 3.1.

Appendix B.11 and B.12 show the mean and standard deviations for intra- and inter-day variability for the urine method. The mean intra-day variability of the analytical method was 1.1%, 1.0%, 1.6%, while the mean inter-day variability was 2.0%, 2.7%, and 5.2% for 1000 pg/µl, 100 pg/µl, and 10 pg/µl PAH and OH-PAH spikes, respectively.

3.3.6 Estimated detection limits (EDLs)

Appendix B.2 shows the PAH and OH-PAH EDLs for the entire analytical method in urine and $PM_{2.5}$. The PAH EDLs in urine ranged from 3.0 pg/ml for Phen to 90 pg/ml for BaP and were comparable to those reported by Campo et al.[127] The OH-PAH EDLs in urine ranged from 6.0 pg/ml for 4-OH-Phen to 181 pg/ml for 2,3-OH-Nap. These detection limits were slightly higher than those reported by Onyemauwa et al.[37], Itoh et al.[51], Luan et al.[52] and Romanoff et al.[53], but lower than those reported by Campo et al.[127] and Matarozzi et al.[143]

3.3.7 NIST urine SRM analysis for OH-PAH and PAH

Two NIST urine SRMs, SRM 3672 (Organic Contaminants in Smoker's Urine) and SRM 3673 (Organic Contaminants in Non-Smoker's Urine), were analyzed using the urine analytical method shown in Figure 3.1. The measured mean OH-PAH and PAH concentrations, and corresponding standard deviations, are presented in Appendix B.13. In both SRM samples, the following compounds were measured above the EDL: 1-OH-Nap, 2-OH-Nap, 1,5-OH-Nap, 2-OH-Flu, 3-OH-Flu, 9-OH-Flu, 1-OH-Phen, 2-OH-Phen, 3-OH-Phen, 4-OH-Phen, 1-OH-Pyr, Phen, Flt, Pyr, and Ret. One compounds, 1,6-OH-Nap, was only measured in SRM 3672. With the exception of 1-OH-Nap, the OH-PAH concentrations were higher in the smoker's urine compared to the non-smoker's urine. Urinary PAH concentrations were not statistically different between the smoker's and

non-smoker's urine. We measured 1,5-OH-Nap, 1,6-OH-Nap, Nap, Flu, and Pyr in the NIST urine SRMs that were not analyzed for by NIST.

The percent differences between our measured OH-PAH concentrations and the NIST OH-PAH concentrations ranged from 9% for 2-OH-Flu in SRM 3672[144] to 1460% for 9-OH-Flu in SRM 3673[145] (Appendix B.13). The high percent differences for some of the measured OH-PAH concentrations between our measurement and NIST's measurement of the NIST urine SRMs may be due to the absence of corresponding labeled surrogates for all of the OH-PAHs in our method. Because of cost limitations, only 5 out of 16 PAH and OH-PAH compounds detected above the EDL in our method had corresponding labeled surrogates (Appendix B.13). However, the NIST method had corresponding labeled surrogates for all of the OH-PAH detected above the EDL.

3.3.8 PAH and OH-PAH Concentrations in Operators' Urine from Fish Smoking Activities and Comparison to NHANES

Figure 3.3 shows the OH-PAH and PAH concentrations measured in the urine samples collected before and during the CTUIR fish smoking activities for the different types of wood (apple and alder), as well as the different smoking structures (tipi and smoke shed). The OH-PAH concentrations were normalized to the creatinine concentration of each urine sample to account for dilution[146].

At the time zero the individual urinary OH-PAH concentrations ranged from 0.022 µg/g creatinine (4-OH-Phen) for the tipi (TP) operator to 11.2 µg/g creatinine (2-OH-Nap) for the TP operator. After the operating the smoking structures for 4 to 5 hours, urinary OH-PAHs concentrations increased 1.6 fold (4-OH-Phen, Apple-TP-U-4.3) to 25.4 fold (1-OH-Nap, Apple-TP-U-4.3), with a concentration range of 0.034 µg/g creatinine (4-OH-Phen, Apple-TP-U-4.3) to 18.5 µg/g creatinine (2-OH-Nap, Apple-TP-

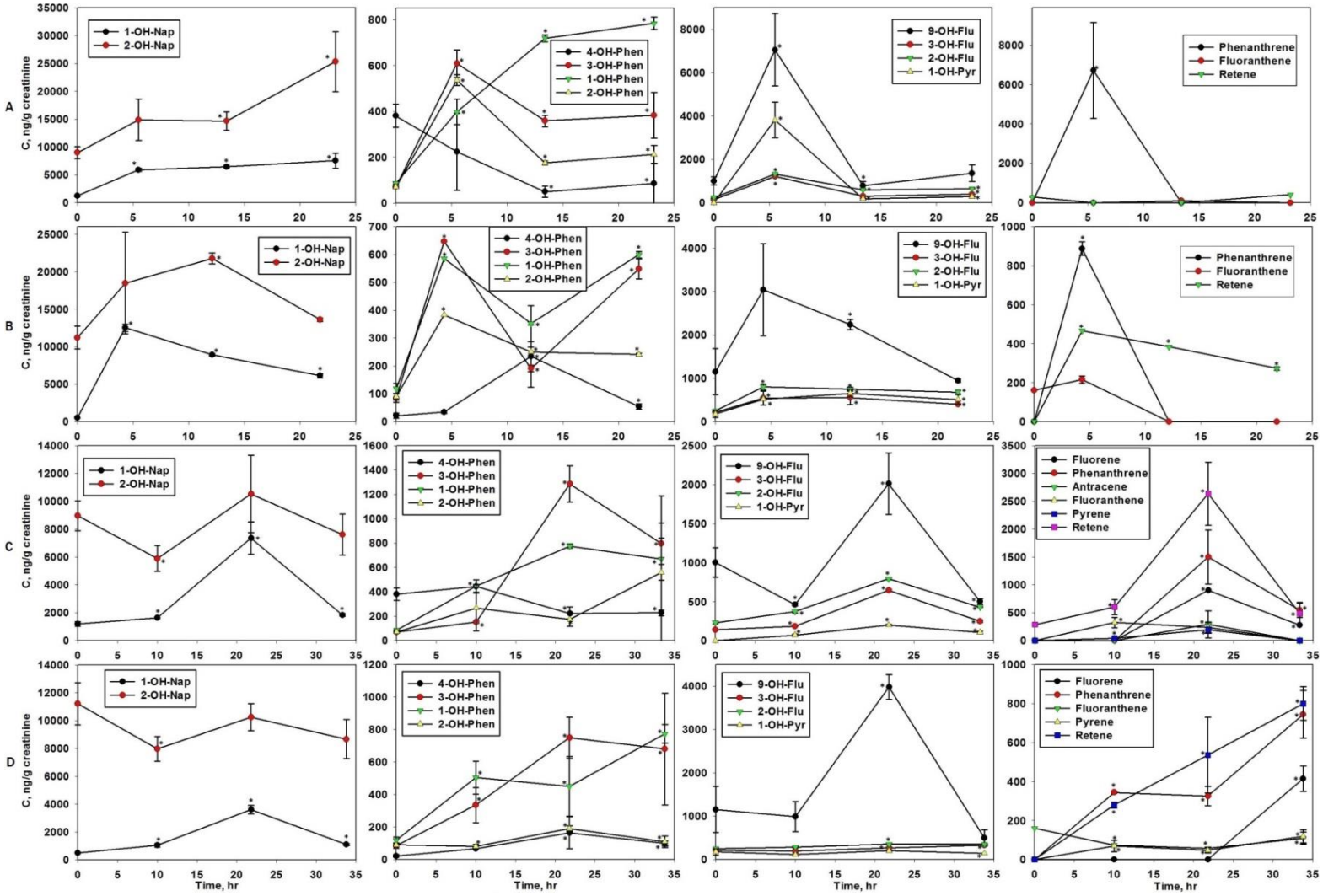


Figure 3.3 Mean OH- and PAH concentrations in urine for A) Apple × Smoke Shed, B) Apple × Tipi, C) Alder × Smoke Shed, D) Alder × Tipi.
Three independent extractions, error bars represent standard deviation.

U-4.3). After twelve to thirteen hours of operating the smoking structures with apple wood, most OH-PAH concentrations in urine decreased by 2 to 95%, with the exception of 1-OH-Nap (Apple-SS-13.4), 1-OH-Phen (Apple-SS-13.4), 2-OH-Nap (Apple-TP-12.1), 4-OH-Phen (Apple-TP-12.1), 3-OH-Flu (Apple-TP-12.1), and 1-OH-Pyr (Apple-TP-12.1). These concentrations were higher than the time zero concentrations with exception of 4-OH-Phen (Apple-SS-13.4), and 9-OH-Flu (Apple-SS-13.4). This fluctuation in urinary OH-PAH over the first thirteen hours of operating a smoking structure likely reflects the need to spend more time in the smoking structures starting and maintaining the fires at the start of a smoking event, compared to the middle and end of the fish smoking activity when the fires are established. Figure 3.3 shows that, 22 to 23 hours after the start of the fish smoking, the urinary OH-PAH concentrations continued to be higher than the pre-exposure concentrations (except 4-OH-Phen, Apple-SS-U-23.2 and 9-OH-Flu, Apple-TP-U-21.8).

For the second batch of salmon which was smoked using alder wood, urinary OH-PAH concentrations increased 1.1 fold (2-OH-Flu, Alder-TP-U-10.0) to 5.2 fold (1-OH-Phen, Alder-SS-U-10.0) after 10 hours of exposure and ranged from 0.066 µg/g creatinine (4-OH-Phen, Alder-TP-U-10.0) to 8.0 µg/g creatinine (2-OH-Nap, Alder-TP-U-10.0). After 21.8 hours of operating the smoking structures with alder wood, most urinary OH-PAHs concentrations increased even further from 1.2 (for 2-OH-Flu, Alder-TP-U-21.8) to 8.4 fold (3-OH-Phen, Alder-SS-U-21.8), with a concentration range from 0.16 µg/g creatinine (1-OH-Nap, Alder-TP-U-21.8) to 10 µg/g creatinine (2-OH-Nap, Alder-SS-U-21.8). Thirty-three of operating the smoking structures with alder wood, most urinary OH-PAH concentrations decreased, except for 4-OH-Phen (Alder-SS-33.3),

2-OH-Phen (Alder-SS-33.3), 1-OH-Phen (Alder-TP-33.8), 3-OH-Flu (Alder-TP-33.8), and 2-OH-Flu (Alder-TP-33.8). This pattern, which was also observed on the first day with apple wood, is likely due to decreased amount of time spent in the smoking structures once the fires have been established or because the fires are not as strong towards the end of the smoking process. Since the dihydroxynaphthalenes (1,3-; 2,3-; 2,7-; and 2,6-), hydroxyanthraquinones (2- and 2,6-), hydroxyfluorenones (1- and 2-), 3-hydroxyfluoranthene, 2-hycroxybenzo(a)anthracene, 3-hydroxybenzo(c)pnenanthrene, hydroxybenzo(a)pyrenes (3-, 7-, 9-, 10- and 12-), hydroxychrysenes (1-, 2-, 3-, 4-, and 6-), and 11-hydroxybenzo(b)fluoranthene were not detected above the EDL in the urine samples or in the NIST SRM urine samples, we conclude they are not predominant human metabolites of PAHs.

Creatinine-adjusted OH-PAH concentrations measured in the urine samples collected in this study were compared to creatinine-adjusted OH-PAH concentrations reported in the National Health and Nutrition Examination Survey (NHANES) for the U.S. population[147] (Figure 3.4). The average time zero concentrations for all measured OH-PAHs, except for 2-OH-Nap and 9-OH-Flu, were in the 25^{th}-to-75^{th} percentile of NHANES values (Figure 3.4, grey bars). The median urinary OH-PAH concentrations measured after time zero were higher than the NHANES median concentration. However, the highest urinary 1-OH-Nap, 3-OH-Flu, and 2-OH-Flu concentrations in the CTUIR urine samples were lower than the highest NHANES concentration. Both the median and the highest concentrations of 1-hydroxypyrene in the CTUIR urine samples collected after time zero were higher than the median and the highest NHANES values. On

average, the mean CTUIR urinary OH-PAH concentrations were 4.4 times higher than the mean NHANES concentrations.

Compared to time zero measurements, inhalation exposure that occurred as a result of smoking salmon led to elevated urinary PAH concentrations (Figure 3.3). After 5.5 hr from the start of fish smoking activities with apple wood, urinary phenanthrene concentrations increased from <EDL to 6.7 µg/g creatinine for the smoke shed operator and to 0.89 µg/g creatinine for the tipi operator. With apple wood, the urinary fluoranthene and retene concentrations also increased in the tipi operator after 4.3 hr from the start of fish smoking from 0.16 to 0.22 µg/g creatinine and from <EDL to 0.47 µg/g creatinine, respectively. In all of the urine samples collected after time zero when the operator was burning apple wood, the urinary PAH concentrations decreased over time after an initial spike in concentration after the initial exposure (Figure 3.3).

Compared to time zero, after 10 hr from the start of smoking salmon with alder wood, urinary PAHs increased for both operators (pyrene [from <EDL to 0.043 µg/g creatinine and from <EDL to 0.07 µg/g creatinine], retene [from 0.029 to 0.06 µg/g creatinine and from <EDL to 0.028 µg/g creatinine]), although fluoranthene concentrations only increased in the smoke shed operator's urine (from <EDL to 0.032 µg/g creatinine, Figure 3.3). With alder wood, the retene concentrations were higher than the other PAH concentrations in operators' urine (2.6 and 0.8 µg/g creatinine, respectively).

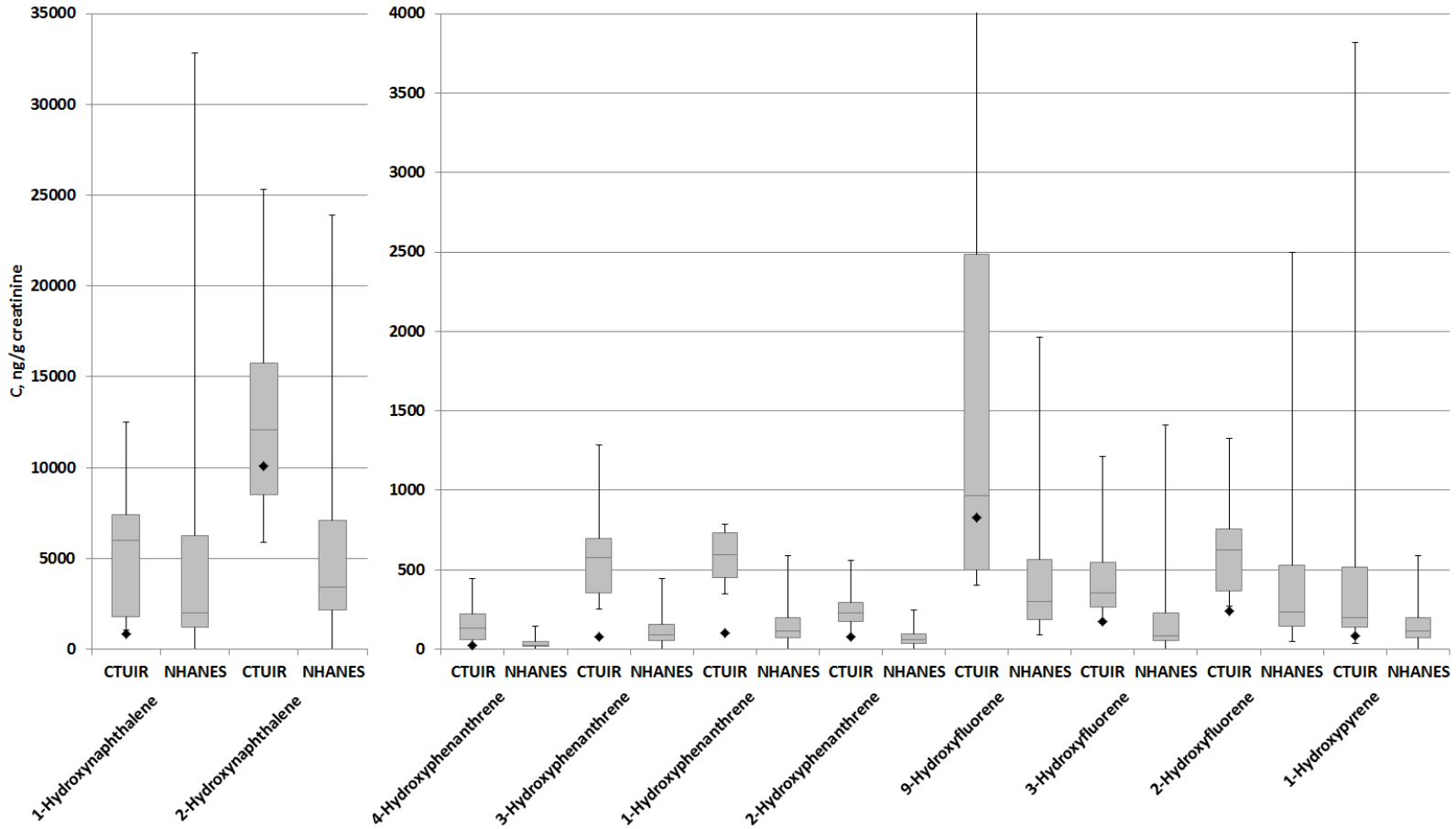


Figure 3.4 The boxplots of urinary OH-PAH concentrations for the CTUIR fish smoking activities and for NHANES values from 2007-2008 survey years.
The grey rectangle represents the first and third quartile (25th and 75th percentile), the line inside is the median, and whiskers are minimum and maximum values. The black diamond is pre-exposure value for CTUIR samples.

3.3.9 PAH Concentrations in Personal $PM_{2.5}$ Air Samples Collected During Fish Smoking Activities

Figure 3.5 shows PAH, high molecular weight PAHs (MW>302), and oxy-PAH concentrations measured in personal $PM_{2.5}$ samples. OH-PAHs were not detected in the personal $PM_{2.5}$ samples above the EDLs. When apple wood was burned, personal $PM_{2.5}$ PAH concentrations were, on average, 16.8 fold higher in the smoke shed than in the tipi (ranging from 5.0 fold for dibenzo[a,c]anthracene to 75.4 fold for 1-methylnaphthalene). The sum of the lower MW PAH concentrations in the personal $PM_{2.5}$ samples when apple wood was burned was 9.1-fold higher in the smoke shed than in the tipi (6209 ng/m^3 vs 683 ng/m^3, respectively). When alder wood was burned, the personal $PM_{2.5}$ PAH concentrations were, on average, 4.6-fold higher in the smoke shed than in the tipi (ranging from 1.2 fold for phenanthrene to 8.0 fold for benzo[k]fluoranthene).

The sum of the higher MW PAH (MW>302) concentrations were 424 ng/m^3 for the smoke shed vs 55 ng/m^3 for the tipi when apple wood was burned and 259 ng/m^3 for the smoke shed vs 12 ng/m^3 for the tipi when alder wood was burned (Figure 3.5). The higher MW PAH concentrations in the personal $PM_{2.5}$ samples were 2.5% to 12% of the lower MW PAH concentrations in the samples. The average concentration of higher MW PAH in the personal $PM_{2.5}$ samples was higher for apple wood compared to alder wood (7.7-fold vs 21.2-fold, respectively).

Appendix B.14 shows the concentration profile of the lower MW PAHs, oxy-PAHs and higher MW PAHs in the personal $PM_{2.5}$ samples across the four combinations of wood and smoking structure types. The lower MW PAHs with the in highest concentration were phenanthrene, fluoranthene and pyrene. Although the concentration of fluoranthene was the highest in all personal $PM_{2.5}$ samples, a metabolite of

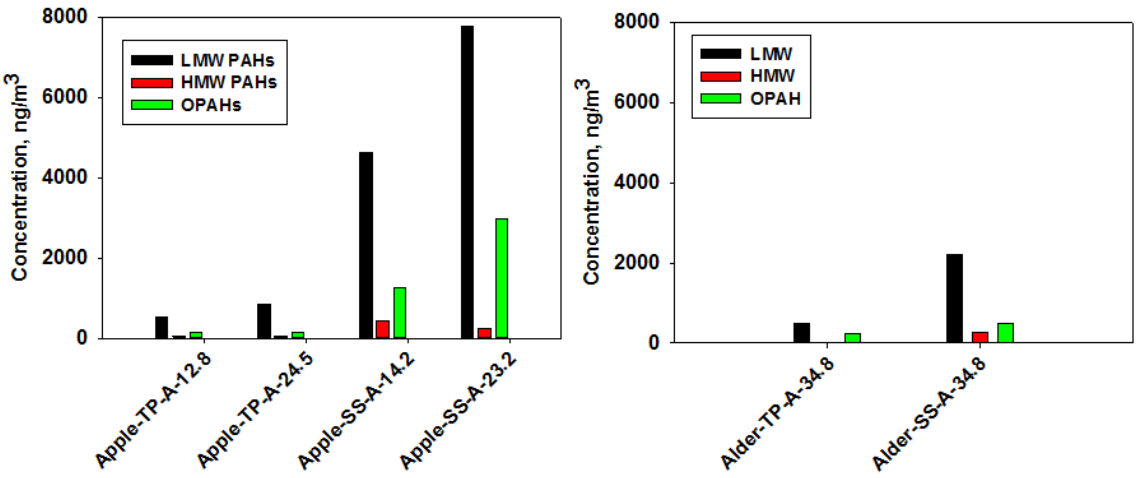


Figure 3.5 Concentrations of low molecular weight (LMW) PAHs, high molecular weight (HMW) PAHs, and oxy-PAHs (OPAHs) in the tipi and the smoke shed personal $PM_{2.5}$ samples.

fluoranthene (3-OH-Flt) was not identified in any urine samples, suggesting that 3-OH-Flt is not a major metabolite of fluoranthene. This is in agreement with Chetiyanukornkul et al.[48] who did not detect urinary 3-OH-fluoranthene in an exposed population in Taiwan. Four urinary metabolites of phenanthrene (4-, 3-, 2-, and 1-OH-hydroxyphenanthrene) and one urinary metabolite of pyrene (1-hydroxypyrene) were identified (Figure 3.3). Although the highest urinary metabolite concentrations were for the hydroxynaphthalenes, the naphthalene concentrations in the personal $PM_{2.5}$ samples were below the EDL. This discrepancy is likely due to the partitioning of naphthalene primarily to the gas phase and exposure to naphthalene via the gas phase rather than $PM_{2.5}$[148]. The sum of all urinary OH-PAH concentrations followed the pattern Apple × SS ≈ Apple × TP > Alder × SS > Alder × TP, while the sum of PAHs in the personal $PM_{2.5}$ samples followed the pattern Apple × SS > Alder x SS > Apple × TP > Alder × TP.

Smoking of fish and meats to preserve them is a cultural tradition among the CTUIR, as is eating large amounts of fish. While this activity cannot, therefore, be reduced, the information provided in this manuscript will allow the CTUIR to craft health advice for those engaged in this activity. The partnership between the university and Tribe benefits both the scientific community (advances in analytical methodology) and the Tribe (provides useful health-related information).

3.4 Acknowledgements

This publication was made possible in part by grant number P30ES00210 from the National Institute of Environmental Health Sciences (NIEHS), NIH and NIEHS Grant P42 ES016465. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of the NIEHS, NIH. We thank Alex Brewer and Dr. Jennifer Field for conducting creatinine measurements. We thank Steven Wise from NIST for providing urine SRM samples.

4. METABOLISM AND EXCRETION RATES OF PARENT AND HYDROXY-PAHs IN URINE COLLECTED AFTER CONSUMPTION OF TRADITIONALLY SMOKED SALMON IN NATIVE AMERICANS

OLEKSII MOTORYKIN[1], LISANDRA SANTIAGO-DELGADO[1], DIANA ROHLMAN[2], JILL E. SCHRLAU[3], BARBARA HARPER[4], STUART HARRIS[4], ANNA HARDING[2], MOLLY L. KILE[2], STACI L. MASSEY SIMONICH[1,3*]

[1]Department of Chemistry, Oregon State University, Corvallis, Oregon 97331, USA

[2]School of Biological and Population Health Sciences, College of Public Health and Human Sciences, Oregon State University, Corvallis, Oregon 97331, USA

[3]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon 97331, USA

[4]Department of Science and Engineering, Confederated Tribes of the Umatilla Indian Reservation, Pendleton, Oregon 97801, USA

Will be submitted to Science of the Total Environment

*Corresponding author, E-mail: staci.simonich@oregonstate.edu, fax: 541-737-0497.

ABSTRACT

A limited amount of data has been published about excretion rates of polycyclic aromatic hydrocarbons (PAHs) and hydroxy-polycyclic aromatic hydrocarbons (OH-PAHs) following oral exposure. This study investigated excretion rates and half-lives of 4 PAHs and 10 OH-PAHs after the consumption of Native American traditionally smoked fish. Nine members of the Confederated Tribes of the Umatilla Indian Reservation consumed smoked fish for breakfast and urine samples were collected during the following 24 hours. The concentrations of OH-PAHs increased to 349 ng/g creatinine for 1-OH-Pyr to 43.9 µg/g creatinine for 2-OH-Nap after 3 to 6 hr post-consumption. The lowest half-life was for retene (1.4 hr) and the highest was for 3-hydroxyfluorene (7.0 hr). The excretion of PAHs was slightly faster than OH-PAHs, and it was shown that frequent urination may decrease the amount of OH-PAHs produced by eliminating PAHs from the human body. The results showed a significant positive correlation between the ration of OH-PAH-to-PAH and creatinine concentrations, indicating the relationship between hydration state and rates of metabolism.

4.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread organic pollutants[4, 23]. They are formed in a process of incomplete combustion of any carbon-based matter, such as wood[116] (while burning), coal[149] (smoldering), meat[13, 114] (barbequing, charcoaling, grilling), and others[1]. Humans are exposed to PAHs mainly through ingestion or inhalation[141, 150, 151], but dermal exposure is also possible[152]. Once PAHs are inside the human body, they are metabolized by the family of CYP-450 enzymes to more water-soluble hydroxy-PAHs (OH-PAHs) and excreted via urine[124, 153]. Some portion of

unmetabolized PAHs are also excreted via urine[45], however the main route of excretion is feces[153, 154] (especially for the higher molecular weight PAHs).

PAHs and OH-PAHs pose a threat to human health because some are toxic, carcinogenic[155, 156], and/or mutagenic[68]. The US Environmental Protection Agency (EPA) priority pollutant list includes 16 PAHs, and some of these have been classified as mutagens and animal carcinogens[9]. The World Health Organization also ranked some of the PAHs as possible or probable human carcinogens[157]. Some hydroxy-PAHs are more toxic than the parent PAHs and can bind to the DNA, causing genetic mutations and tumor growth[36].

Limited numbers of animal and human studies have been conducted to investigate the fate of orally ingested PAHs. Laurent at el.[158] studied the pig's blood levels of BaP and Phen after oral exposure to spiked milk. It was shown that the peak absorption to the blood occurred at 6 hr and 5 hr for BaP and Phen, respectively. The elimination of Phen, Pyr, and BaP via milk, urine and feces of lactating goats was studied by Grova et al.[159] It was shown that 40.4%, 11.4%, and 6.3% of the total amount of Phen, Pyr, and BaP was excreted via urine, respectively. Buckley and Lioy[160] investigated the excretion kinetics of 1-OH-Pyr after oral exposure to BaP. The estimated half-life of 1-OH-Pyr was 4.4 hr (with a range from 3.1 to 5.9 hr). Zhang et al.[150] studied dietary and inhalation exposure to PAHs in Beijing population and found a significant positive correlation ($p<0.01$) between ingested amount of phenanthrene and urinary levels of 2-hydroxyphenanthrenen, and between ingested amount of pyrene and urinary levels of 1-hydroxypyrene. Li et al.[161] studied the excretion rates and half-lives of 10 PAH metabolites after oral ingestion of barbequed chicken. It was found that the half-lives ranged from 2.5 hr for 2-OH-Nap

to 6.1 hr for 3-OH-Flu. Additionally, it was shown that the maximum levels of urinary 1-OH-Pyr after oral exposure were 8 times higher than those of heavy smokers (over 20 cigarettes per day) and at similar levels as coke oven workers or graphite electrode plant workers. All these indicate the need of controlling dietary exposure to PAHs and more research on the elimination kinetics of PAHs.

The tradition of smoking game and fish as a food source is prominent in many Native American communities. There are different ways to smoke game or fish, including a traditional tipi (Figure S1). The fish fillets are hung approximately 2 m above the fire in the tipi and, as the smoke rises from the fire, the meat is cooked. Depending on the wood type and fire, it may take 24 to 48 hr to completely smoke and cook the fish. Gas and particulate phase PAHs are emitted from the fire and deposit onto the surface of the fish. Due to the long cooking time, the PAH concentration sorbed by the fish can be significant and can be up to 300 times higher compared to uncooked or commercially smoked fish[113]. A study by Duedahl-Olesen et al.[14] showed that smoking increased the total PAH load in the fish meat from 6 µg/kg to 32 µg/kg, however the skin contained the highest PAH concentration of 392 µg/kg (a 65 fold increase compared to raw fish). Because smoked fish is a significant portion of Native American diet, it is important to understand their metabolism of PAHs.

In this study, non-smoking members of the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) consumed 50 g of traditionally smoked salmon and provided 5 urine samples over a 24 hour period for analysis of PAH and OH-PAH. The objective of this study was to investigate the metabolism and excretion rates of PAHs and OH-PAHs in members of this Native American community. To the best of our

knowledge, this is the first study to investigate the metabolism of PAHs in a Native American community after the consumption of traditionally smoked food.

4.2 Experimental section

4.2.1 Reagents and materials

All PAH standards, including Naphthalene (Nap), Acenaphtylene (Acy), Acenaphthene (Ace), Fluorene (Flo), Phenanthrene (Phen), Anthracene (Ant), Fluoranthene (Flt), Pyrene (Pyr), Retene (Ret), Benzo(a)anthracene (BaA), Chrysene (Chr), Triphenylene (TriPh), Benzo(b)fluoranthene (BbFlt), Benzo(k)fluoranthene (BkFlt), Benzo(e)pyrene (BeP), Benzo(a)pyrene (BaP), Indeno(1,2,3-cd)pyrene (I(1,2,3-cd)Pyr), Dibenz(a,h)anthracene (BahA), Benzo(ghi)perylene (BghiPer), and all and OH-PAH standards, including 1-Hydroxynaphthalene (1-OH-Nap), 2-Hydroxynaphthalene (2-OH-Nap), 2,3-Dihydroxynaphthalene (2,3-OH-Nap), 1,3-Dihydroxynaphthalene (1,3-OH-Nap), 1,5-Dihydroxynaphthalene (1,5-OH-Nap), 1,6-Dihydroxynaphthalene (1,6-OH-Nap), 2,7-Dihydroxynaphthalene (2,7-OH-Nap), 2,6-Dihydroxynaphthalene (2,6-OH-Nap), 9-Hydroxyfluorene (9-OH-Flo), 3-Hydroxyfluorene (3-OH-Flo), 2-Hydroxyfluorene (2-OH-Flo), 1-Hydroxy-9-fluorenone (1-OH-Flon), 2-Hydroxy-9-fluorenone (2-OH-Flon), 2-Hydroxyanthraquinone (2-OH-AntQn), 4-Hydroxyphenanthrene (4-OH-Phen), 3-Hydroxyphenanthrene (3-OH-Phen), 2-Hydroxyphenanthrene (2-OH-Phen), 1-Hydroxyphenanthrene (1-OH-Phen), 3-Hydroxyfluoranthene (3-OH-Flt), 1-Hydroxypyrene (1-OH-Pyr), 2-Hydroxyenzo(a)anthracene (2-OH-BaA), 3-hydroxybenzo(c)pnenanthrene (3-OH-BcPhen), 10-Hydroxybenzo(a)pyrene (10-OH-BaP), 12-Hydroxybenzo(a)pyrene (12-OH-BaP), 7-Hydroxybenzo(a)pyrene (7-OH-BaP), 9-Hydroxybenzo(a)pyrene (9-OH-

BaP), 3-Hydroxybenzo(a)pyrene (3-OH-BaP), 4-Hydroxychrysene (4-OH-Chr), 6-Hydroxychrysene (6-OH-Chr), 3-Hydroxychrysene (3-OH-Chr), 2-Hydroxychrysene (2-OH-Chr), 1-Hydroxychrysene (1-OH-Chr), 2,6-Hydroxyanthraquinone (2.6-OH-AntQn), 11-Hydroxybenzo(b)fluoranthene (11-OH-BbFlt) (Appendix B.2) were purchased from AccuStandards, Inc. (New Haven, CT), Sigma-Aldrich (Milwaukee, WI), MRI Global (Kansas City, MO), VWR international, Inc (Radnor, PA), or TCI America (Portland, OR). The isotopically labeled PAH standards, including [$^{2}H_{10}$]-Fluorene, [$^{2}H_{10}$]-Phenanthrene, [$^{2}H_{10}$]-Pyrene, [$^{2}H_{12}$]-Triphenylene, [$^{2}H_{12}$]-Benzo(a)pyrene, [$^{2}H_{12}$]-Benzo(ghi)perylene, and isotopically labeled OH-PAH standards, inclusing 1-Hydroxy[$^{2}H_{7}$]naphthalene, 2-Hydroxy[$^{2}H_{9}$]fluorene, 4-Hydroxy[$^{13}C_{6}$]phenanthrene, 1-Hydroxy[$^{13}C_{6}$]pyrene, 1-Hydroxy[$^{13}C_{6}$]benzo(a)anthracene, 3-Hydroxy[$^{13}C_{6}$]benzo(c)phenanthrene, 3-Hydroxy[$^{13}C_{6}$]chrysene, (Appendix B.2) were purchased from Cambridge Isotope Laboratories (Andover, MA), Santa Cruz Biotechnology Inc. (Santa Cruz, CA), MRI Global (Kansas City, MO), or C/D/N isotopes Inc. (Pointe-Claire, Quebec, Canada). The SPE cartridges, including Bond Elut Plexa (60 mg, 3 mL), Bond Elut C18 (100 mg, 3 mL), and Bond Elut Si (500 mg, 3mL), were purchased from Agilent Technologies (New Castle, DE). All solvents (methanol, hexane (Hex), ethyl acetate (EA), acetonitrile (ACN), and dichlromethane (DCM)) were optima grade solvents and were purchased from Thermo Fisher Scientific (Santa Clara, CA), as well as 20 ml clear glass vial. Glass urine collection cups were purchased from VWR International (San Francisco, CA). Acetate buffer (pH=5.5, APHA) was purchased from Ricca Chemical Company (Arlington, TX) and β-glucuronidase/arylsulfatase was purchased from Roche Diagnostics Corporation (Indianapolis, IN). Toluene (>=99.9%)

and MTBSTFA (>97%) was purchased from Sigma-Aldrich (Milwaukee, WI). The GC amber vials (1.5 mL) and inserts (300 μL) were purchased from VWR International (San Francisco, CA). Standard Reference Materials (SRMs) were kindly provided by National Institute for Standards and Technology (NIST) (Gaithersburg, MD). Portable coolers (5 L) and freeze packs were purchased through Amazon (Seattle, WA).

4.2.2 Selection of Study Participants

The study was approved by the Institutional Review Board of Oregon State University, the CTUIR Health Commission, and Portland Area Indian Health Board.

The participants were non-smoker adults with no occupational PAH exposure. There were total of 9 participants (2 males and 7 females). This sample size is similar to other non-occupational exposure studies, including the study by Li et. al.[130]

4.2.3 Preparation of Smoked Salmon

Freshly caught spring Chinook salmon from the Columbia River was purchased from a commercial Native American fisherman near Celilo, Oregon and smoked in a traditional tipi three days prior to the start of the experiment. The fish was stored in a refrigerator at 5 °C. Two fish fillets were homogenized and 50±1 g of salmon was weighed for each participant to consume with breakfast on the first day of observation.

4.2.4 Fish Consumption and Collection of Urine Samples

Urine samples were collected from each participant after the consumption of traditionally smoked salmon in June 2014. Three days before the experiment, the participants were invited to an informational session where the purpose of the study and their responsibilities as the participants were explained. Upon signing a consent forms, each participants was given a list of foods to avoid, including fried, broiled, charcoaled,

roasted or toasted foods, for the duration of the observation period, beginning one day prior to consumption of smoked salmon. On the day of the study, each participant was given a urine collection kit and a survey to evaluate alternative exposures to PAHs. All participants were assigned an anonymous code in order to keep their information private.

The first urine sample was collected before the consumption of the smoked salmon (8:00 am) and was used to measure background PAH and OH-PAH concentrations for each participant. The urine was collected into a 250 ml plastic cup using mid-stream collection technique, and an aliquot was transferred to a 60 ml amber glass urine collection container. After the initial urine sample collection, the participants ate a breakfast that included the pre-weighed 50g of traditionally smoked fish, along with other approved (non-smoked) food items. Four additional urine samples were collected at approximately 3, 6, 12, and 24 hr (8:00 am next day) after the smoked fish consumption. The participants stored their urine samples in a cloth cooler containing two ice packs at approximately +5 °C. The coolers were transported to the Oregon State University on the same day the last urine sample was collected. An aliquot (10 ml) of each sample was transferred to a clear 20 ml glass vial for creatinine measurements and the rest was stored at -70°C until analysis. All samples were extracted for PAH and OH-PAH analysis within 2 weeks of sample collection.

4.2.5 Analytical Method

The analytical method developed for the determination of PAH and OH-PAH in urine is shown in Figure 3.1 and described in Motorykin et al.[130] Briefly, a 3 ml aliquot of urine was taken and mixed with 5 mL of acetate buffer (pH=5.5). Fifteen labeled surrogates were spiked into the mixture for quantitation and 10 μl of β-

glucuronidase/arylsulfatase was added. The mixture was incubated overnight (37 ºC, 16-17 hours) to hydrolyze OH-PAHs. Bond Elut Plexa and Bond Elut C18 solid phase extraction cartridges were used, in series, extract PAHs and OH-PAHs from the urine matrix. Bond Elute Silica cartridges were then used to fractionate the extract. The extract was loaded onto the silica column and eluted with 5 ml of 5% EA in Hex (the PAH fraction), and with 5 ml of 20% of EA in Hex (the OH-PAH fraction). The two fractions were concentrated separately to ~20µl under a gentle stream of nitrogen in an amber GC vial. Internal standards (10 µL, 1 mg/L) and 20 µl of toluene were spiked to both fractions. The OH-PAH fraction was solvent-exchanged to acetonitrile (addition of 100 µl and evaporation to ~20 µl) and 30 µl of MTBSTFA was added to it. The OH-PAH fraction was then incubated at 65 °C for 25 minutes and both fractions were analyzed separately by GC-MS[130]. All urine samples were extracted and analyzed in triplicate. The estimated method detection limits in urine was calculated based on response factors according to the US EPA method 8280A[140] and are shown in Appendix B.2. Creatinine was measured in the urine samples at Peace Health Labs (Springfield, OR) using a colorimetric method[162].

4.2.6 Pharmacokinetic Model

To calculate the PAH and OH-PAH excretion rate constants and half-lives, the creatinine adjusted PAH and OH-PAH concentrations were used. For every time point, starting at 3 hrs post smoked salmon consumption, the means of the individual PAH and OH-PAH concentrations for all 9 participants were calculated and the following nonlinear mixed effects model was used to the estimate first order excretion kinetics[161]:

$$C_i = C_{0i} + a_i * e^{-k_i(t_i-3)} \quad (1)$$

Where C_i is the metabolite *i* concentration at time *t* (mg/g creatinine), C_{0i} is the background concentration of the metabolite *i* (mg/g creatinine), a_i is the initial increase in the concentration of metabolite (mg/g creatinine), t_i is the time of sample collection (hrs), and k_i is the first-order elimination rate constant (hrs^{-1}). After estimating k_i, the half-life of each metabolite was estimated using equation 2[161]:

$$t_{1/2i} = \frac{\ln(2)}{k_i} \qquad (2)$$

4.3 Results and Discussion

4.3.1 Creatinine, PAH and OH-PAH Concentrations

The mean creatinine concentration for all samples was 115±66 mg/dL, with a range of 16 mg/dL (sample P-2 6 hr) to 249 mg/dL (sample P-5 24 hr, Appendix C.1). For most participants (except P-3), the creatinine concentrations were highest in the morning, decreased after 3 hr, and then increased again after 6 hr (P-3, P-4, P-5, P-6, and P-8), 12 hr (P-7 and P-9), or 24 hr (P-1 and P-2).

The urinary PAH and OH-PAH concentrations were creatinine adjusted and plotted against time (Figure 1, Appendices C.2 and C3). Appendix C.4 shows the median, highest and lowest PAH and OH-PAH concentrations for all participants and Figure 4.2 shows the comparison of these concentrations to the concentrations we measured in urine for our previous study on the inhalation of PAH from $PM_{2.5}$ during fish smoking[130] and the 2008 National Health and Nutrition Examination Survey (NHANES) values[147]. The mean OH-PAH pre-exposure concentrations ranged from 56 ng/g creatinine for 2-OH-Phen to 6.9 µg/g creatinine for 2-OH-Nap and were in the range of the 25th to 75th percentile of NHANES concentrations. The highest post-consumption OH-PAH

concentrations ranged from 349 ng/g creatinine for 1-OH-Pyr to 43.9 µg/g creatinine for 2-OH-Nap. The concentrations of most OH-PAHs were highest three hours post smoked fish consumption, however some participants had peak concentrations at six and nine hours post-consumption (Figure 4.1, Appendices C.2 and C3). With the exception of 1-OH-Pyr, the median urinary OH-PAH concentrations were higher in this study compared to the NHANES concentrations. The maximum concentrations of OH-PAHs were also higher in this study compared to the NHANES concentrations, except for 1-OH-Nap, 3-OH-Flu and 1-OH-Pyr.

We compared the results from this study to the result of our previous study, where the inhalation exposure to the smoke from smoking fish was investigated[130] (Figure 4.2). The pre-exposure concentrations were similar for all metabolites, except 4-OH-Phen. The median urinary concentrations were similar for 2-OH-Nap, 4-OH-Phen, 3-OH-Flu, and 9-OH-Flu. The inhalation exposure resulted in higher median concentrations for other metabolites, including 1-OH-Nap, 3-OH-Phen, 1-OH-Phen, 2-OH-Phen, and 1-OH-Pyr. The maximum concentrations, however, were higher for ingestion exposure for most of metabolites, including 1-OH-Nap, 2-OH-Nap, 4-OH-Phen, 1-OH-Phen, 2-OH-Phen, 9-OH-Flu, and 2-OH-Flu. The 3-OH-Phen, 3-OH-Flu, and 1-OH-Pyr had higher maximum urinary concentrations for inhalation exposure.

4.3.2 The ratio of the OH-PAH to PAH

The ratio of the OH-PAH to PAH concentration in urine was used to better understand the efficiency of PAH metabolism. Appendix C.5 shows the ratio of the sum of all hydroxyphenanathrenes to the phenanthrene for all participants. A ratio greater than one indicates that the concentration of OH-PAHs is higher than the concentration of

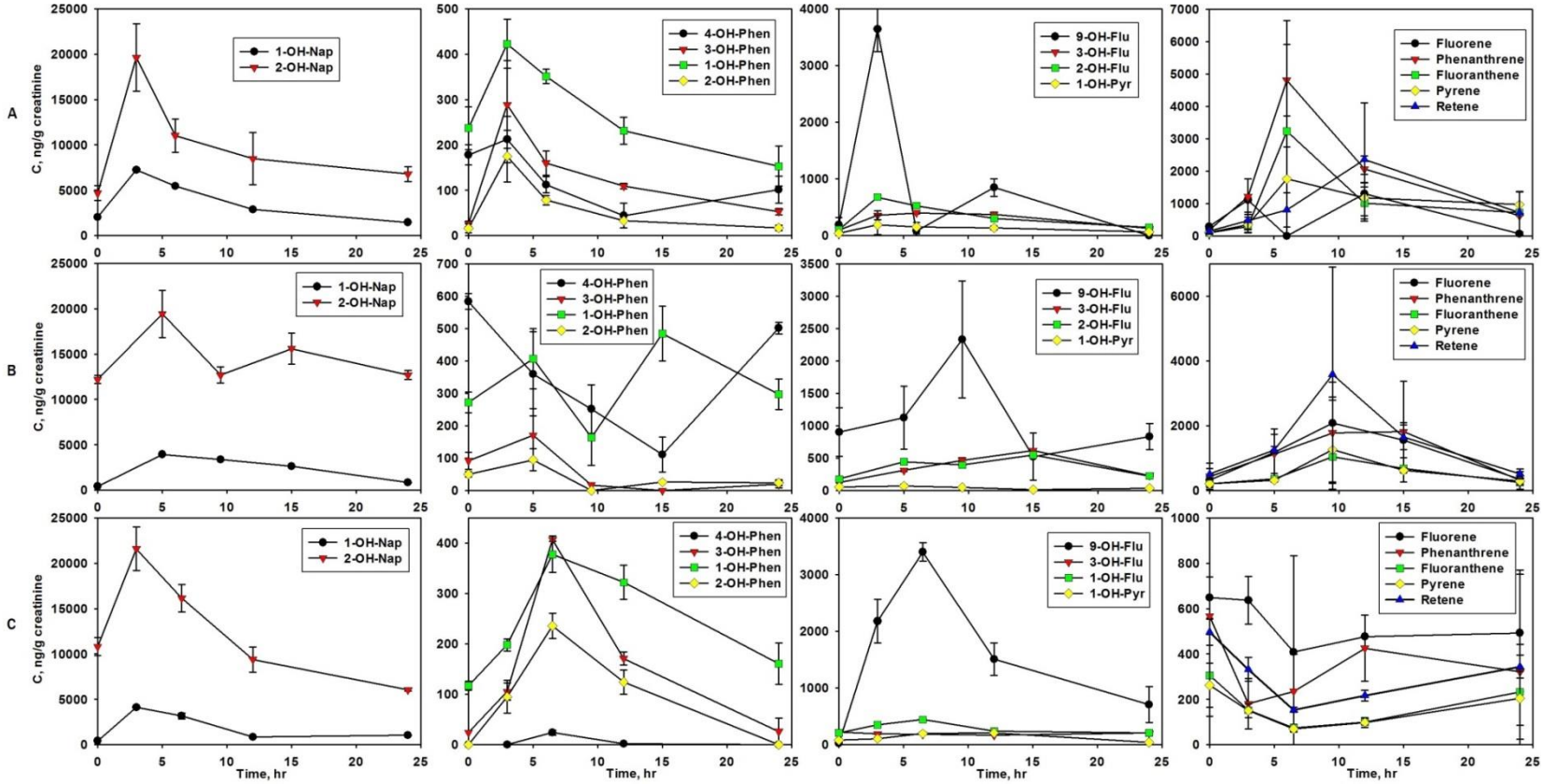


Figure 4.1. The mean creatinine adjusted PAH and OH-PAH concentrations over 24 hr time period.
Three independent extractions, the error bars represent standard deviation. The consumption of fish occurred right at time zero.
A) P-1, B) P-2, C) P-3.

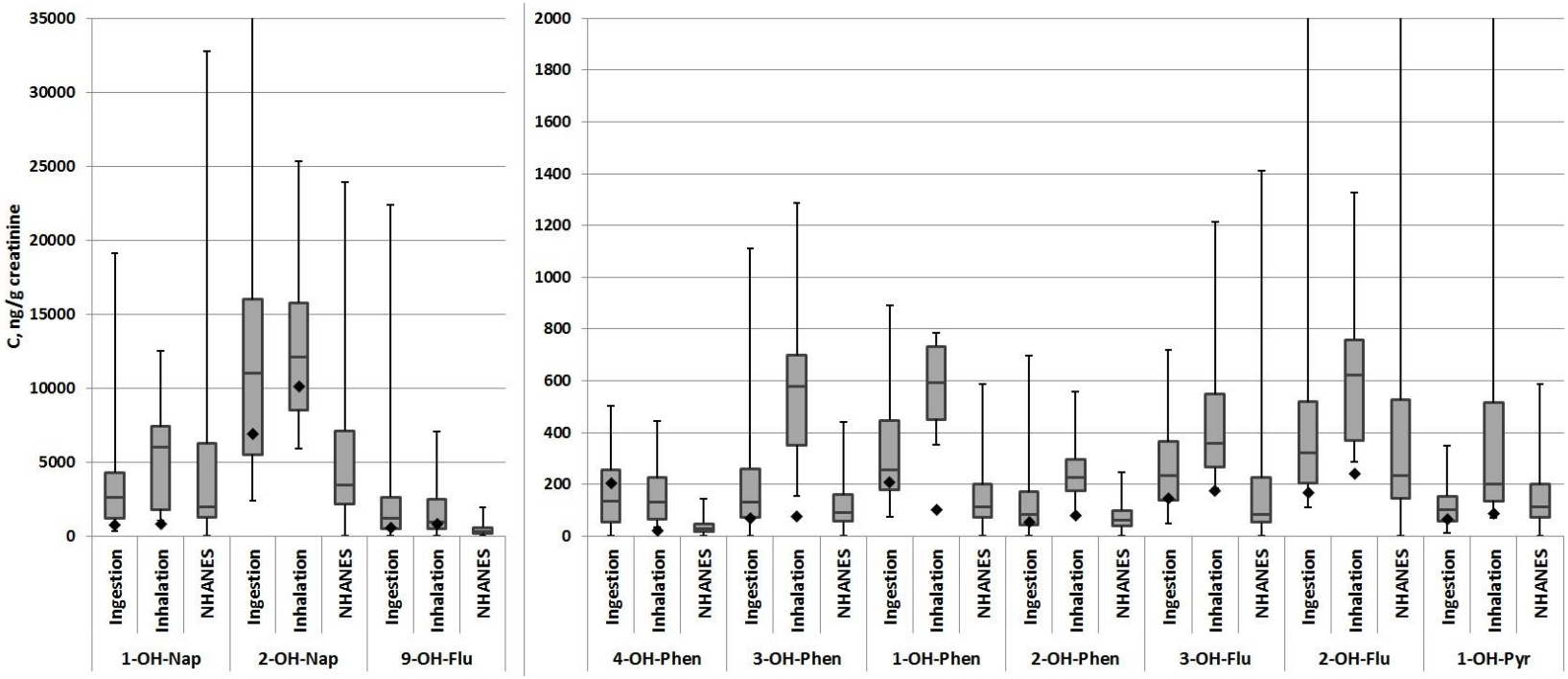


Figure 4.2 The boxplots of urinary OH-PAH concentrations for this study, the CTUIR inhalation study[130] and for NHANES values from 2007-2008 survey years[163].
The grey rectangle represents the first and third quartile (25th and 75th percentile), the line inside is the median, and whiskers are minimum and maximum values. The black diamond is pre-exposure value for ingestion and inhalation studies.

PAHs, and the increase in the ratio means that the rate of excretion of metabolites is higher than the parent compounds. Most of the participants (6 out of 9) had in increased ratio at 3 to 12 hr post-consumption, where the excretion of hydroxy-PAHs is the highest compared to PAHs (and the ratio is higher than one). After the peak the ratio decreased, indicating the slower excretion of OH-PAHs compared to PAHs. For 3 participants (P-1, P-2, P-9), no peak was observed and the ratio was lower or close to 1 during the 12 hr post-consumption. This may be due to different hydration between participants. Participants P-1, P-2, and P-9 had the lowest creatinine concentration during the day (average 45 mg/dl vs average 133 mg/dl for all other participants), indicating that they drank a lot of fluids and urinated more often than the others. Frequent urination can accelerate the excretion of PAHs, not giving them enough time to be converted to more toxic OH-PAHs.

Appendix C.5 also shows the ratio of 1-hydroxypyrene to pyrene for 6 participants (the other three participants did not have enough data points to make a ratio graph). The ratio peaked at 3-6 hr post-consumption for all participants. For P-1, P-2, and P-9 the ratio was always less that one, even at its peak, and for P-3, P-4, and P-5 the ratio was greater than one at its peak. This also indicates faster excretion of PAHs compared to OH-PAHs for P-1, P-2, and P-9.

4.3.3 Pharmacokinetics and Half-Life Estimates

Table 4 lists the modeled pharmacokinetics parameters for 5 parent PAHs and 10 OH-PAH and Figure 4.3 shows the first order elimination curves. The modeled background concentrations were similar to the measured pre-exposure background concentrations (Table 4.1), and ranged from 41 ng/g creatinine for 1-OH-Pyr to 5.3 µg/g

creatinine for 2-OH-Nap. The smallest and largest percent difference between the modeled and measured background concentrations was 0.2% for 3-OH-Phen and 66.6% for 4-OH-Phen, respectively.

The median half-lives for the OH-PAHs varied from 1.7 hr for 9-OH-Flu to 7.0 hr for 3-OH-Flu, and were in the same range as the study by Li et al.[161] (Table 4). The half-lives were statistically significantly lower in this study, compared to the Li et al study[161], for 3-OH Phen (3.6 hr vs 4.1 hr), 1-OH-Phen (3.1 hr vs 5.1 hr) and 9-OH-Flu (1.7 hr vs 3.1 hr). The median half-lives for the PAHs ranged from 1.4 hr for retene and 3.3 hr for pyrene. The median half-life for phenanthrene was 2.2 hr, compared to 2.6 hr for 3-OH-Phen, 2.9 hr for 4-OH-Phen, 3.1 hr for 1-OH-Phen, and 3.7 hr for 2-OH-Phen. The median half-life for pyrene was 3.3 hr, compared to 4.4 hr for 1-OH-Pyr. These excretion half-lives suggest that PAHs are excreted more rapidly than OH-PAHs from the human body. In addition, most of the PAH and OH-PAH concentrations in urine returned to the background concentrations 24-hours post smoked fish consumption.

4.4 Acknowledgements

This publication was made possible in part by grant number P30ES00210 from the National Institute of Environmental Health Sciences (NIEHS), NIH and NIEHS Grant P42 ES016465. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of the NIEHS, NIH.

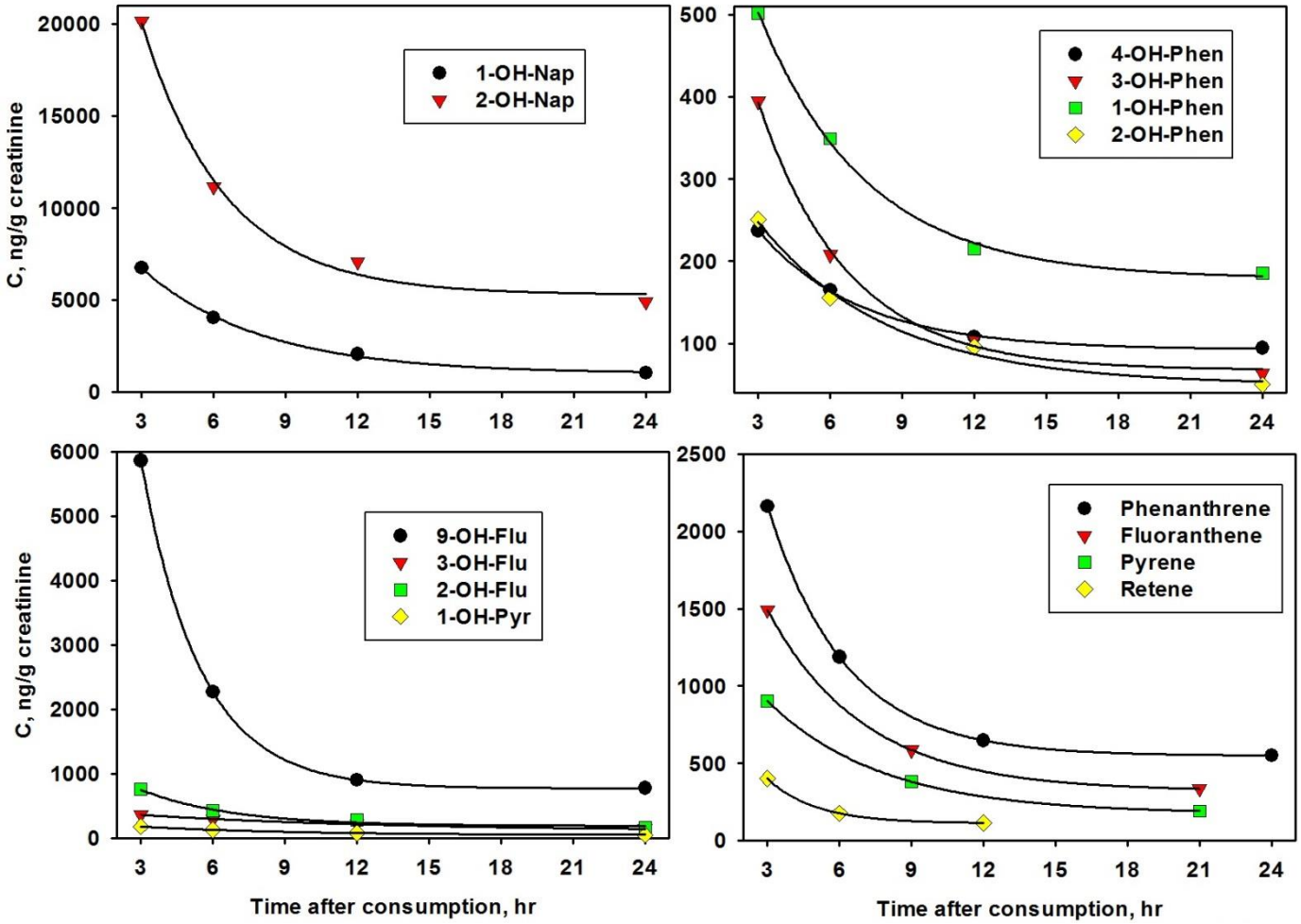


Figure 4.3 Urinary PAH and OH-PAH first order elimination curves.
Fish consumption occurred at time zero.

Table 4. Measured and modeled parameters for the elimination of PAHs and OH-PAHs.
* - statistically different (p-value<0.05), NM – not measured.

		Modeled parameters			Li et al.[130]
	Mean measured background level, ng/g creatinine, $*10^{-2}$	Mean background level, C_0, ng/g creatinine, $*10^{-2}$	Mean elimination rate constant (±SE), *k*, 1/hr	Median half-life (95% CI), hr	Median half-life (95% CI), hr
1-Hydroxynaphthalene	7.6±7.4	10±1.7	0.20±0.02	3.4 (3.1−3.8)	4.3 (3.3−6.2)
2-Hydroxynaphthalene	69±47	52±8.1	0.29±0.06	2.4 (2.0−3.0)	2.5 (2.0−3.4)
4-Hydroxyphenanthrene	2.8±1.9	0.93±0.02	0.24±0.01	2.9 (2.8−3.1)	3.5 (2.7−4.8)
3-Hydroxyphenanthrene	0.68±0.45	0.68±0.11	0.27±0.03	2.6 (2.3−2.9)	4.1 (3.3−5.6)*
1-Hydroxyphenanthrene	2.1±0.91	1.8±0.09	0.22±0.02	3.1 (2.8−3.4)	5.1 (4.3−6.1)*
2-Hydroxyphenanthrene	0.56±0.39	0.50±0.15	0.19±0.04	3.7 (3.0−4.9)	3.9 (3.4−4.6)
9-Hydroxyfluorene	6.0±6.9	7.8±0.04	0.41±0.001	1.7 (1.7−1.7)	3.1 (2.6−3.8)*
3-Hydroxyfluorene	1.5±0.70	1.2±0.12	0.10±0.01	7.0 (6.3−7.9)	6.1 (4.9−8.1)
2-Hydroxyfluorene	1.7±0.48	1.9±0.47	0.26±0.08	2.6 (2.0−3.8)	2.9 (2.3−4.0)
1-Hydroxypyrene	0.67±0.16	0.49±0.08	0.16±0.03	4.4 (3.7−5.3)	3.9 (3.0−5.7)
Phenanthrene	8.6±4.9	5.5±0.02	0.309	2.2	NM
Fluoranthene	1.5±0.87	3.2	0.248	2.8	NM
Pyrene	1.6±0.58	1.8	0.211	3.3	NM
Retene	2.7±1.8	1.1	0.499	1.4	NM

5. CONCLUSIONS

In Chapter 2 the correlation between PAH emissions (their BaP equivalent concentrations) and lung cancer mortality rates (estimated number of lung cancer deaths per 100,000 and age standardized lung cancer death rate per 100,000) was investigated for the 136 countries in the world. Simple and multiple linear regression models were built with regards to the socioeconomic level of the country – low, low middle, upper middle, and high. It was shown that, even after accounting for smoking, price of a pack of cigarettes, GDP per capita, and body mass index, PAH emissions significantly correlated with lung cancer mortality rates for the high (in case of ED100000) and the combination of upper middle and high (in case of both ED100000 and ASDR100000) socioeconomic country groups. The results showed that a 10% increase in mean BaP equivalent emissions was associated with 0.81% and 0.70% increase in estimated number of lung cancer deaths per 100,000 for the high and the combination of upper middle and high socioeconomic country groups. Additionally, a 10% increase in mean BaP equivalent emissions was associated with 0.58% increase in median age standardized deaths rate per 100,000 for the combination of upper middle and high socioeconomic country groups. The absence of correlation for the low and low middle country groups can be explained by average life expectancy in the different socioeconomic country groups. For the low, low middle, upper middle and high socioeconomic country groups, the mean life expectancy was 53.9, 69.3, 72.1, and 79.0 years, respectively. Generally, for the lung cancer to grow to measurable size it takes 20-25 years, so the population with lower life expectancy may not develop detectable lung cancer in their life time.

It was also show that smoking highly significantly positively correlated with lung cancer for all groups, except for the high (ASDR100000). A 10% increase in smoking prevalence was associated with 4.3% to 10.6% increase in median lung cancer mortality rates (for ASDR100000 for the combination of low and low middle, and for ED100000 for the upper middle socioeconomic country groups, respectively). This may be due to different smoking habits. It was shown that people with high income tend to smoke less and have higher quit rates.

The price of the pack of cigarettes was significantly negatively correlated with lung cancer mortality rates. A 10% increase in the mean price was associated with up to 3.2% decrease in median ED100000 for the combination of low and low middle country groups. Body mass index was also shown to be significantly negatively correlated with lung cancer rates for all socioeconomic country groups, except for the low and high (both ED100000 and ASDR100000) and the combination of low and low-middle (ED100000). A 10% increase in mean BMI was associated with up to 46% decrease in median ED100000. This is in agreement with other studies that showed lower lung cancer rates with higher BMI.

In Chapter 2 it was concluded that reducing PAH emissions in high socioeconomic countries and with high PAH emissions could reduce the estimated lung cancer deaths per 100,000 people. Additionally, reducing smoking prevalence in countries in low socioeconomic countries and with high smoking prevalence could significantly reduce the estimated lung cancer deaths per 100,000 people, as well as the age standardized lung cancer mortality rate per 100,000 people.

To further investigate human exposure to PAHs, in Chapter 3 we developed an analytical method to measure parent and hydroxylated PAHs in human urine and atmospheric particulate matter. Hydroxy-PAHs were derivatized with MTBSTFA and both PAHs and OH-PAHs were analyzed by GC-MS in selected ion monitoring mode. The reliability and variability of the method was tested and the method was validated using two NIST Standard Reference Materials. The mean urinary recoveries were 69% for PAHs 61% for OH-PAHs. The mean $PM_{2.5}$ recoveries were 105% for PAHs and 92% for OH-PAHs. The urine samples were stable for up to 3-6 days at -20°C and the derivatized product was stable for up to one to two weeks at -20°C. The maximum intraday variability of the method was 1.6% and the maximum inter-day variability was 5.2%. The estimated detection limits in urine ranged from 6.0 to 181 pg/ml for OH-PAHs and from 3.0 to 90 pg/ml for PAHs, and, in $PM_{2.5}$, they ranged from 5.2 to 155 pg/m^3 for OH-PAHs and from 2.5 to 77 pg/m^3 for PAHs.

Using the new method we investigated the metabolism of PAHs in human body after non-occupational inhalation of smoke from burning wood during traditional Native American fish smoking. Two fish smoking operators collected urine samples before and during fish smoking activities and they also wore personal air samplers to collect $PM_{2.5}$ in near breathing region. Later, the urine and particulate matter was analyzed using presented method and the lifetime cancer risk associated with breathing smoke during fish smoking activities was estimated. There were two smoking structures (tipi and smoked shed) and two wood types (apple and alder). It was shown that urinary OH-PAH concentrations increased up to 18 µg/g creatinine, and urinary PAHs increased up to 6.7 µg/g creatinine during the fish smoking activity. The median urinary OH-PAH

concentrations after the start of the fish smoking activities were, on average, 3.9 times higher than the reported by National Health and Nutrition Examination Survey (NHANES) for general US population. The exposure in the smoke shed was higher than in the tipi and smoke from burning apple wood had higher PAH concentrations than burning alder.

The $PM_{2.5}$ was analyzed for low molecular weight PAHs, high molecular weight PAHs, oxy-PAHs and nitro-PAHs. The highest concentrations of lower MW PAHs were for phenanthrene, fluoranthene and pyrene. When apple wood was used, the sum of all PAHs was 5.3 $\mu g/m^3$ and 0.68 $\mu g/m^3$ for the smoked shed and tipi, respectively. When alder wood was used, the sum of all PAHs was 2.5 $\mu g/m^3$ and 0.50 $\mu g/m^3$ for the smoked shed and tipi, respectively.

In Chapter 4 we used the method developed in Chapter 3 to measure parent and hydroxy-PAHs in human urine after ingestion of Native American traditionally smoked salmon. Nine participants had 50g of smoked salmon for breakfast and collected urine samples before the consumption and after 3, 6, 12, and 24 hr. Their diet was controlled 24 hr prior to the experiment and during urine collection to minimize the bias of the experiment. It was shown that the concentration of urinary OH-PAHs increased up to 44 µg/g creatinine and reached maximum after 3 to 9 hr post-consumption. The concentration of urinary PAHs also increased up to 6.1 µg/g creatinine. The pre-exposure values for all metabolites (except 1-OH-Nap and 4-OH-Phen) were in the range of 25^{th} to 75^{th} percentile of NHANES values. With the exception of 1-OH-Pyr, the median urinary OH-PAH concentrations were higher in this study compare to NHANES. The maximum

levels of metabolites were also higher in this study, except for 1-OH-Nap, 3-OH-Flu and 1-OH-Pyr.

The ratio of hydroxy-to-parent PAHs was also investigated. It was shown participants who had lowest creatinine (high hydration) had the ratio lower than 1, indicating lower rates of excretion for OH-PAHs compared to PAHs. The correlation between the ratio and creatinine concentration showed that hydration state play a role in excretion kinetics. The higher the hydration state the faster PAHs are eliminated and the less OH-PAHs are produced. This is important conclusion because OH-PAHs are more toxic than PAHs and the minimization of their creation is the key to lowering the health risks.

The rates of elimination were estimated in Chapter 4. Of all OH-PAHs, the 9-hydroxyfluorene had the lowest half-life of 1.7 hr, while 3-hydroxyfluorene had the highest – 7.0 hr. For the parent PAHs half-lives were higher and ranged from 1.4 hr for retene to 3.3 hr for pyrene. This shows that, in general, PAHs and OH-PAHs are eliminated relatively fast from human body, however PAHs are eliminated with higher rate. After 24 hr of the consumption of the smoked fish, the urinary concentrations return to their pre-exposure values.

The results from Chapter 3 and 4 showed that the maximum urinary OH-PAH concentrations were higher after consumption of smoked fish than after inhalation of smoke from smoking the fish. This result indicates that oral exposure has higher risk compared with inhalation. This is most likely due to the higher mass amount of PAHs in the smoked fish than in the air.

The next steps of this research will be applying developed method to measure urinary PAH and OH-PAH concentrations after different human exposure. Another possibility is to study the mass balance of PAHs that come in the human body and then excreted as unmetabolized or metabolized compounds.

BIBLIOGRAPHY

(1) Baek, S. O., Field, R. A., Goldstone, M. E., Kirk, P. W., Lester, J. N., and Perry, R. (1991) A REVIEW OF ATMOSPHERIC POLYCYCLIC AROMATIC-HYDROCARBONS - SOURCES, FATE AND BEHAVIOR. *Water Air Soil Pollut.*, *60*, 279-300.

(2) Jaffrezo, J. L., Clain, M. P., and Masclet, P. (1994) POLYCYCLIC AROMATIC-HYDROCARBONS IN THE POLAR ICE OF GREENLAND - GEOCHEMICAL USE OF THESE ATMOSPHERIC TRACERS. *Atmos. Environ.*, *28*, 1139-1145.

(3) Kukucka, P., Lammel, G., Dvorska, A., Klanova, J., Moller, A., and Fries, E. (2010) Contamination of Antarctic snow by polycyclic aromatic hydrocarbons dominated by combustion sources in the polar region. *Environmental Chemistry*, *7*, 504-513.

(4) Usenko, S., Landers, D. H., Appleby, P. G., and Simonich, S. L. (2007) Current and historical deposition of PBDEs, pesticides, PCBs, and PAHs to rocky mountain national park. *Environ. Sci. Technol.*, *41*, 7235-7241.

(5) Jung, K. H., Yan, B. Z., Chillrud, S. N., Perera, F. P., Whyatt, R., Camann, D., Kinney, P. L., and Miller, R. L. (2010) Assessment of Benzo(a)pyrene-equivalent Carcinogenicity and Mutagenicity of Residential Indoor versus Outdoor Polycyclic Aromatic Hydrocarbons Exposing Young Children in New York City. *Int. J. Environ. Res. Public Health*, *7*, 1889-1900.

(6) United States Environmental Protection Agency. Water: CWA Methods. Priority pollutants.

(7) International Agency for Research on Cancer (IARC). (1983) Benzo[a]pyrene. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans.Polynuclear Aromatic Compounds. Part 1. Chemical, Environmental and Experimental Data. *World Health Organization, Lyon, France*, *32*, 33-224.

(8) WHO, and IARC. (2010) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, World Health Organization.

(9) U.S. EPA. (1992) Integrated Risk Information System (IRIS), US Environmental Protection Agency.

(10) Agency for Toxic Substances and Disease Registry. (1995) Toxicological profile for polycyclic aromatic hydrocarbons, Department of Health and Human Services, Public Health Service, Atlanta, GA, U.S.

(11) Hecht, S. S. (2003) Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nature Reviews Cancer*, *3*, 733-744.

(12) International Agency for Research on Cancer (IARC). (2004) Working Groupon the Evaluation of Carcinogenic Risks to Humans: Tobacco smoke and involuntary smoking. *IARC Monogr Eval Carcinog Risks Hum*, *83*, 1-1438.

(13) Akpambang, V. O. E., Purcaro, G., Lajide, L., Amoo, I. A., Conte, L. S., and Moret, S. (2009) Determination of polycyclic aromatic hydrocarbons (PAHs) in commonly consumed Nigerian smoked/grilled fish and meat. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment*, *26*, 1096-1103.

(14) Duedahl-Olesen, L., Christensen, J. H., Hojgard, A., Granby, K., and Timm-Heinrich, M. (2010) Influence of smoking parameters on the concentration of polycyclic aromatic hydrocarbons (PAHs) in Danish smoked fish. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment*, *27*, 1294-1305.

(15) Essumang, D. K., Dodoo, D. K., and Adjei, J. K. (2012) Polycyclic aromatic hydrocarbon (PAH) contamination in smoke-cured fish products. *Journal of Food Composition and Analysis*, *27*, 128-138.

(16) Wretling, S., Eriksson, A., Eskhult, G. A., and Larsson, B. (2010) Polycyclic aromatic hydrocarbons (PAHs) in Swedish smoked meat and fish. *Journal of Food Composition and Analysis*, *23*, 264-272.

(17) European Commission. (2005) Commission Regulation (EC) No. 208/(2005) of 4 February (2005) amending Regulation No. 466/(2001) as regards polycyclic aromatic hydrocarbons, (Off J Eur Union L., Ed.) pp 34:33–35.

(18) Veyrand, B., Sirot, V., Durand, S., Pollono, C., Matchand, P., Dervilly-Pinel, G., Tard, A., Leblanc, J.-C., and Le Bizec, B. (2013) Human dietary exposure to polycyclic aromatic hydrocarbons: Results of the second French Total Diet Study. *Environment International*, *54*, 11-17.

(19) Williams, E. S., Mahler, B. J., and Van Metre, P. C. (2013) Cancer Risk from Incidental Ingestion Exposures to PAHs Associated with Coal-Tar-Sealed Pavement. *Environ. Sci. Technol.*, *47*, 1101-1109.

(20) Wang, W. T., Jariyasopit, N., Schrlau, J., Jia, Y. L., Tao, S., Yu, T. W., Dashwood, R. H., Zhang, W., Wang, X. J., and Simonich, S. L. M. (2011) Concentration and Photochemistry of PAHs, NPAHs, and OPAHs and Toxicity of PM2.5 during the Beijing Olympic Games. *Environ. Sci. Technol.*, *45*, 6887-6895.

(21) Zimmermann, K., Jariyasopit, N., Simonich, S. L. M., Tao, S., Atkinson, R., and Arey, J. (2013) Formation of Nitro-PAHs from the Heterogeneous Reaction of Ambient Particle-Bound PAHs with N2O5/NO3/NO2. *Environ. Sci. Technol.*, *47*, 8434-8442.

(22) Primbs, T., Piekarz, A., Wilson, G., Schmedding, D., Higginbotham, C., Field, J., and Simonich, S. M. (2008) Influence of Asian and Western United States urban areas and fires on the atmospheric transport of polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and fluorotelomer alcohols in the Western United States. *Environ. Sci. Technol.*, *42*, 6385-6391.

(23) Usenko, S., Smonich, S. L. M., Hageman, K. J., Schrlau, J. E., Geiser, L., Campbell, D. H., Appleby, P. G., and Landers, D. H. (2010) Sources and Deposition of Polycyclic Aromatic Hydrocarbons to Western US National Parks. *Environ. Sci. Technol.*, *44*, 4512-4518.

(24) Obiri, S., Cobbina, S. J., Armah, F. A., and Luginaah, I. (2013) Assessment of cancer and noncancer health risks from exposure to PAHs in street dust in the Tamale Metropolis, Ghana. *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering*, *48*, 408-416.

(25) Olsson, A. C., Fevotte, J., Fletcher, T., Cassidy, A., t Mannetje, A., Zaridze, D., Szeszenia-Dabrowska, N., Rudnai, P., Lissowska, J., Fabianova, E., Mates, D., Bencko, V., Foretova, L., Janout, V., Brennan, P., and Boffetta, P. (2010) Occupational exposure to polycyclic aromatic hydrocarbons and lung cancer risk:

a multicenter study in Europe. *Occupational and Environmental Medicine*, *67*, 98-103.

(26) Svecova, V., Topinka, J., Solansky, I., Rossner, P., Jr., and Sram, R. J. (2013) Personal exposure to carcinogenic polycyclic aromatic hydrocarbons in the Czech Republic. *Journal of Exposure Science and Environmental Epidemiology*, *23*, 350-355.

(27) Zhang, Y. X., and Tao, S. (2009) Global atmospheric emission inventory of polycyclic aromatic hydrocarbons (PAHs) for 2004. *Atmos. Environ.*, *43*, 812-819.

(28) Wang, J., Chen, S. J., Tian, M., Zheng, X. B., Gonzales, L., Ohura, T., Mai, B. X., and Simonich, S. L. M. (2012) Inhalation Cancer Risk Associated with Exposure to Complex Polycyclic Aromatic Hydrocarbon Mixtures in an Electronic Waste and Urban Area in South China. *Environ. Sci. Technol.*, *46*, 9745-9752.

(29) Xia, Z., Duan, X., Tao, S., Qiu, W., Liu, D., Wang, Y., Wei, S., Wang, B., Jiang, Q., Lu, B., Song, Y., and Hu, X. (2013) Pollution level, inhalation exposure and lung cancer risk of ambient atmospheric polycyclic aromatic hydrocarbons (PAHs) in Taiyuan, China. *Environmental Pollution*, *173*, 150-156.

(30) Zhou, B., and Zhao, B. (2012) Population inhalation exposure to polycyclic aromatic hydrocarbons and associated lung cancer risk in Beijing region: Contributions of indoor and outdoor sources and exposures. *Atmos. Environ.*, *62*, 472-480.

(31) Kriek, E., Vanschooten, F. J., Hillebrand, M. J. X., Vanleeuwen, F. E., Denengelse, L., Delooff, A. J. A., and Dijkmans, A. P. G. (1993) DNA ADDUCTS AS A MEASURE OF LUNG-CANCER RISK IN HUMANS EXPOSED TO POLYCYCLIC AROMATIC-HYDROCARBONS. *Environ. Health Perspect.*, *99*, 71-75.

(32) Mollerup, S., Berge, G., Baera, R., Skaug, V., Hewer, A., Phillips, D. H., Stangeland, L., and Haugen, A. (2006) Sex differences in risk of lung cancer: Expression of genes in the PAH bioactivation pathway in relation to smoking and bulky DNA adducts. *Int. J. Cancer*, *119*, 741-744.

(33) Pavanello, S., and Lotti, M. (2013) Internal exposure to carcinogenic polycyclic aromatic hydrocarbons and DNA damage. *Archives of Toxicology*, *87*, 551-553.

(34) Shields, P. G., Bowman, E. D., Harrington, A. M., Doan, V. T., and Weston, A. (1993) POLYCYCLIC AROMATIC HYDROCARBON-DNA ADDUCTS IN HUMAN LUNG AND CANCER SUSCEPTIBILITY GENES. *Cancer Res.*, *53*, 3486-3492.

(35) Veglia, F., Matullo, G., and Vineis, P. (2003) Bulky DNA adducts and risk of cancer: A meta-analysis. *Cancer Epidemiol. Biomarkers Prev.*, *12*, 157-160.

(36) Wang, L. R., Wang, Y., Chen, J. W., and Guo, L. H. (2009) A structure-based investigation on the binding interaction of hydroxylated polycyclic aromatic hydrocarbons with DNA. *Toxicology*, *262*, 250-257.

(37) Onyemauwa, F., Rappaport, S. M., Sobus, J. R., Gajdosova, D., Wu, R. A., and Waidyanatha, S. (2009) Using liquid chromatography-tandem mass spectrometry to quantify mono hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine. *J. Chromatogr. B*, *877*, 1117-1125.

(38) Kuang, D., Zhang, W., Deng, Q., Zhang, X., Huang, K., Guan, L., Hu, D., Wu, T., and Guo, H. (2013) Dose-Response Relationships of Polycyclic Aromatic Hydrocarbons Exposure and Oxidative Damage to DNA and Lipid in Coke Oven Workers. *Environ. Sci. Technol.*, *47*, 7446-7456.

(39) Eom, S.-Y., Yim, D.-H., Moon, S. I., Youn, J.-W., Kwon, H.-J., Oh, H. C., Yang, J. J., Park, S. K., Yoo, K.-Y., Kim, H. S., Lee, K.-S., Chang, S.-H., Kim, Y. D., Kang, J.-W., and Kim, H. (2013) Polycyclic Aromatic Hydrocarbon-induced Oxidative Stress, Antioxidant Capacity, and the Risk of Lung Cancer: A Pilot Nested Case-control Study. *Anticancer Res.*, *33*, 3089-3097.

(40) Barbeau, D., Marques, M., and Maitre, A. (2009) 3-hydroxybenzo[a]pyrene as a new biomarker of exposure to carcinogenic polycyclic aromatic hydrocarbons. *Toxicol. Lett.*, *189*, S160-S160.

(41) Jongeneelen, F. J. (2001) Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. *Ann. Occup. Hyg.*, *45*, 3-13.

(42) Levin, J. O. (1995) FIRST INTERNATIONAL WORKSHOP ON HYDROXYPYRENE AS A BIOMARKER FOR PAH EXPOSURE IN MAN - SUMMARY AND CONCLUSIONS. *Sci. Total Environ.*, *163*, 165-168.

(43) Grova, N., Salquebre, G., Schroeder, H., and Appenzeller, B. M. R. (2011) Determination of PAHs and OH-PAHs in Rat Brain by Gas Chromatography Tandem (Triple Quadrupole) Mass Spectrometry. *Chem. Res. Toxicol.*, *24*, 1653-1667.

(44) Li, Z., Pittman, E. N., Trinidad, D. A., Romanoff, L. C., Mulholland, J., and Sjodin, A. (2010) Determination of 43 polycyclic aromatic hydrocarbons in air particulate matter by use of direct elution and isotope dilution gas chromatography/mass spectrometry. *Anal. Bioanal. Chem.*, *396*, 1321-1330.

(45) Campo, L., Fustinoni, S., Buratti, M., Cirla, P. E., Martinotti, I., and Foa, V. (2007) Unmetabolized polycyclic aromatic hydrocarbons in urine as biomarkers of low exposure in asphalt workers. *J. Occup. Environ. Hyg.*, *4*, 100-110.

(46) Moret, S., Amici, S., Bortolomeazzi, R., and Lercker, G. (1995) DETERMINATION OF POLYCYCLIC AROMATIC-HYDROCARBONS IN WATER AND WATER-BASED ALCOHOLIC BEVERAGES. *Z. Lebensm.-Unters.-Forsch.*, *201*, 322-326.

(47) Jia, Y. L., Stone, D., Wang, W. T., Schrlau, J., Tao, S., and Simonich, S. L. M. (2011) Estimated Reduction in Cancer Risk due to PAH Exposures If Source Control Measures during the 2008 Beijing Olympics Were Sustained. *Environ. Health Perspect.*, *119*, 815-820.

(48) Chetiyanukornkul, T., Toriba, A., Kameda, T., Tang, N., and Hayakawa, K. (2006) Simultaneous determination of urinary hydroxylated metabolites of naphthalene, fluorene, phenanthrene, fluoranthene and pyrene as multiple biomarkers of exposure to polycyclic aromatic hydrocarbons. *Anal. Bioanal. Chem.*, *386*, 712-718.

(49) Fan, R. F., Wang, D. L., Ramage, R., and She, J. W. (2012) Fast and Simultaneous Determination of Urinary 8-Hydroxy-2 '-deoxyguanosine and Ten Monohydroxylated Polycyclic Aromatic Hydrocarbons by Liquid Chromatography/Tandem Mass Spectrometry. *Chem. Res. Toxicol.*, *25*, 491-499.

(50) Klotz, K., Schindler, B. K., and Angerer, J. (2011) 1,2-Dihydroxynaphthalene as biomarker for a naphthalene exposure in humans. *Int. J. Hyg. Environ. Health.*, *214*, 110-114.

(51) Itoh, N., Tao, H., and Ibusuki, T. (2006) In-tube silylation in combination with thermal desorption gas chromatography-mass spectrometry for the determination of hydroxy polycyclic aromatic hydrocarbons in water. *Anal. Chim. Acta*, *555*, 201-209.

(52) Luan, T. G., Fang, S. H., Zhong, Y., Lin, L., Chan, S. M. N., Lan, C. Y., and Tam, N. F. Y. (2007) Determination of hydroxy metabolites of polycyclic aromatic hydrocarbons by fully automated solid-phase microextraction derivatization and gas chromatography-mass spectrometry. *J. Chromatogr. A*, *1173*, 37-43.

(53) Romanoff, L. C., Li, Z., Young, K. J., Blakely, N. C., Patterson, D. G., and Sandau, C. D. (2006) Automated solid-phase extraction method for measuring urinary polycyclic aromatic hydrocarbon metabolites in human biomonitoring using isotope-dilution gas chromatography high-resolution mass spectrometry. *J. Chromatogr. B*, *835*, 47-54.

(54) Schummer, C., Delhomme, O., Appenzeller, B. M. R., Wennig, R., and Millet, M. (2009) Comparison of MTBSTFA and BSTFA in derivatization reactions of polar compounds prior to GC/MS analysis. *Talanta*, *77*, 1473-1482.

(55) Smith, C. J., Huang, W. L., Walcott, C. J., Turner, W., Grainger, J., and Patterson, D. G. (2002) Quantification of monohydroxy-PAH metabolites in urine by solid-phase extraction with isotope dilution-GC-MS. *Anal. Bioanal. Chem.*, *372*, 216-220.

(56) Hagedorn, H. W., Scherer, G., Engl, J., Riedel, K., Cheung, F., Errington, G., Shepperd, J., and McEwan, M. (2009) Urinary Excretion of Phenolic Polycyclic Aromatic Hydrocarbons (OH-PAH) in Nonsmokers and in Smokers of Cigarettes with Different ISO Tar Yields. *J. Anal. Toxicol.*, *33*, 301-309.

(57) Kishikawa, N., Morita, S., Wada, M., Ohba, Y., Nakashima, K., and Kuroda, N. (2004) Determination of hydroxylated polycyclic aromatic hydrocarbons in airborne particulates by high-performance liquid chromatography with fluorescence detection. *Anal. Sci.*, *20*, 129-132.

(58) Rossbach, B., Preuss, R., Letzel, S., Drexler, H., and Angerer, J. (2007) Biological monitoring of occupational exposure to polycyclic aromatic hydrocarbons (PAH) by determination of monohydroxylated metabolites of phenanthrene and pyrene in urine. *Int. Arch. Occup. Environ. Health*, *81*, 221-229.

(59) Jacob, P., Wilson, M., and Benowitz, N. L. (2007) Determination of phenolic metabolites of polycyclic aromatic hydrocarbons in human urine as their pentafluorobenzyl ether derivatives using liquid chromatography-tandem mass spectrometry. *Anal. Chem.*, *79*, 587-598.

(60) Strickland, P., and Kang, D. H. (1999) Urinary 1-hydroxypyrene and other PAH metabolites as biomarkers of exposure to environmental PAH in air particulate matter. *Toxicol. Lett.*, *108*, 191-199.

(61) Garcia, M., Jemal, A., Ward, E., Center, M., Hao, Y., Siege, R., and Thun, M. (2007) Global Cancer Facts & Figures 2007. *American Cancer Society*.

(62) WHO. (2008) The world health report 2008 : primary health care now more than ever, World Health Organization.

(63) Jemal, A., Murray, T., Ward, E., Samuels, A., Tiwari, R., Ghafoor, A., Feuer, E., and Thun, M. (2005) Cancer statistics 2005. *CA Cancer J Clin*, *55*.

(64) Thun, M. J., Hannan, L. M., Adams-Campbell, L. L., Boffetta, P., Buring, J. E., Feskanich, D., Flanders, W. D., Jee, S. H., Katanoda, K., Kolonel, L. N., Lee, I. M., Marugame, T., Palmer, J. R., Riboli, E., Sobue, T., Avila-Tang, E., Wilkens, L. R., and Samet, J. M. (2008) Lung Cancer Occurrence in Never-Smokers: An Analysis of 13 Cohorts and 22 Cancer Registry Studies. *PLos Med.*, *5*, 1357-1371.

(65) Grant, W. B. (2009) Air Pollution in Relation to US Cancer Mortality Rates: An Ecological Study; Likely Role of Carbonaceous Aerosols and Polycyclic Aromatic Hydrocarbons. *Anticancer Res.*, *29*, 3537-3545.

(66) Tonne, C. C., Whyatt, R. M., Camann, D. E., Perera, F. P., and Kinney, P. L. (2004) Predictors of personal polycyclic aromatic hydrocarbon exposures among pregnant minority women in New York City. *Environ. Health Perspect.*, *112*, 754-759.

(67) Xu, S. S., Liu, W. X., and Tao, S. (2006) Emission of polycyclic aromatic hydrocarbons in China. *Environ. Sci. Technol.*, *40*, 702-708.

(68) Bostrom, C. E., Gerde, P., Hanberg, A., Jernstrom, B., Johansson, C., Kyrklund, T., Rannug, A., Tornqvist, M., Victorin, K., and Westerholm, R. (2002) Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environ. Health Perspect.*, *110*, 451-488.

(69) Hainaut, P., and Hollstein, M. (2000) p53 and human cancer: The first ten thousand mutations. *Adv.Cancer Res.*, *77*, 81-137.

(70) Neff, J. M. (1978) Polycyclic aromatic hydrocarbons in the aquatic environment and cancer risk to aquatic organisms and man. *Symposium: Carcinogenic Polynuclear Aromatic Hydrocarbons in the Marine Environment*, 385-409.

(71) Farrelly, M. C., Nimsch, C. T., Hyland, A., and Cummings, M. (2004) The effects of higher cigarette prices on tar and nicotine consumption in a cohort of adult smokers. *Health Econ.*, *13*, 49-58.

(72) Kollarova, H., Machova, L., Horakova, D., Cizek, L., Janoutova, G., and Janout, V. (2008) Is obesity a preventive factor for lung cancer? *Neoplasma*, *55*, 71-73.

(73) Leung, C. C., Lam, T. H., Yew, W. W., Chan, W. M., Law, W. S., and Tam, C. M. (2011) Lower lung cancer mortality in obesity. *Int. J. Epidemiol.*, *40*, 174-182.

(74) Hack, K. M., Meza, J. L., Kessinger, M. A., and Ganti, A. K. (2009) Effect of diabetes mellitus on the epidemiology of lung cancer. *J. Thorac. Oncol.*, *4*, S429-S429.

(75) Hatlen, P., Gronberg, B. H., Langhammer, A., Carlsen, S. M., and Amundsen, T. (2011) Prolonged Survival in Patients with Lung Cancer with Diabetes Mellitus. *J. Thorac. Oncol.*, *6*, 1810-1817.

(76) Turan, O., and Akkoclu, A. (2009) May diabetes mellitus affect the development of lung cancer? *J. Thorac. Oncol.*, *4*, S652-S653.

(77) Lee, M. Y., Lin, K. D., Hsiao, P. J., and Shin, S. J. (2012) The association of diabetes mellitus with liver, colon, lung, and prostate cancer is independent of hypertension, hyperlipidemia, and gout in Taiwanese patients. *Metab.-Clin. Exp.*, *61*, 242-249.

(78) Luo, J. H., Chlebowski, R., Wactawski-Wende, J., Schlecht, N. F., Tinker, L., and Margolis, K. L. (2012) Diabetes and Lung Cancer Among Postmenopausal Women. *Diabetes Care*, *35*, 1485-1491.

(79) Shieh, S. H., Probst, J. C., Sung, F. C., Tsai, W. C., Li, Y. S., and Chen, C. Y. (2012) Decreased survival among lung cancer patients with co-morbid tuberculosis and diabetes. *BMC Cancer*, *12*, 174.

(80) Chen, M. Y., Lin, F. L., and Chang, C. K. (2009) Relations between health care expenditure and income: an application of local quantile regressions. *Appl. Econ. Lett.*, *16*, 177-181.

(81) Swift, R. (2011) THE RELATIONSHIP BETWEEN HEALTH AND GDP IN OECD COUNTRIES IN THE VERY LONG RUN. *Health Econ.*, *20*, 306-322.

(82) Chen, W. Q., Clements, M., Rahman, B., Zhang, S. W., Qiao, Y. L., and Armstrong, B. K. (2010) Relationship between cancer mortality/incidence and ambient ultraviolet B irradiance in China. *Cancer Causes & Control*, *21*, 1701-1709.

(83) Mohr, S. B., Garland, C. F., Gorham, E. D., Grant, W. B., and Garland, F. C. (2008) Could ultraviolet B irradiance and vitamin D be associated with lower incidence rates of lung cancer? *J. Epidemiol. Community Health*, *62*, 69-74.

(84) Zhang, Y. X., Tao, S., Shen, H. Z., and Ma, J. M. (2009) Inhalation exposure to ambient polycyclic aromatic hydrocarbons and lung cancer risk of Chinese population. *Proc. Natl. Acad. Sci. U. S. A.*, *106*, 21063-21067.

(85) Lewtas, J. (2007) Air pollution combustion emissions: Characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects. *Mutat. Res.-Rev. Mutat. Res.*, *636*, 95-133.

(86) WHO. (2008) The global burden of disease: 2004 update, World Health Organization, Geneva.

(87) WHO. Age-standardized death rates per 100,000 by cause, World Health Organization.

(88) World Bank. (2012) World Bank Analytical Classifications, The World Bank.

(89) WHO. (2009) Report on the Global Tobacco Epidemic, 2009: Implementing smoke-free environments, World Health Organization.

(90) Franks, P., Jerant, A. F., Leigh, J. P., Lee, D., Chiem, A., Lewis, I., and Lee, S. (2007) Cigarette prices, smoking, and the poor: Implications of recent trends. *Am. J. Public Health*, *97*, 1873-1877.

(91) Kozlowski, L. T., Heatherton, T. F., and Ferrence, R. G. (1989) PACK SIZE, REPORTED CIGARETTE-SMOKING RATES, AND THE HEAVINESS OF SMOKING. *Can. J. Public Health-Rev. Can. Sante Publ.*, *80*, 266-270.

(92) WHO. (2012) Global Database on Body Mass Index. An interactive surveillance tool for monitoring nutrition transition World Health Organization.

(93) WHO. (2012) Country and regional data on diabetes, WHO.

(94) Loeb, L. A. (2001) A mutator phenotype in cancer. *Cancer Res.*, *61*, 3230-3239.

(95) Akaike, H. (1981) CITATION CLASSIC - A NEW LOOK AT THE STATISTICAL-MODEL IDENTIFICATION. *Current Contents/Engineering Technology & Applied Sciences*, 22-22.

(96) Townsend, J., Roderick, P., and Cooper, J. (1994) CIGARETTE-SMOKING BY SOCIOECONOMIC GROUP, SEX, AND AGE - EFFECTS OF PRICE, INCOME, AND HEALTH PUBLICITY. *Br. Med. J.*, *309*, 923-927.

(97) Etter, J. F. (2010) Smoking prevalence, cigarette consumption and advice received from physicians: Change between 1996 and 2006 in Geneva, Switzerland. *Addict. Behav.*, *35*, 355-358.

(98) Filion, K. B., Steffen, L. M., Duval, S., Jacobs, D. R., Blackburn, H., and Luepker, R. V. (2012) Trends in Smoking Among Adults From 1980 to 2009: The Minnesota Heart Survey. *Am. J. Public Health*, *102*, 705-713.

(99) Fukuda, Y., Nakamura, K., and Takano, T. (2005) Socioeconomic pattern of smoking in Japan: Income inequality and gender and age differences. *Ann. Epidemiol.*, *15*, 365-372.

(100) Nagelhout, G. E., de Boer, D. D., Kunst, A. E., van der Meer, R. M., de Vries, H., van Gelder, B. M., and Willemsen, M. C. (2012) Trends in socioeconomic inequalities in smoking prevalence, consumption, initiation, and cessation between 2001 and 2008 in the Netherlands. Findings from a national population survey. *BMC Public Health*, *12*.

(101) Whitlock, G., VanderHoorn, S., Davis, P., Jackson, R., and Norton, R. (1997) Socioeconomic distribution of smoking in a population of 10 529 New Zealanders. *N. Z. Med. J.*, *110*, 327-330.

(102) Kabat, G. C., and Wynder, E. L. (1992) BODY-MASS INDEX AND LUNG-CANCER RISK. *Am. J. Epidemiol.*, *135*, 769-774.

(103) Knekt, P., Heliovaara, M., Rissanen, A., Aromaa, A., Seppanen, R., Teppo, L., and Pukkala, E. (1991) LEANNESS AND LUNG-CANCER RISK. *Int. J. Cancer*, *49*, 208-213.

(104) Albanes, D., Jones, D. Y., Micozzi, M. S., and Mattson, M. E. (1987) ASSOCIATIONS BETWEEN SMOKING AND BODY-WEIGHT IN THE UNITED-STATES POPULATION - ANALYSIS OF NHANES-II. *Am. J. Public Health*, *77*, 439-444.

(105) Garn, S. M., Rosenberg, K. R., and Schaefer, A. E. (1983) RELATIONSHIP BETWEEN FATNESS LEVEL AND SIZE ATTAINMENT IN CENTRAL AMERICA. *Ecol. Food Nutr.*, *13*, 157-165.

(106) Billet, S., Abbas, I., Le Goff, J., Verdin, A., Andre, V., Lafargue, P. E., Hachimi, A., Cazier, F., Sichel, F., Shirali, P., and Garcon, G. (2008) Genotoxic potential of Polycyclic Aromatic Hydrocarbons-coated onto airborne Particulate Matter (PM(2.5)) in human lung epithelial a549 cells. *Cancer Lett.*, *270*, 144-155.

(107) Phillips, D. H., Hewer, A., Martin, C. N., Garner, R. C., and King, M. M. (1988) CORRELATION OF DNA ADDUCT LEVELS IN HUMAN-LUNG WITH CIGARETTE-SMOKING. *Nature*, *336*, 790-792.

(108) Schwartz, A. G., Prysak, G. M., Bock, C. H., and Cote, M. L. (2007) The molecular epidemiology of lung cancer. *Carcinogenesis*, *28*, 507-518.

(109) Central Intelligence Agency (CIA). (2012) The World Factbook, Central Intelligence Agency.

(110) Orourke, M. A., Feussner, J. R., Feigl, P., and Laszlo, J. (1987) AGE TRENDS OF LUNG-CANCER STAGE AT DIAGNOSIS - IMPLICATIONS FOR LUNG-

CANCER SCREENING IN THE ELDERLY. *JAMA-J. Am. Med. Assoc.*, *258*, 921-926.

(111) Landers, D. H., Simonich, S. M., Jaffe, D., Geiser, L., Campbell, D. H., Schwindt, A., Schreck, C., Kent, M., Hafner, W., Taylor, H. E., Hageman, K., Usenko, S., Ackerman, L., Schrlau, J., Rose, N., Blett, T., and Erway, M. M. (2010) The Western Airborne Contaminant Assessment Project (WACAP): An Interdisciplinary Evaluation of the Impacts of Airborne Contaminants in Western US National Parks. *Environ. Sci. Technol.*, *44*, 855-859.

(112) Guillen, M. D., Sopelana, P., and Partearroyo, M. A. (1997) Food as a source of polycyclic aromatic carcinogens. *Reviews on environmental health*, *12*, 133-146.

(113) Forsberg, N. D., Stone, D., Harding, A., Harper, B., Harris, S., Matzke, M. M., Cardenas, A., Waters, K. M., and Anderson, K. A. (2012) Effect of Native American Fish Smoking Methods on Dietary Exposure to Polycyclic Aromatic Hydrocarbons and Possible Risks to Human Health. *J. Agric. Food Chem.*, *60*, 6899-6906.

(114) Alomirah, H., Al-Zenki, S., Al-Hooti, S., Zaghloul, S., Sawaya, W., Ahmed, N., and Kannan, K. (2011) Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs) from grilled and smoked foods. *Food Control*, *22*, 2028-2035.

(115) Adetona, O., Sjodin, A., Zheng, L., Romanoff, L. C., Aguilar-Villalobos, M., Needham, L. L., Hall, D. B., Luis, A., Cassidy, B. E., Simpson, C. D., and Naeher, L. P. (2012) Personal Exposure to PM2.5 and Urinary Hydroxy-PAH Levels in Bus Drivers Exposed to Traffic Exhaust, in Trujillo, Peru. *J. Occup. Environ. Hyg.*, *9*, 217-229.

(116) Li, Z., Sjoedin, A., Romanoff, L. C., Horton, K., Fitzgerald, C. L., Eppler, A., Aguilar-Villalobos, M., and Naeher, L. P. (2011) Evaluation of exposure reduction to indoor air pollution in stove intervention projects in Peru by urinary biomonitoring of polycyclic aromatic hydrocarbon metabolites. *Environment International*, *37*, 1157-1163.

(117) Zhong, Y., Carmella, S. G., Upadhyaya, P., Hochalter, J. B., Rauch, D., Oliver, A., Jensen, J., Hatsukami, D., Wang, J., Zimmerman, C., and Hecht, S. S. (2011) Immediate Consequences of Cigarette Smoking: Rapid Formation of Polycyclic Aromatic Hydrocarbon Diol Epoxides. *Chem. Res. Toxicol.*, *24*, 246-252.

(118) Motorykin, O., Matzke, M. M., Waters, K. M., and Simonich, S. L. M. (2013) Association of Carcinogenic Polycyclic Aromatic Hydrocarbon Emissions and Smoking with Lung Cancer Mortality Rates on a Global Scale. *Environ. Sci. Technol.*, *47*, 3410-3416.

(119) Xu, X., Hu, H., Kearney, G. D., Kan, H., and Sheps, D. S. (2013) Studying the effects of polycyclic aromatic hydrocarbons on peripheral arterial disease in the United States. *Sci. Total Environ.*, *461–462*, 341-347.

(120) Jerina, D. M., Daly, J. W., Witkop, B., Zaltzman.P, and Udenfrie.S. (1970) 1,2-NAPHTHALENE OXIDE AS AN INTERMEDIATE IN MICROSOMAL HYDROXYLATION OF NAPHTHALENE. *Biochemistry*, *9*, 147-&.

(121) Wei, Y., Lin, Y., Zhang, A.-Q., Guo, L.-H., and Cao, J. (2010) Evaluation of the noncovalent binding interactions between polycyclic aromatic hydrocarbon metabolites and human p53 cDNA. *Sci. Total Environ.*, *408*, 6285-6290.

(122) Xu, X., Cook, R. L., Ilacqua, V. A., Kan, H., Talbott, E. O., and Kearney, G. (2010) Studying associations between urinary metabolites of polycyclic aromatic hydrocarbons (PAHs) and cardiovascular diseases in the United States. *Sci. Total Environ.*, *408*, 4943-4948.

(123) Han, Y., Xia, Y., Zhu, P., Qiao, S., Zhao, R., Jin, N., Wang, S., Song, L., Fu, G., and Wang, X. (2010) Reproductive hormones in relation to polycyclic aromatic hydrocarbon (PAH) metabolites among non-occupational exposure of males. *Sci. Total Environ.*, *408*, 768-773.

(124) Jacob, J., and Seidel, A. (2002) Biomonitoring of polycyclic aromatic hydrocarbons in human urine. *Journal of Chromatography B*, *778*, 31-47.

(125) Becher, G., and Bjorseth, A. (1983) DETERMINATION OF EXPOSURE TO POLYCYCLIC AROMATIC-HYDROCARBONS BY ANALYSIS OF HUMAN-URINE. *Cancer Lett.*, *17*, 301-311.

(126) Campo, L., Addario, L., Buratti, M., Scibetta, L., Longhi, O., Valla, C., Cirla, P. E., Martinotti, I., Foa, V., and Fustinoni, S. (2006) Biological monitoring of exposure to polycyclic aromatic hydrocarbons by determination of unmetabolized compounds in urine. *Toxicol. Lett.*, *162*, 132-138.

(127) Campo, L., Rossella, F., Pavanello, S., Mielzynska, D., Siwinska, E., Kapka, L., Bertazzi, P. A., and Fustinoni, S. (2010) Urinary profiles to assess polycyclic aromatic hydrocarbons exposure in coke-oven workers. *Toxicol. Lett.*, *192*, 72-78.

(128) Mucha, A. P., Hryhorczuk, D., Serdyuk, A., Nakonechny, J., Zvinchuk, A., Erdal, S., Caudill, M., Scheff, P., Lukyanova, E., Shkiryak-Nyzhnyk, Z., and Chislovska, N. (2006) Urinary 1-hydroxypyrene as a biomarker of PAH exposure in 3-year-old Ukrainian children. *Environ. Health Perspect.*, *114*, 603-609.

(129) Leroyer, A., Jeandel, F., Maitre, A., Howsam, M., Deplanque, D., Mazzuca, M., and Nisse, C. (2010) 1-Hydroxypyrene and 3-hydroxybenzo[a]pyrene as biomarkers of exposure to PAH in various environmental exposure situations. *Sci. Total Environ.*, *408*, 1166-1173.

(130) Motorykin, O., Schrlau, J. E., Jia, Y., Harper, B., Harris, S., Harding, A., Stone, D., Kile, M. L., Sudakin, D., and Simonich, S. L. (2014) Determination of Parent and Hydroxy PAHs in Personal PM2.5 and Urine Samples Collected During Native American Fish Smoking Activities. *Sci. Total Environ.*, *XX*, YYYY-YYYY.

(131) Li, Z., Sandau, C. D., Romanoff, L. C., Caudill, S. P., Sjodin, A., Needham, L. L., and Patterson, D. G. (2008) Concentration and profile of 22 urinary polycyclic aromatic hydrocarbon metabolites in the US population. *Environ. Res.*, *107*, 320-331.

(132) St Helen, G., Goniewicz, M. L., Dempsey, D., Wilson, M., Jacob, P., and Benowitz, N. L. (2012) Exposure and Kinetics of Polycyclic Aromatic Hydrocarbons (PAHs) in Cigarette Smokers. *Chem. Res. Toxicol.*, *25*, 952-964.

(133) Förster, K., Preuss, R., Roßbach, B., Brüning, T., Angerer, J., and Simon, P. (2008) 3-Hydroxybenzo[a]pyrene in the urine of workers with occupational exposure to polycyclic aromatic hydrocarbons in different industries. *Occupational and Environmental Medicine*, *65*, 224-229.

(134) Li, Z., Mulholland, J. A., Romanoff, L. C., Pittman, E. N., Trinidad, D. A., Lewin, M. D., and Sjodin, A. (2010) Assessment of non-occupational exposure to

polycyclic aromatic hydrocarbons through personal air sampling and urinary biomonitoring. *J. Environ. Monit.*, *12*, 1110-1118.

(135) de Alda, M. J. L., and Barcelo, D. (2001) Review of analytical methods for the determination of estrogens and progestogens in waste waters. *Fresenius J. Anal. Chem.*, *371*, 437-447.

(136) Moon, J., Smith, T. J., Tamaro, S., Enarson, D., Fadl, S., Davison, A. J., and Weldon, L. (1986) Trace metals in scalp hair of children and adults in three Alberta indian villages. *Sci. Total Environ.*, *54*, 107-125.

(137) Schaeffer, D. J., Dellinger, J. A., Needham, L. L., and Hansen, L. G. (2006) Serum PCB profiles in Native Americans from Wisconsin based on region, diet, age, and gender: Implications for epidemiology studies. *Sci. Total Environ.*, *357*, 74-87.

(138) Xue, J., Zartarian, V. G., Liu, S. V., and Geller, A. M. (2012) Methyl mercury exposure from fish consumption in vulnerable racial/ethnic populations: Probabilistic SHEDS-Dietary model analyses using 1999–2006 NHANES and 1990–2002 TDS data. *Sci. Total Environ.*, *414*, 373-379.

(139) U.S. Department of Health and Human Services. National Institutes of Health. (2012) Clean catch urine sample, U.S. National Library of Medicine.

(140) U.S. EPA. (1996) The analysis of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans by high-resolution gas chromatography/low resolution mass spectrometry (HRGC/LRMS). Method 8280A, (U.S. Environmental Protection Agency, Ed.), Washington, DC.

(141) Wang, J., Chen, S., Tian, M., Zheng, X., Gonzales, L., Ohura, T., Mai, B., and Simonich, S. L. M. (2012) Inhalation Cancer Risk Associated with Exposure to Complex Polycyclic Aromatic Hydrocarbon Mixtures in an Electronic Waste and Urban Area in South China. *Environ. Sci. Technol.*, *46*, 9745-9752.

(142) Chiaia, A. C., Banta-Green, C., and Field, J. (2008) Eliminating Solid Phase Extraction with Large-Volume Injection LC/MS/MS: Analysis of Illicit and Legal Drugs and Human Urine Indicators in US Wastewaters. *Environ. Sci. Technol.*, *42*, 8841-8848.

(143) Mattarozzi, M., Musci, M., Careri, M., Mangia, A., Fustinoni, S., Campo, L., and Bianchi, F. (2009) A novel headspace solid-phase microextraction method using in situ derivatization and a diethoxydiphenylsilane fibre for the gas chromatography-mass spectrometry determination of urinary hydroxy polycyclic aromatic hydrocarbons. *J. Chromatogr. A*, *1216*, 5634-5639.

(144) NIST. (2013) SRM 3672 - Organic Contaminants in Smokers' Urine (Frozen).

(145) NIST. (2013) SRM 3673 - Organic Contaminants in Non-Smokers' Urine (Frozen).

(146) Viau, C., Lafontaine, M., and Payan, J. P. (2004) Creatinine normalization in biological monitoring revisited: the case of 1-hydroxypyrene. *Int. Arch. Occup. Environ. Health*, *77*, 177-185.

(147) Centers for Disease Control and Prevention (CDC). Fourth National Report on Human Exposure to Environmental Chemicals. Updated Tables, March, 2013.

(148) Radonic, J., Miloradov, M. V., Sekulic, M. T., Kiurski, J., Djogo, M., and Milovanovic, D. (2011) The octanol-air partition coefficient, K-OA as a predictor of gas-particle partitioning of polycyclic aromatic hydrocarbons and

polychlorinated biphenyls at industrial and urban sites. *J. Serb. Chem. Soc.*, *76*, 447-458.

(149) Simoneit, B. R. T., Bi, X. H., Oros, D. R., Medeiros, P. M., Sheng, G. Y., and Fu, J. M. (2007) Phenols and Hydroxy-PAHs (Arylphenols) as tracers for coal smoke particulate matter: Source tests and ambient aerosol assessments. *Environ. Sci. Technol.*, *41*, 7294-7302.

(150) Zhang, Y. Y., Ding, J. N., Shen, G. F., Zhong, J. J., Wang, C., Wei, S. Y., Chen, C. Q., Chen, Y. C., Lu, Y., Shen, H. Z., Li, W., Huang, Y., Chen, H., Su, S., Lin, N., Wang, L., Liu, W. X., and Tao, S. (2014) Dietary and inhalation exposure to polycyclic aromatic hydrocarbons and urinary excretion of monohydroxy metabolites - A controlled case study in Beijing, China. *Environmental Pollution*, *184*, 515-522.

(151) Suzuki, K., and Yoshinaga, J. (2007) Inhalation and dietary exposure to polycyclic aromatic hydrocarbons and urinary 1-hydroxypyrene in non-smoking university students. *Int. Arch. Occup. Environ. Health*, *81*, 115-121.

(152) McClean, M. D., Rinehart, R. D., Ngo, L., Eisen, E. A., Kelsey, K. T., and Herrick, R. F. (2004) Inhalation and dermal exposure among asphalt paving workers. *Ann. Occup. Hyg.*, *48*, 663-671.

(153) Ramesh, A., Walker, S. A., Hood, D. B., Guillen, M. D., Schneider, K., and Weyand, E. H. (2004) Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. *International Journal of Toxicology*, *23*, 301-333.

(154) Bouchard, M., and Viau, C. (1998) Urinary and biliary excretion kinetics of 1-hydroxypyrene following intravenous and oral administration of pyrene in rats. *Toxicology*, *127*, 69-84.

(155) Flowers, L., Rieth, S. H., Cogliano, V. J., Foureman, G. L., Hertzberg, R., Hofmann, E. L., Murphy, D. L., Nesnow, S., and Schoeny, R. S. (2002) Health assessment of polycyclic aromatic hydrocarbon mixtures: Current practices and future directions. *Polycycl. Aromat. Compd.*, *22*, 811-821.

(156) Pufulete, M., Battershill, J., Boobis, A., and Fielder, R. (2004) Approaches to carcinogenic risk assessment for polycyclic aromatic hydrocarbons: a UK perspective. *Regul. Toxicol. Pharmacol.*, *40*, 54-66.

(157) WHO. (1998) International Programme on Chemical Safety (IPCS) Environmental Health Criteria 202. Selected Non-Heterocyclic Polycyclic Aromatic Hydrocarbons. Appendix I.

(158) Laurent, C., Feidt, C., Lichtfouse, E., Grova, N., Laurent, F., and Rychen, G. (2001) Milk−Blood Transfer of 14C-Tagged Polycyclic Aromatic Hydrocarbons (PAHs) in Pigs. *J. Agric. Food Chem.*, *49*, 2493-2496.

(159) Grova, N., Feidt, C., Laurent, C., and Rychen, G. (2002) [14C] Milk, urine and faeces excretion kinetics in lactating goats after an oral administration of [14C]polycyclic aromatic hydrocarbons. *International Dairy Journal*, *12*, 1025-1031.

(160) Buckley, T. J., and Lioy, P. J. (1992) AN EXAMINATION OF THE TIME COURSE FROM HUMAN DIETARY EXPOSURE TO POLYCYCLIC AROMATIC-HYDROCARBONS TO URINARY ELIMINATION OF 1-HYDROXYPYRENE. *British Journal of Industrial Medicine*, *49*, 113-124.

(161) Li, Z., Romanoff, L., Bartell, S., Pittman, E. N., Trinidad, D. A., McClean, M., Webster, T. F., and Sjodin, A. (2012) Excretion Profiles and Half-Lives of Ten Urinary Polycyclic Aromatic Hydrocarbon Metabolites after Dietary Exposure. *Chem. Res. Toxicol.*, *25*, 1452-1461.

(162) Husdan, H., and Rapoport, A. (1968) ESTIMATION OF CREATININE BY JAFFE REACTION - A COMPARISON OF 3 METHODS. *Clin. Chem.*, *14*, 222-&.

(163) Crinnion, W. J. (2010) The CDC Fourth National Report on Human Exposure to Environmental Chemicals: What it Tells Us About our Toxic Burden and How it Assists Environmental Medicine Physicians. *Alternative Medicine Review*, *15*, 101-108.

(164) Xu, X., Zhang, J. F., Zhang, L., Liu, W. L., and Weisel, C. P. (2004) Selective detection of monohydroxy metabolites of polycyclic aromatic hydrocarbons in urine using liquid chromatography/triple quadrupole tandem mass spectrometry. *Rapid Commun. Mass Spectrom.*, *18*, 2299-2308.

APPENDICES

APPENDIX A

Appendix A.1 Independent and dependent variables used in the regression models.
Country – name of the country; population – population of the country. '000; gdp/cap – Gross Domestic Product per capita, $US'000; bapeq – Benzo[a]Pyrene equivalents of PAH emissions, Mt/year; SP – smoking prevalence, %; price – price of the pack of most popular cigarettes, $US; bmi – average body mass index, kg/m^2; diabetes – percent of people with diabetes, %; ed100000 – estimated lung cancer deaths per 100,000; asdr100000 – age standardized lung cancer death rate per 100,000; income – level of income in the country (low, low middle, upper middle, and high).

Country	population	gdp/cap	bapeq	smoking	price	bmi	diabetes	ed100000	asdr100000	Income
Albania	3188	1470	6.90E-01	21	1.18	25.69	3.12	36	37	low mid
Algeria	32373	1981	2.10E+00	14	0.98	25.06	1.64	9.3	17	low mid
Argentina	38226	7511	1.70E+01	25	1.11	27.0433	4.09	25	23	up mid
Armenia	3050	975	1.30E-01	29	1.63	25.735	4.31	49	41	low mid
Australia	20120	22074	9.30E+01	18	6.65	26.8	5.16	36	24	high
Austria	8115	24674	1.20E+01	41	5.57	25.5283	3.15	41	25	high
Bahrain	725	13250.18	5.50E+00	6	1.6	27.7267	6.24	11	28	high
Bangladesh	140494	396	4.20E+01	23	0.38	20.1283	3.03	12	25	low
Barbados	272	9913.603	4.00E-02	10	5.5	27.3817	4.58	6.9	6.2	up mid
Belarus	9832	1516	4.00E+00	38	0.86	25.9717	7.73	38	26	low mid
Belgium	10405	23134	6.30E+00	25	5.79	25.815	3.23	67	38	high
Belize	283	3669	6.40E-02	4	3.5	27.8767	2.24	6.5	13	up mid
Benin	6890	389	1.20E+01	8	1.06	22.7233	1.61	2	5.1	low
Bolivia	8986	1036	8.50E+01	29	0.78	25.3067	2.83	6.7	11	low mid
Bosnia and Herzegovina	3836	1384	9.40E+00	38	1.42	26.1017	3.13	45	31	low mid
Brazil	178718	3675	2.90E+02	14	1.03	25.4083	3.05	12	16	up mid
Bulgaria	7780	1951	5.30E+00	37	1.98	25.7567	6.04	42	26	low mid
Burkina Faso	12387	257	1.70E+01	14	1.06	21.115	1.29	4.1	10	low
Cambodia	13630	328	2.90E+01	24	0.3	21.0483	1.01	7.9	16	low
Cameroon	16400	651	2.80E+01	6	1.06	23.8017	0.51	3.2	6.9	low
Canada	31902	24712	9.70E+01	15	6.48	26.8317	6.93	55	36	high
Cape Verde	481	1328	1.30E+00	8	2.52	23.67	1.93	1.7	3.5	low mid
Chad	8823	277	2.00E+01	7	1.06	21.3417	1.36	2.9	6.5	low
Chile	15956	5448	1.30E+01	36	2.07	26.9983	3.56	15	15	up mid
China	1326544	1162	2.80E+03	31	0.73	22.7483	1.78	29	31	low mid

Appendix A.1 (Continued)

Country	population	gdp/cap	bapeq	smoking	price	bmi	diabetes	ed100000	asdr100000	Income
Congo	54775	89	1.30E+02	6	0.94	20.5217	0.03	2	4.6	low
Cook Islands	21	6413.571	6.00E-02	33	6.02	32.2767	3.71	6	9.3	up mid
Costa Rica	4061	4534	7.90E-01	6	1.35	26.285	2.40	6.6	9	up mid
Cote d'Ivoire	17142	583	1.30E+01	6	1.49	22.825	1.83	6.6	14	low
Croatia	4508	4857	1.80E+00	30	2.91	25.5867	3.51	61	36	up mid
Cuba	11365	2845.332	3.20E+00	34	0.3	25.3067	4.66	40	31	low mid
Czech Republic	10183	6148	1.70E+01	25	3	27.0317	3.44	56	36	up mid
Denmark	5397	30930	7.40E+00	26	6.24	25.2933	3.09	65	38	high
Dominica	71	3534	2.80E-03	14	1.4	26.3133	4.41	17	19	up mid
Ecuador	13213	1435	2.00E+00	4	2.2	25.9817	3.17	5.7	8	low mid
Egypt	68738	1663	1.90E+01	14	0.49	27.9433	4.61	6	8.9	low mid
Eritrea	4477	163	1.10E+00	6	1.63	20.7717	1.33	1.7	4.8	low
Estonia	1345	5170	2.30E+00	29	2.88	25.3667	3.39	51	31	up mid
Ethiopia	69961	113	8.40E+01	3	0.44	20.1783	1.33	2.3	5.4	low
Federated States of Micronesia	127	1745	3.50E-01	20	1.75	29.1067	4.78	1.3	2.4	low mid
Fiji	848	2232	2.30E+00	10	1.3	27.1983	4.91	0.67	1	low mid
Finland	5215	25107	8.10E+00	21	6.12	25.9483	3.22	36	20	high
France	59991	23157	5.60E+01	27	7.38	25.1817	3.06	50	31	high
Gambia, The	1449	344	2.00E+00	15	0.36	22.6567	1.88	4.4	9.8	low
Georgia	4521	897	2.70E+00	28	0.6	25.5917	4.49	18	13	low mid
Germany	82631	23209	5.30E+01	27	6.55	26.2233	3.36	50	27	high
Ghana	21053	285	1.70E+01	4	1.16	23.12	1.78	2.4	4.9	low
Greece	11075	11885	6.20E+00	30	4.18	25.4133	7.97	57	32	high
Guatemala	12628	1676	1.50E+01	4	1.29	25.6033	1.43	6.6	13	low mid
Hungary	10072	5339	6.20E+00	34	3.02	26.2133	3.36	81	52	up mid
Iceland	290	32449	2.80E+00	20	5.52	26.225	2.34	40	29	high
India	1079721	538	1.60E+03	15	1.65	20.9983	3.53	6.3	9.9	low
Indonesia	217588	906	2.90E+02	29	1.14	22.0283	4.66	16	23	low mid

Appendix A.1 (Continued)

Country	population	gdp/cap	bapeq	smoking	price	bmi	diabetes	ed100000	asdr100000	Income
Iran	66928	1812	2.40E+01	14	1.32	25.7833	4.00	4.8	8.4	low mid
Iraq	24700	719.6222	2.40E+00	11	0.63	27.2483	3.43	14	32	low
Ireland	4019	29118	4.70E+00	24	11.27	26.8117	2.38	42	32	high
Israel	6798	17752	5.40E-01	21	5	26.81	4.26	21	19	high
Italy	57573	19344	3.30E+01	23	5.98	25.4933	7.65	57	28	high
Jamaica	2665	2975	8.20E-01	13	5.05	25.2667	3.58	13	15	low mid
Japan	127764	39195	3.90E+01	25	3.31	22.595	5.52	47	21	high
Jordan	5440	1908	2.40E-01	36	1.97	28.14	4.77	8.1	18	low mid
Kazakhstan	14958	1822	1.50E+01	22	0.75	25.9533	3.21	33	35	low mid
Kenya	32447	343	2.60E+01	11	1.54	22.0583	0.69	2.1	5.8	low
Korea	22745	568.5456	2.90E+01	28	0.14	23.4483	1.77	15	14	low
Kuwait	2460	16970.62	7.10E-01	18	1.7	29.58	5.39	2.7	9.6	high
Kyrgyzstan	5099	324	6.40E-01	21	0.61	24.9667	2.25	11	17	low
Latvia	2303	4502	4.10E+00	32	2.93	25.6333	3.61	47	28	up mid
Lebanon	4554	4358	3.40E+00	17	1.33	27.05	3.89	14	17	up mid
Lithuania	3439	4398	2.70E+00	29	1.83	26.135	3.44	41	27	up mid
Luxembourg	450	47926	5.30E-01	31	4.45	26.45	2.93	45	30	high
Malawi	11182	165	1.50E+01	11	1.03	22.065	0.57	1.8	4.8	low
Malaysia	25209	4221	1.70E+01	23	2.6	24.6767	4.55	16	27	up mid
Maldives	300	2693	1.90E-01	24	1.56	24.2983	2.84	31	67	low mid
Mali	11937	260	2.00E+01	9	1.49	21.865	1.47	2.2	4.9	low
Malta	401	9508	2.50E-02	23	5.29	27.1667	10.32	33	22	up mid
Marshall Islands	60	1738	1.70E-01	18	2.5	29.665	3.78	9.6	15	low mid
Mauritania	2906	396	2.90E+01	18	1.35	24.0017	1.49	2.5	5.2	low
Mauritius	1234	4289	3.40E+00	14	2.05	25.2167	10.31	11	12	up mid
Mexico	103795	5968	3.50E+01	14	2.07	27.6367	2.61	7.8	11	up mid
Moldova	4218	398	8.10E-01	21	0.58	25.3717	4.28	24	20	low
Mongolia	2515	462	5.80E-01	24	0.39	24.8833	1.60	18	35	low

Appendix A.1 (Continued)

Country	population	gdp/cap	bapeq	smoking	price	bmi	diabetes	ed100000	asdr100000	Income
Morocco	30586	1302	3.40E+00	15	2.16	25.56	1.71	8.1	12	low mid
Mozambique	19129	270	3.60E+01	9	0.6	22.1517	0.79	1.3	3.1	low
Namibia	2033	1905	4.10E+00	13	2.47	23.3967	1.46	4.1	9	low mid
Nauru	13	3611.462	3.70E-02	47	3.05	33.6033	17.44	9.8	17	up mid
Nepal	25190	245	4.50E+01	28	0.84	20.5367	2.20	6.4	12	low
Netherlands	16250	23255	1.50E+01	25	6.12	25.4267	2.86	60	38	high
New Zealand	4061	14984	7.30E+00	19	5.9	27.1067	4.83	36	26	high
Nigeria	139824	361	2.90E+02	5	1.89	22.995	0.10	1.8	4.2	low
Norway	4582	39198	1.80E+01	23	10.14	25.9917	3.06	45	28	high
Oman	3508	6185.231	2.30E-01	4	1.56	26.1383	4.10	3.6	9.1	up mid
Pakistan	152061	566	2.10E+02	17	0.23	22.66	4.19	7.2	14	low
Palau	20	6360	3.50E-02	20	3.5	30.28	5.67	7.2	12	up mid
Paraguay	5782	1413	7.00E+00	16	0.2	25.3583	2.28	9.6	16	low mid
Philippines	82987	1079	1.70E+01	27	0.53	22.8983	4.15	11	20	low mid
Poland	38160	4885	9.20E+01	29	1.94	26.0367	3.11	59	42	up mid
Portugal	10436	10395	1.80E+01	21	4.94	26.22	6.62	33	20	high
Romania	21858	2115	1.70E+01	29	2.22	25.05	5.18	40	27	low mid
Russia	142814	2302	1.80E+02	44	0.51	26.1483	3.27	34	24	low mid
Samoa	179	1417	3.10E-01	37	2.69	31.165	2.46	2.3	4	low mid
Sao Tome and Principe	161	342	7.20E-02	15	1.31	23.64	0.70	9.4	19	low
Saudi Arabia	23215	9259	3.50E+00	7	1.6	28.235	4.77	3.6	9.2	up mid
Senegal	10455	504	3.40E+00	8	1.27	22.72	1.72	2.1	4	low
Serbia and Montenegro	8152	1272	8.80E+00	29	0.95	25.8783	4.09	53	37	low mid
Seychelles	85	6573	2.80E-01	15	3.98	26.275	11.14	8.6	10	up mid
Singapore	4335	23636	4.30E-01	15	8.06	23.4617	8.70	27	26	high
Slovakia	5390	4488	5.90E+00	25	2.45	26.3933	3.00	39	29	up mid
Slovenia	1995	10871	3.10E+00	23	3.06	26.7533	3.45	57	35	high
South Africa	45584	3307	1.00E+02	16	2.04	27.2367	1.92	11	17	up mid

Appendix A.1 (Continued)

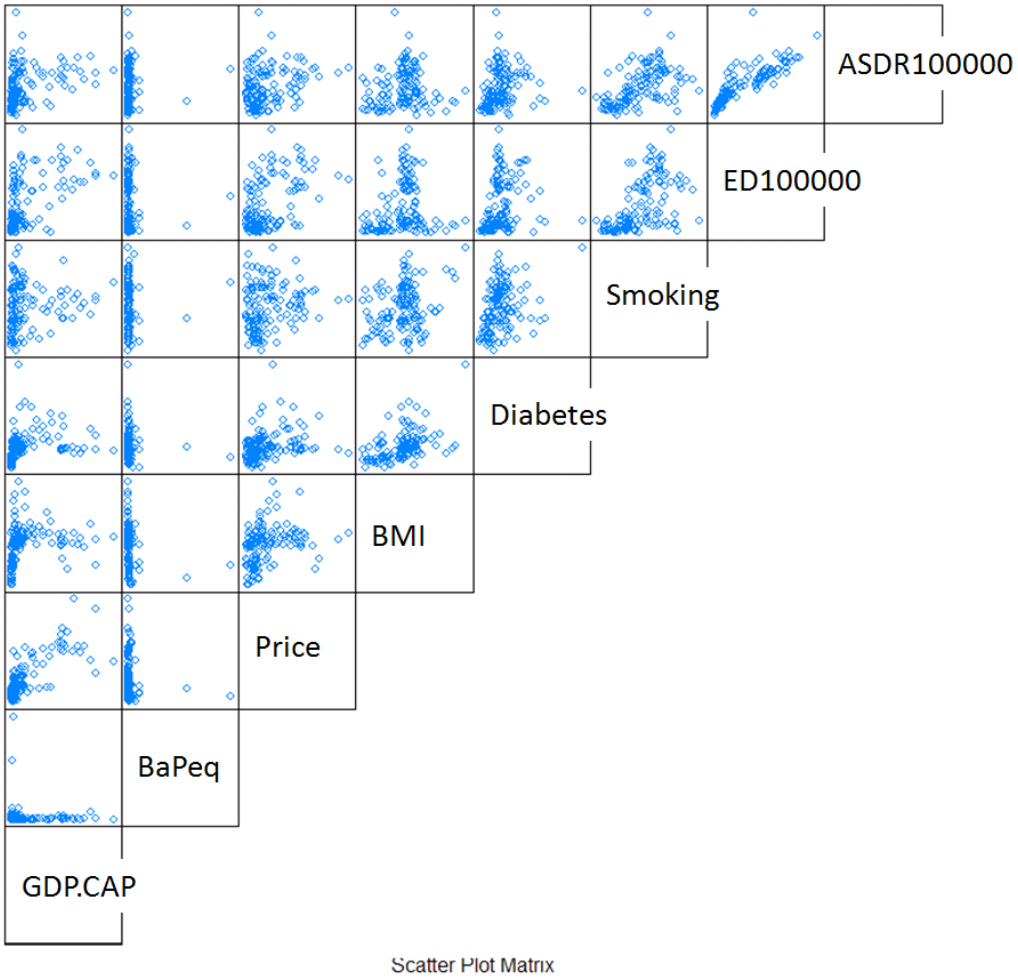
Country	population	gdp/cap	bapeq	smoking	price	bmi	diabetes	ed100000	asdr100000	Income
Spain	41286	15079	2.50E+01	28	4.18	26.61	6.92	47	28	high
Sri Lanka	19444	965	1.20E+01	14	2.83	22.2617	3.96	11	12	low mid
St. Lucia	164	4276	7.60E-03	19	3.7	25.5733	3.54	5.6	6.3	up mid
St. Vincent and the Grenadines	108	3382	4.80E-03	11	2	25.9767	5.12	9.8	13	up mid
Sudan	34356	448	4.60E+01	14	0.97	22.33	1.62	1.8	3.7	low
Suriname	443	2388	1.10E+00	1	1.82	26.1433	2.36	9.8	13	low mid
Swaziland	1120	1356	1.50E+00	10	3.44	25.385	1.26	4	9.6	low mid
Sweden	8985	28912	1.00E+01	15	5.63	25.56	3.42	34	18	high
Switzerland	7382	34190	6.20E+00	22	6.2	25	3.18	40	24	high
Tanzania, United Republic of	36571	322	5.80E+01	11	1.09	22.435	0.70	2.5	6	low
Thailand	62387	2399	2.70E+01	18	1.29	23.42	2.72	21	22	low mid
Tonga	102	1638	1.80E-01	36	3.56	31.86	3.33	7.2	10	low mid
Tunisia	10012	2315	6.90E+00	32	1.3	26.0867	1.95	9.4	13	low mid
Turkey	71727	3197	6.00E+01	30	1.97	27.105	4.72	24	33	low mid
Uganda	25920	285	3.50E+01	9	0.51	26.8067	0.50	1.9	5.4	low
Ukraine	48008	917	3.60E+01	39	0.39	25.4917	3.40	35	22	low mid
United Arab Emirates	4284	18684.11	7.70E+00	8	1.77	28.3583	9.21	1.4	6.9	high
United Kingdom	59405	26506	2.70E+01	28	7.64	22.1083	3.17	57	32	high
United States	293507	36790	2.00E+02	17	4.58	28.025	6.60	55	39	high
Uruguay	3399	5826	1.60E+00	31	1.85	26.0967	4.81	37	28	up mid
Uzbekistan	25930	645	3.40E+00	11	0.5	25.085	2.04	5.6	9.4	low
Vanuatu	215	1110	8.10E-02	26	5.68	26.955	3.47	5.8	13	low mid
Venezuela	26127	4575	1.60E+01	23	3.96	27.2867	2.75	10	15	up mid
Vietnam	82162	500	8.10E+01	18	0.65	20.5883	1.22	15	22	low
Yemen	19763	550	3.70E-01	14	0.75	24.9667	2.30	3	7.7	low
Zambia	10547	366	3.30E+01	10	1.14	21.5467	0.81	2.7	6.7	low
Zimbabwe	13151	379.0674	2.80E+01	15	0.4	23.275	0.98	5.5	12	low

Appendix A.2 Interpolation for *Obesity* and *BMI*

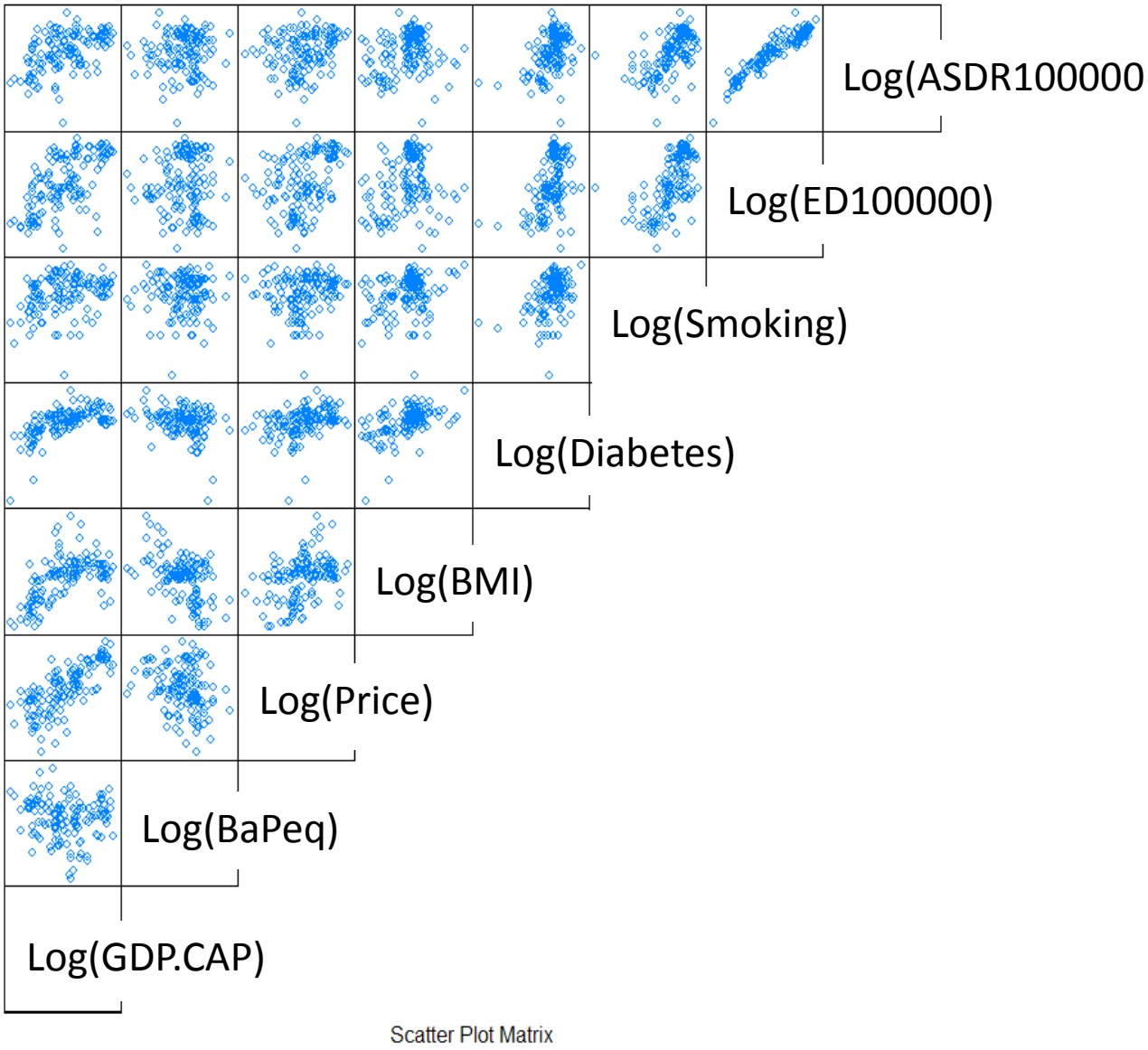
The data on the percentage of people with diabetes was taken for 2000 and 2006 and average BMI was taken for 2000 and an estimate for 2030, and interpolation was needed to calculated the data for 2004. Equation [S1] was used

[S1] $$OB_{2004} = OB_{ly} + \frac{2004-ly}{hy-ly} * (OB_{hy} - OB_{ly})$$

Where OB_{2004} – parameter to interpolate: *Obesity* or *BMI*, ly – low year of interpolation (2000 for both *Obesity* and *BMI*), hy – high year of interpolation (2006 for *Diabetes* and 2030 for *BMI*), OB_{ly} and OB_{hy} are data for corresponding parameters for low and high year, respectively.



Appendix A.3 Scatter plot matrix for untransformed variables.
ASDR100000 – age standardized death rate per 100,000; ED100000 – estimated number of lung cancer deaths per 100,000; GDP – gross domestic product, US$ thousands; Diabetes – % of people diagnosed with diabetes, %; BMI – mean body mass index; Price – price of the pack of most popular cigarettes, US$; Smoking – prevalence of smoking in the country, %; BaP eq – Benzo[a]Pyrene equivalents of emission, Mt/year.



Appendix A.4 Scatter plot matrix for Log-transformed variables.
ASDR100000 – age standardized death rate per 100,000; ED100000 – estimated number of lung cancer deaths per 100,000; GDP – gross domestic product, US$ thousands; Diabetes – % of people with diagnosed diabetes, %; BMI – mean body mass index; Price – price of the pack of most popular cigarettes, US$; Smoking – prevalence of smoking in the country, %; BaP eq – Benzo[a]Pyrene equivalents of emission, Mt/year.
Slope and significance of slope's estimate is shown on each plot (* p-value < 0.05; **p-value<0.01, *** p-value <0.001)

Appendix A.5 Correlation matrix (R^2) for Log_e-transformed variables.

	Log(ED100000)	Log(Smoking)	Log(Diabetes)	Log(BMI)	Log(Price)	Log(BaPeq)	Log(GDP.CAP)
Log(ASDR100000)	0.88***	0.32***	0.18***	0.04*	0.06**	0.00	0.27***
Log(ED100000)		0.38***	0.23***	0.05**	0.14***	0.00	0.40***
Log(Smoking)			0.17***	0.07**	0.02	0.00	0.10***
Log(Diabetes)				0.28***	0.11***	0.09***	0.36***
Log(BMI)					0.15***	0.22***	0.31***
Log(Price)						0.05**	0.56***
Log(BaPeq)							0.02

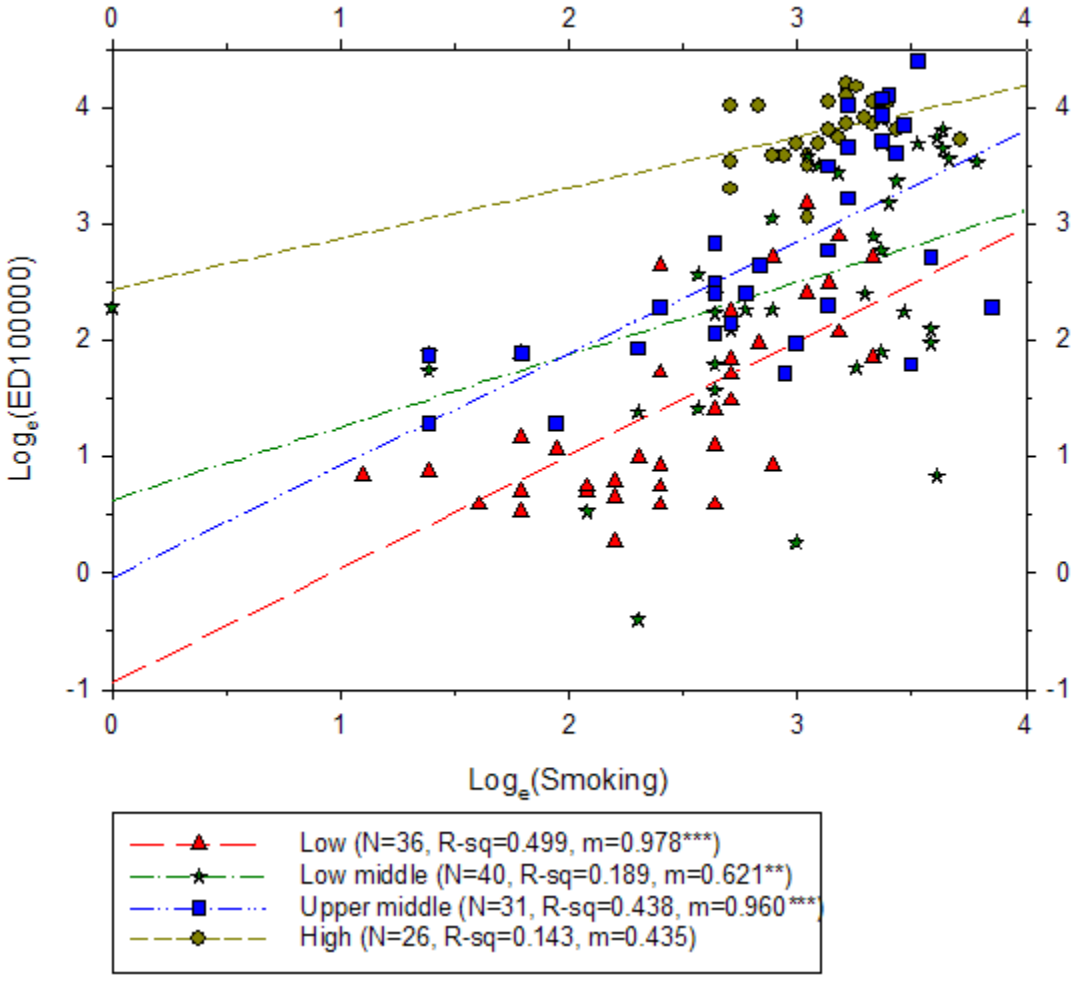
* p-value < 0.05; **p-value<0.01, *** p-value <0.001

Appendix A.6. Regression coefficients for SLR.
Standard error and percent of total regression sum-of-squares due to β_n are given in parenthesis; * p-value < 0.05; **p-value<0.01, *** p-value <0.001.

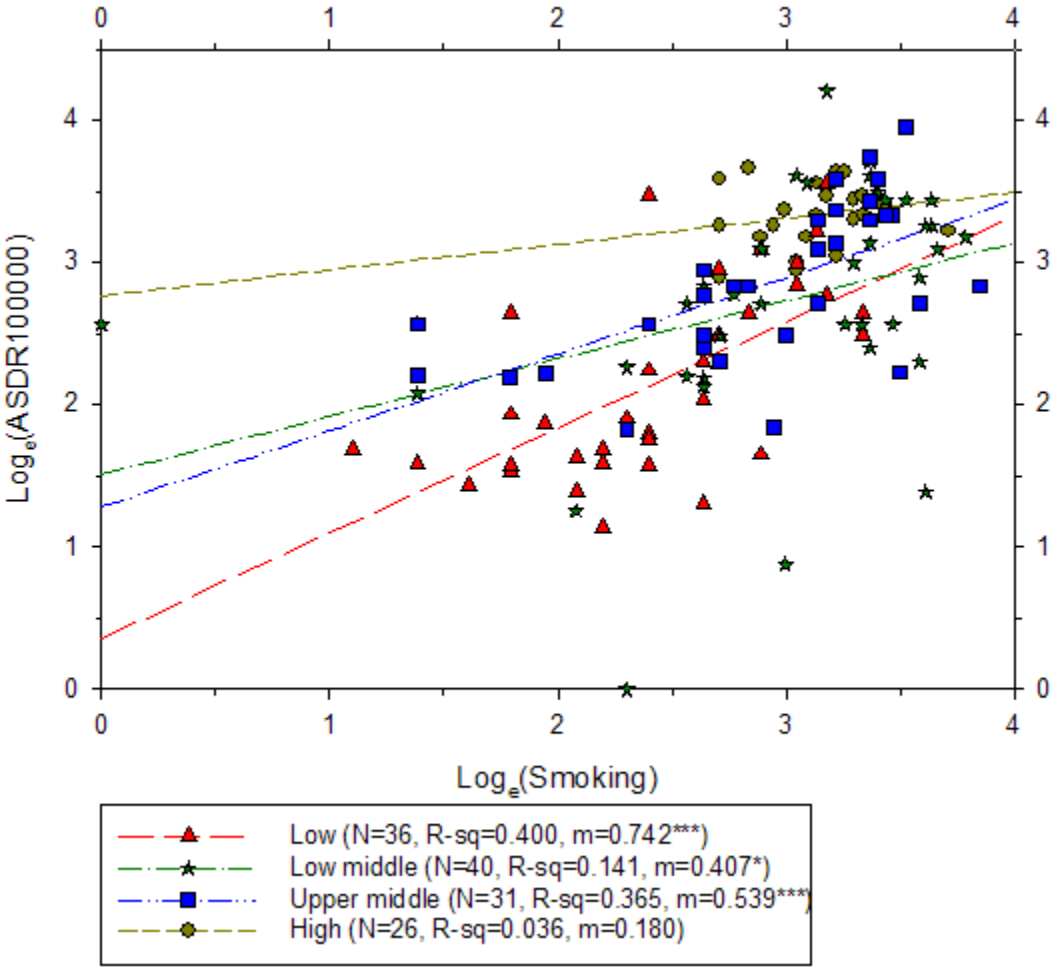
LCMR	Socioeconomic Group	BaPeq	SP	Price	GDP.CAP	BMI	Diabetes
ED100000	Low (N=36)	-0.11 (0.06, 8.3%)	0.98*** (0.18, 44.9%)	-0.69*** (0.19, 27.6%)	0.81** (0.26, 22.0%)	2.5 (1.7, 5.9%)	0.42** (0.13, 23.8%)
	Low Middle (N=40)	0.14 (0.07, 8.4%)	0.62** (0.21, 18.9%)	-0.48* (0.22, 10.9%)	0.02 (0.50, 0.00%)	-3.8 (2.1, 7.8%)	0.72 (0.39, 8.1%)
	Upper Middle (N=31)	0.11* (0.05, 12.7%)	0.96*** (0.20, 43.8%)	-0.02 (0.37, 0.01%)	-0.20 (0.56, 0.43%)	-5.0* (2.3, 13.5%)	-0.18 (0.35, 0.94%)
	High (N=26)	0.08* (0.03, 21.0%)	0.44 (0.22, 14.3%)	-0.10 (0.19, 1.2%)	0.06 (0.14, 0.74%)	-0.43 (1.0, 0.69%)	-0.07 (0.14, 1.1%)
	Low + Low middle (N=76)	-0.03 (0.06, 0.47%)	0.90*** (0.14, 35.3%)	-0.22 (0.17, 2.1%)	0.64*** (0.12, 26.0%)	2.7* (1.2, 6.5%)	0.66*** (0.12, 27.8%)
	Upper Middle + High (N=60)	0.16*** (0.04, 20.0%)	1.2*** (0.19, 40.0%)	0.80*** (0.19, 24.1%)	0.49** (0.14, 17.0%)	-7.3*** (1.7, 24.3%)	-0.39 (0.28, 3.2%)
	Complete (N=136)	0.03 (0.04, 0.35%)	1.1*** (0.12, 37.7%)	0.49*** (0.10, 14.3%)	0.48*** (0.05, 39.7%)	2.8** (1.0, 5.4%)	0.70*** (0.11, 22.8%)
ASDR100000	Low (N=36)	-0.10 (0.05, 10.6%)	0.74*** (0.16, 40.0%)	-0.52** (0.16, 24.0%)	0.61** (0.21, 19.1%)	2.0 (1.4, 5.5%)	0.31** (0.10, 20.5%)
	Low Middle (N=40)	0.08 (0.06, 5.0%)	0.41* (0.16, 14.1%)	-0.28 (0.17, 6.7%)	0.09 (0.38, 0.14%)	-3.3* (1.6, 10.4%)	0.24 (0.31, 1.62%)
	Upper Middle (N=31)	0.08* (0.03, 19.7%)	0.54*** (0.13, 36.5%)	-0.15 (0.22, 1.6%)	-0.34 (0.34, 3.3%)	-2.4 (1.5, 8.1%)	-0.14 (0.21, 1.5%)
	High (N=26)	0.03 (0.03, 3.2%)	0.18 (0.19, 3.6%)	0.06 (0.16, 0.72%)	0.05 (0.12, 0.64%)	0.56 (0.86, 1.7%)	-0.06 (0.11, 1.0%)
	Low + Low middle (N=76)	-0.03 (0.04, 0.77%)	0.59*** (0.11, 28.4%)	-0.18 (0.12, 2.8%)	0.37*** (0.10, 16.7%)	1.2 (0.89, 2.4%)	0.38*** (0.10, 17.8%)
	Upper Middle + High (N=60)	0.10*** (0.02, 24.1%)	0.55*** (0.11, 30.7%)	0.30** (0.11, 11.8%)	0.22** (0.08, 12.2%)	-2.9** (0.96, 13.7%)	-0.18 (0.15, 2.5%)
	Complete (N=136)	0.01 (0.02, 0.23%)	0.64*** (0.08, 31.6%)	0.20** (0.07, 6.3%)	0.25*** (0.04, 27.1%)	1.5* (0.64, 3.8%)	0.39*** (0.07, 17.9%)

Appendix A.7. Regression coefficients for MLR.
Standard error and percent of total regression sum-of-squares due to β_n are given in parenthesis; * p-value < 0.05; **p-value<0.01, *** p-value <0.001.

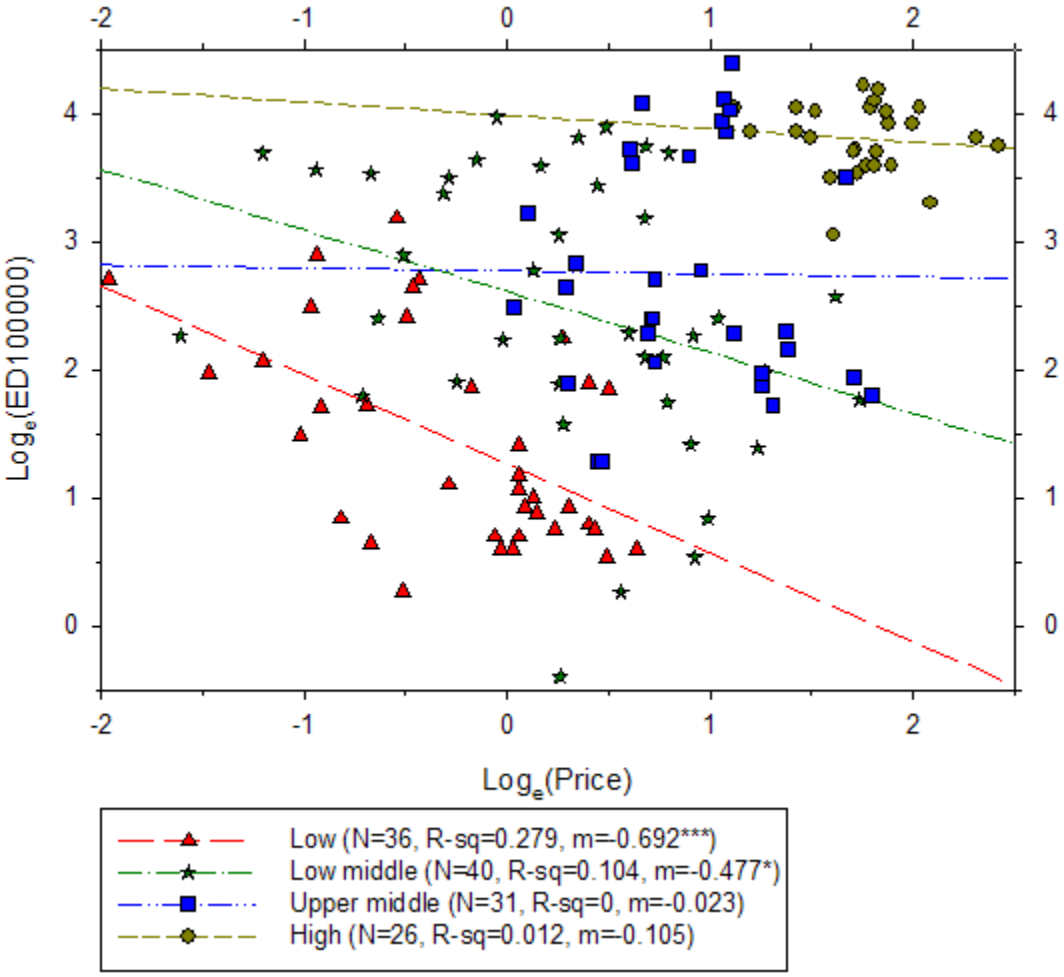
LCMR	Socioeconomic Group	BaPeq	SP	Price	GDP.CAP	BMI	R^2
ED100000	Low	D	0.98*** (0.18, 44.9%)	D	D	D	0.45***
	Low Middle	D	0.67** (0.20, 18.9%)	D	D	-4.4* (1.9, 10.8%)	0.30**
	Upper Middle	D	1.0*** (0.16, 43.8%)	D	D	-6.4*** (1.5, 21.9%)	0.66***
	High	0.08** (0.03, 21.0%)	0.46* (0.19, 16.3%)	D	D	D	0.37**
	Low + Low middle	D	0.61*** (0.14, 35.3%)	-0.34* (0.14, 0.44%)	0.55*** (0.12, 14.0%)	D	0.50***
	Upper Middle + High	0.07* (0.03, 20.0%)	0.89*** (0.15, 30.0%)	0.39** (0.13, 7.9%)	D	-5.1*** (1.2, 10.4%)	0.68***
	Complete	D	0.88*** (0.10, 37.7%)	D	0.46*** (0.05, 21.1%)	-2.9*** (0.78, 3.8%)	0.63***
ASDR100000	Low	D	0.74*** (0.16, 40.0%)	D	D	D	0.40***
	Low Middle	D	0.45** (0.15, 14.1%)	D	D	-3.8* (1.4, 13.3%)	0.27**
	Upper Middle	D	0.59*** (0.12, 36.5%)	D	D	-3.2** (1.1, 14.1%)	0.51***
	High	D	D	D	D	D	D
	Low + Low middle	D	0.44*** (0.11, 28.4%)	-0.22* (0.11, 0.93%)	0.47*** (0.12, 8.65%)	-2.14* (0.99, 3.82%)	0.42***
	Upper Middle + High	0.06** (0.02, 24.1%)	0.46*** (0.10, 20.8%)	D	D	-1.84* (0.79, 4.9%)	0.50***
	Complete	D	0.50*** (0.08, 31.6%)	-0.19* (0.08, 3.0%)	0.32*** (0.05, 12.3%)	-1.6** (0.57, 2.8%)	0.50***



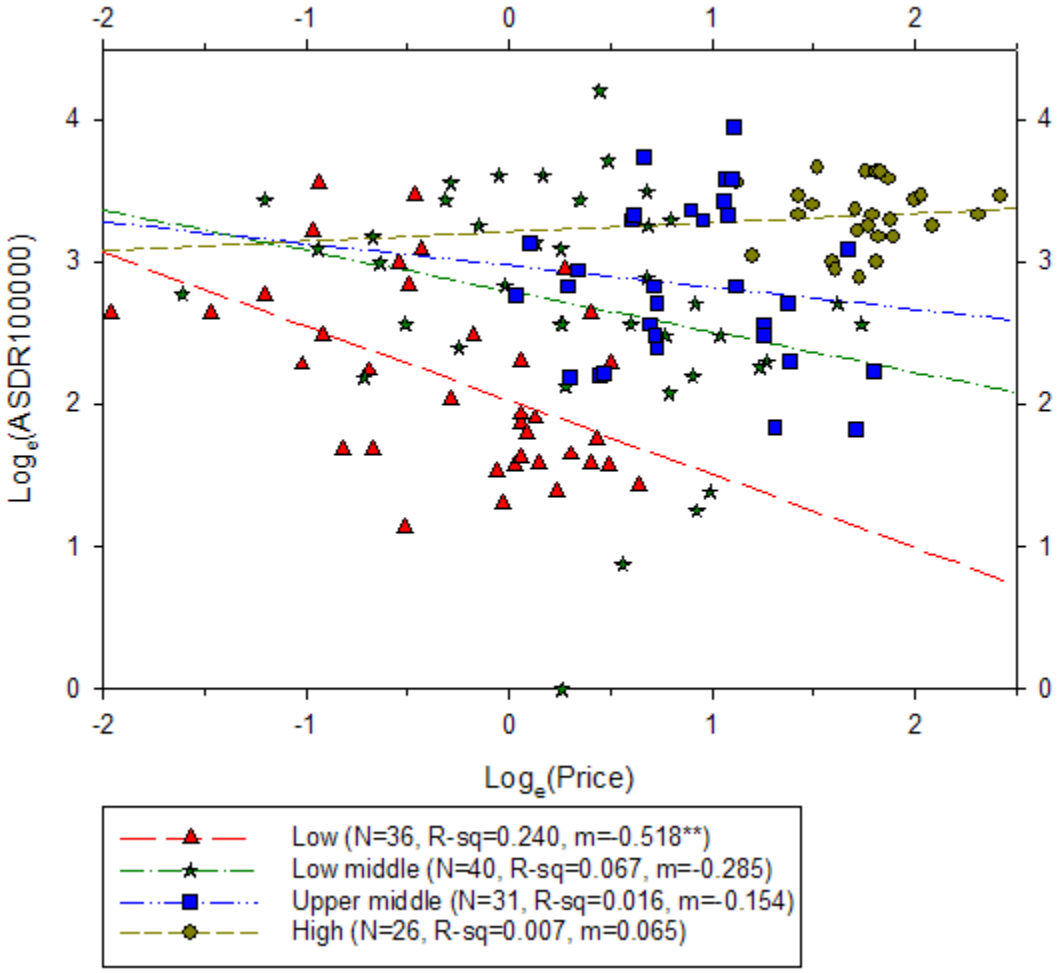
Appendix A.8 Scatter plot between lung cancer mortality rate (ED100000) and smoking prevalence.
Significance of estimates: * p-value<0.05; **p-value<0.01, *** p-value <0.001.



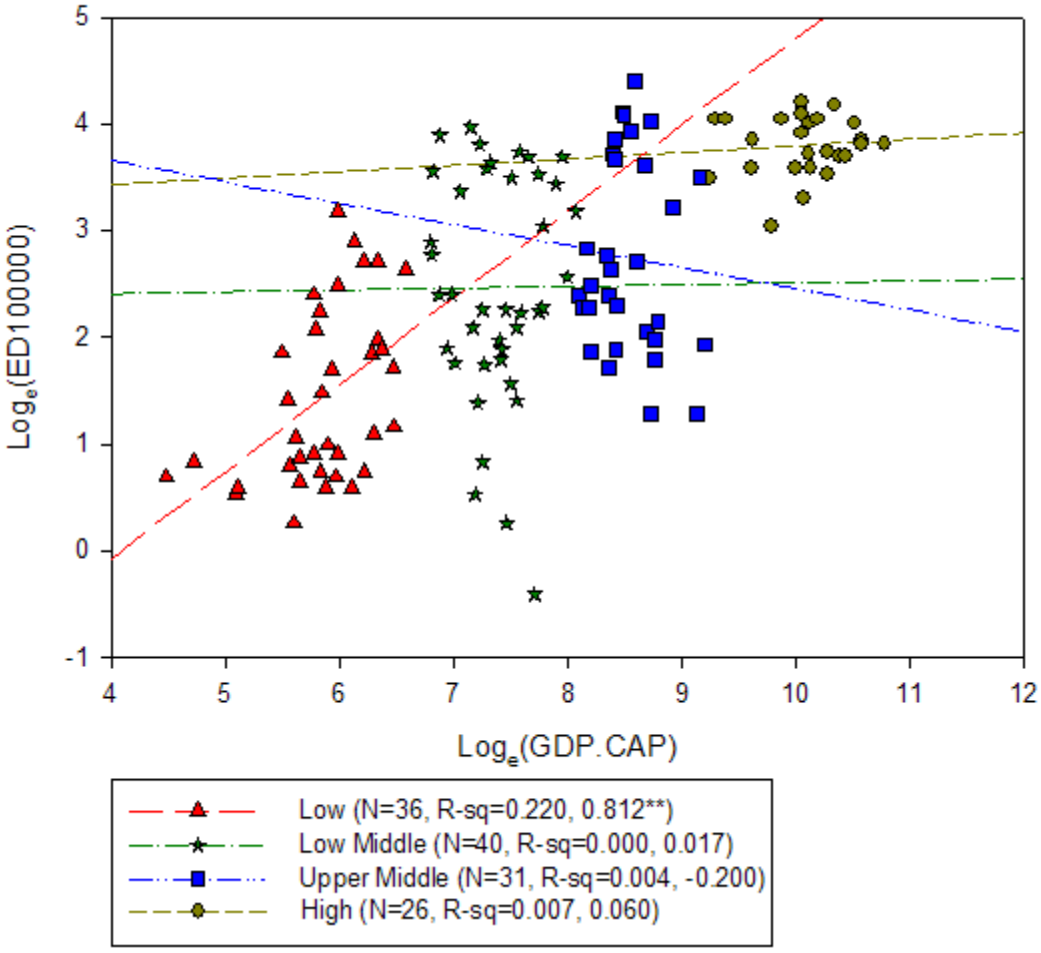
Appendix A.9 Scatter plot between lung cancer mortality rate (ASDR100000) and smoking prevalence.
Significance of estimates: * p-value < 0.05; **p-value<0.01, *** p-value <0.001.



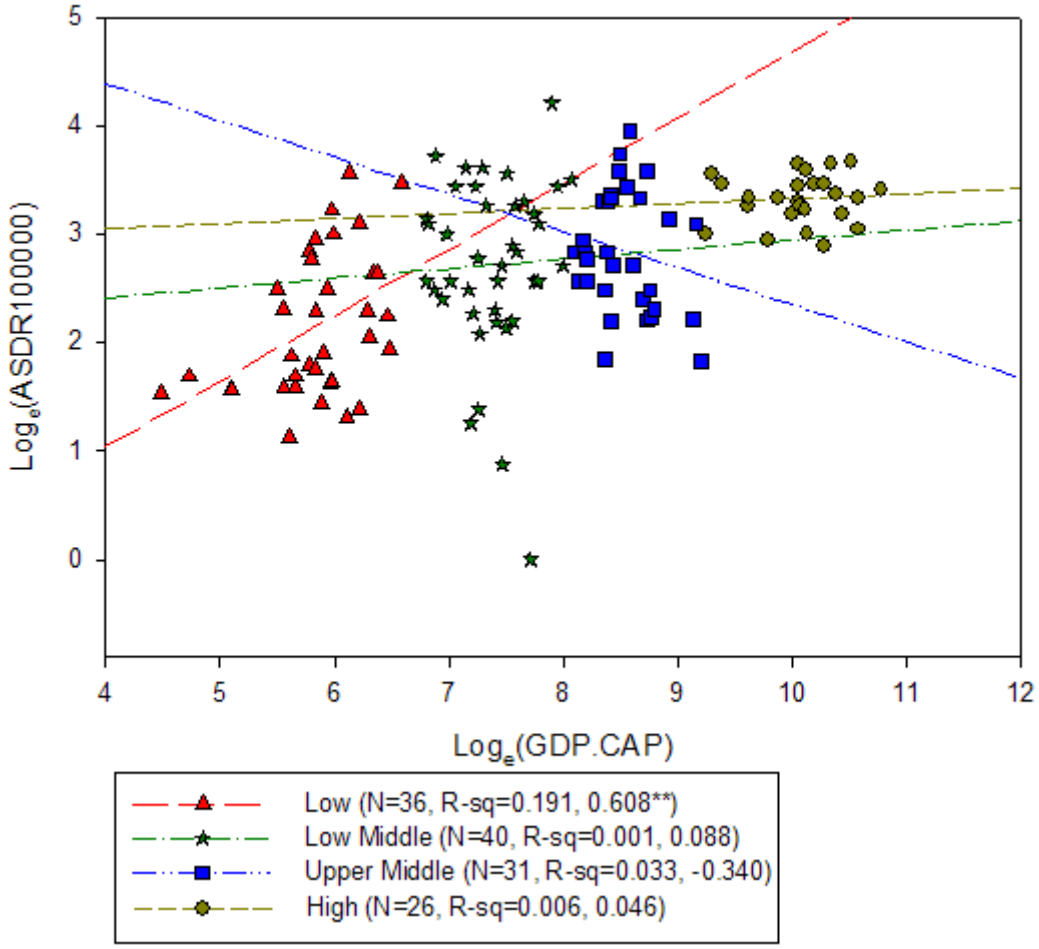
Appendix A.10 Scatter plot between lung cancer mortality rate (ED100000) and cigarette price.
Significance of estimates: * p-value < 0.05; **p-value<0.01, *** p-value <0.001.



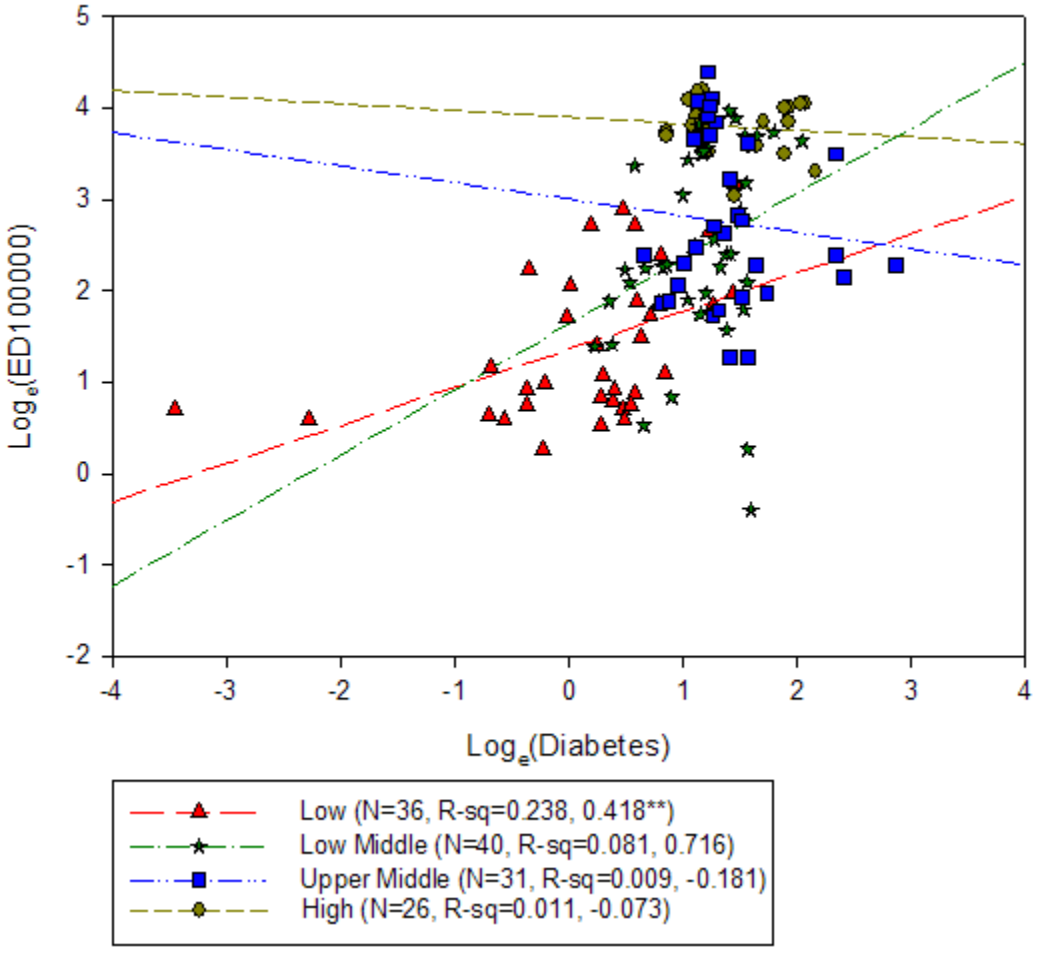
Appendix A.11 Scatter plot between lung cancer mortality rate (ASDR100000) and cigarette price.
Significance of estimates: * p-value < 0.05; **p-value<0.01, *** p-value <0.001.



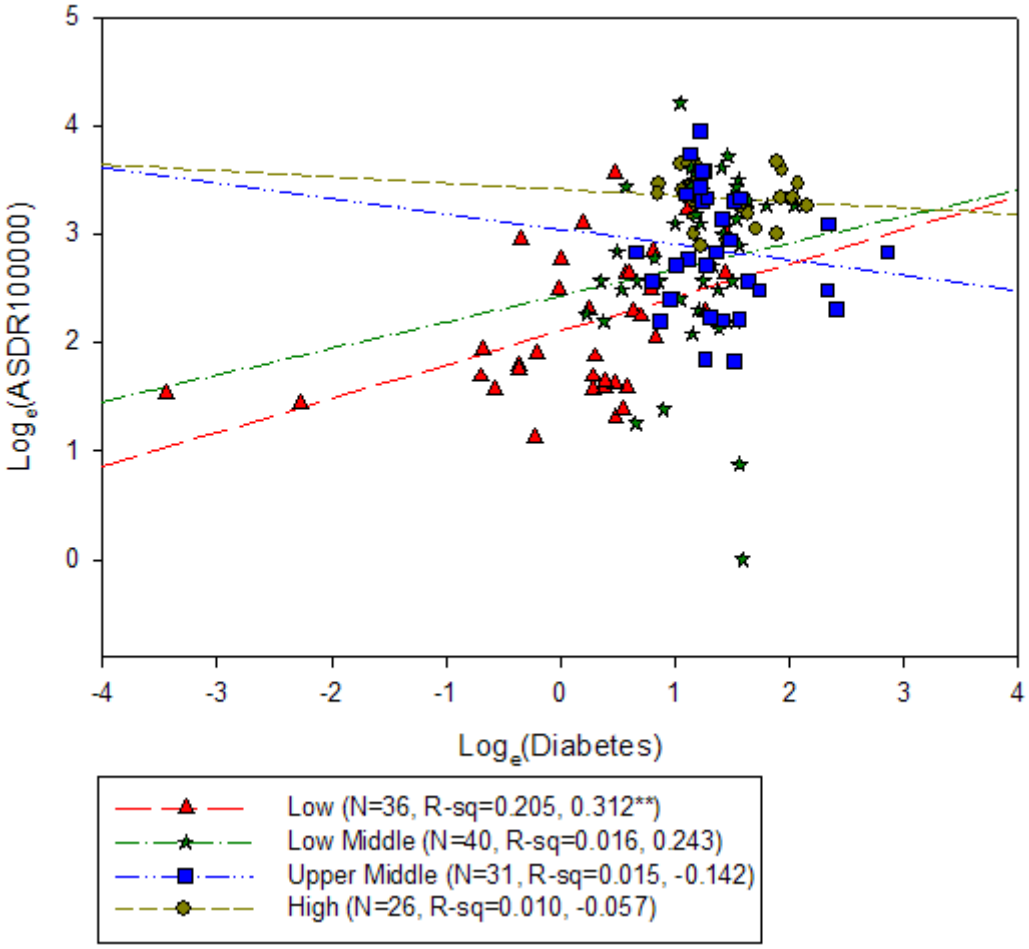
Appendix A.12 Scatter plot between lung cancer mortality rate (ED100000) and GDP per capita.
Significance of estimates: * p-value < 0.05; **p-value<0.01, *** p-value <0.001.



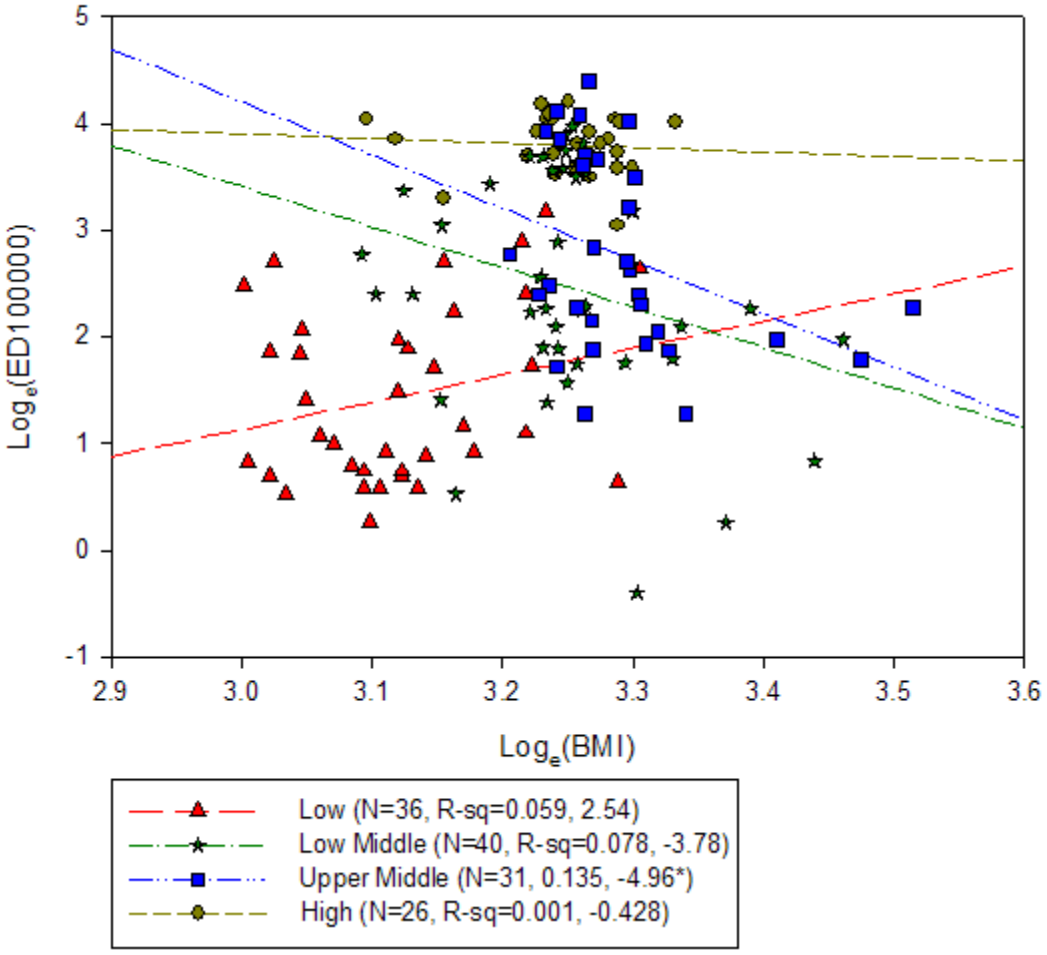
Appendix A.13 Scatter plot between lung cancer mortality rate (ASDR100000) and GDP per capita.
Significance of estimates: * p-value < 0.05; **p-value<0.01, *** p-value <0.001.



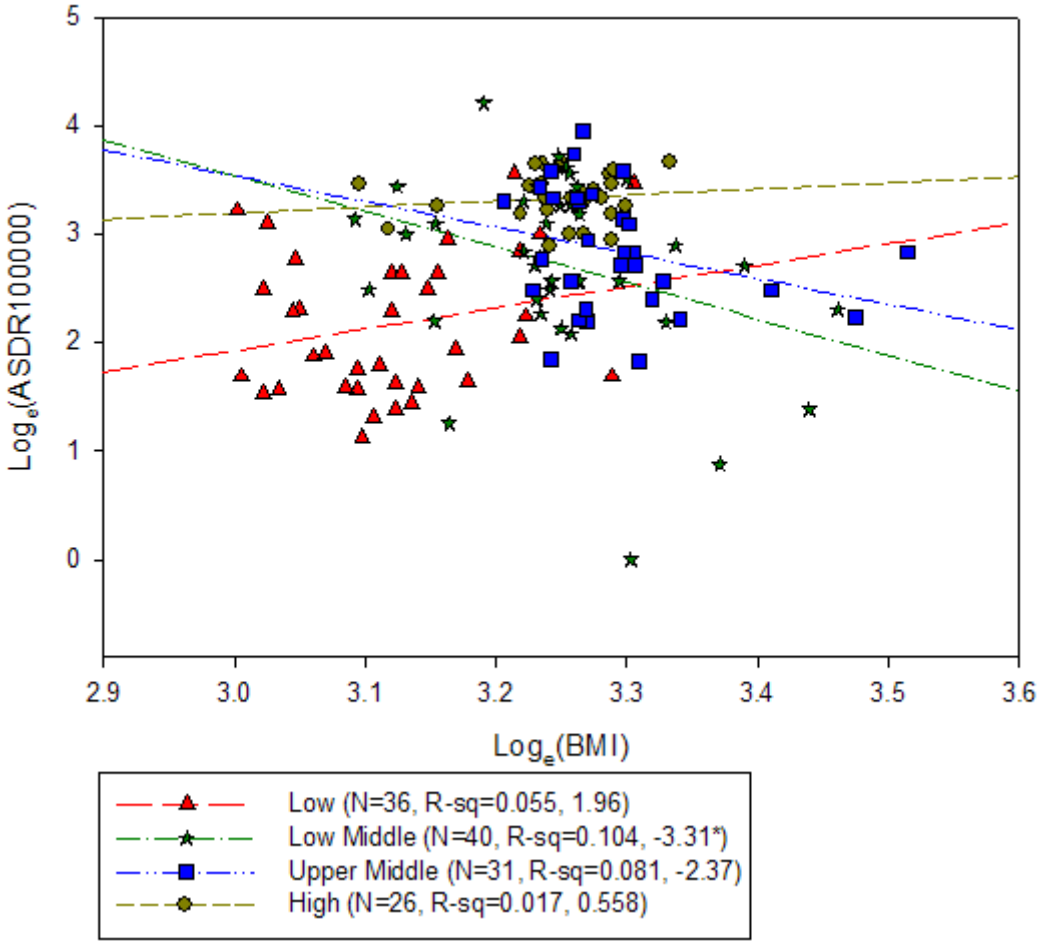
Appendix A.14 Scatter plot between lung cancer mortality rate (ED100000) and percent people with diabetes.
Significance of estimates: * p-value < 0.05; **p-value<0.01, *** p-value <0.001.



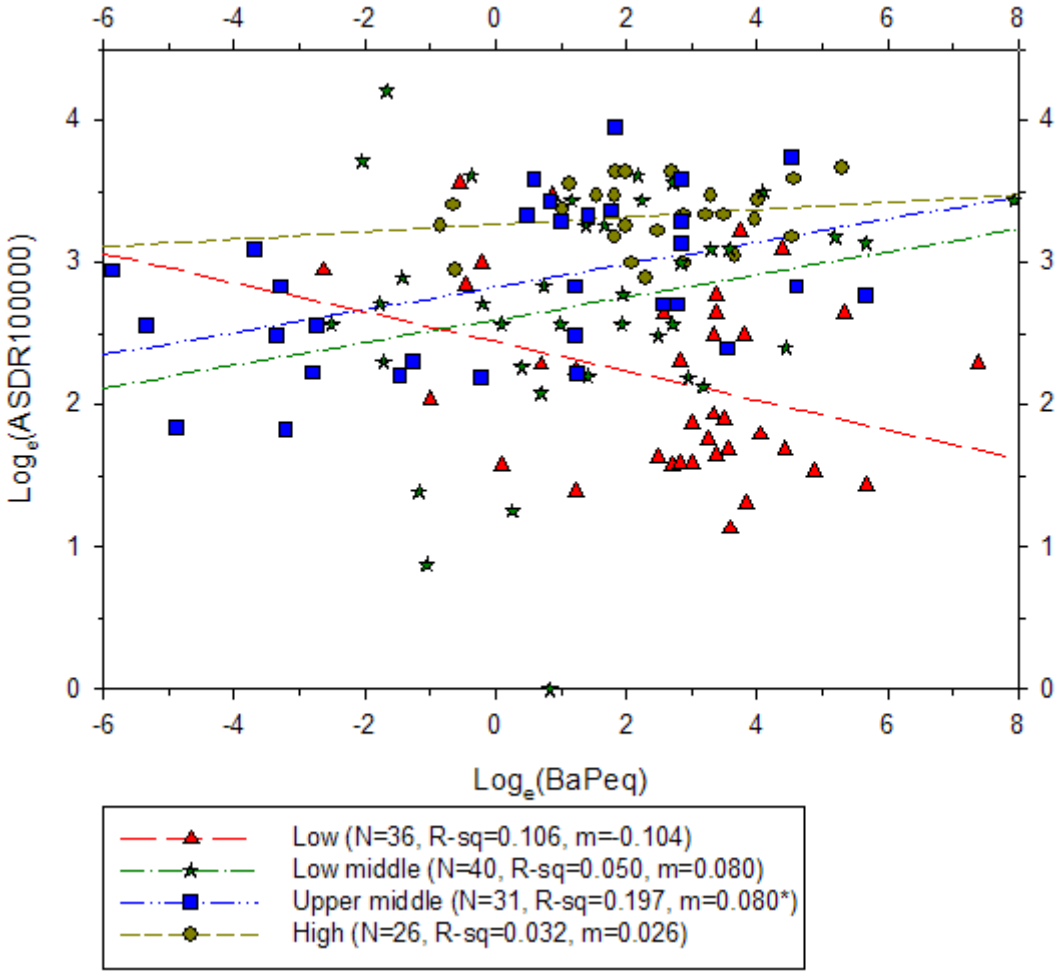
Appendix A.15 Scatter plot between lung cancer mortality rate (ASDR100000) and percent people with diabetes.
Significance of estimates: * p-value < 0.05; **p-value<0.01, *** p-value <0.001.



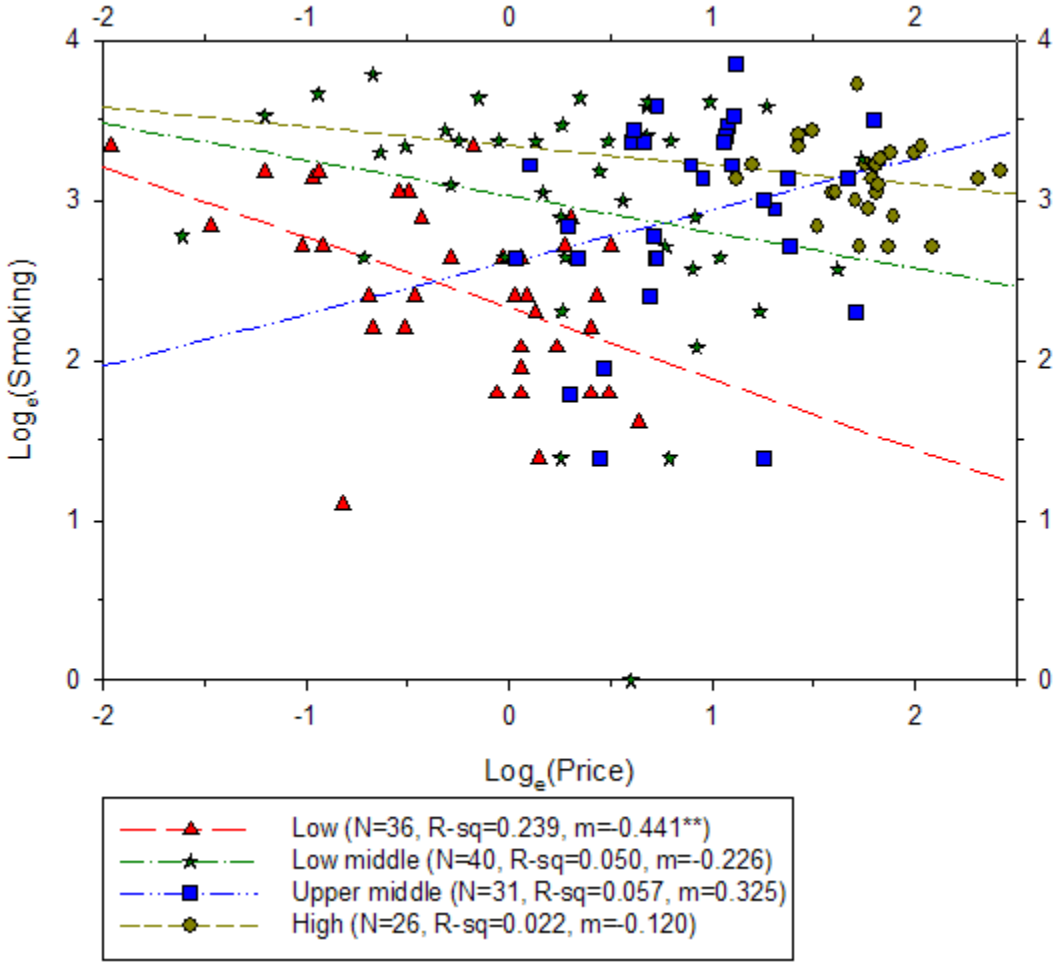
Appendix A.16 Scatter plot between lung cancer mortality rate (ED100000) and body mass index (BMI).
Significance of estimates: * p-value < 0.05; **p-value<0.01, *** p-value <0.001.



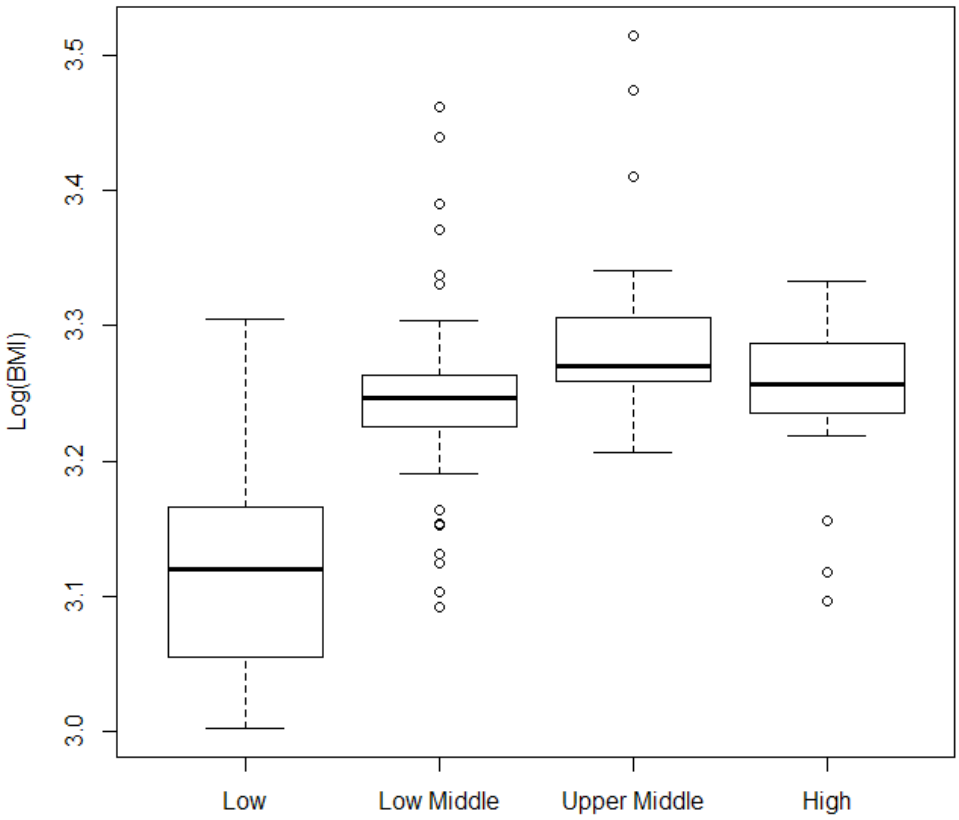
Appendix A.17 Scatter plot between lung cancer mortality rate (ASDR100000) and body mass index (BMI).
Significance of estimates: * p-value < 0.05; **p-value<0.01, *** p-value <0.001.



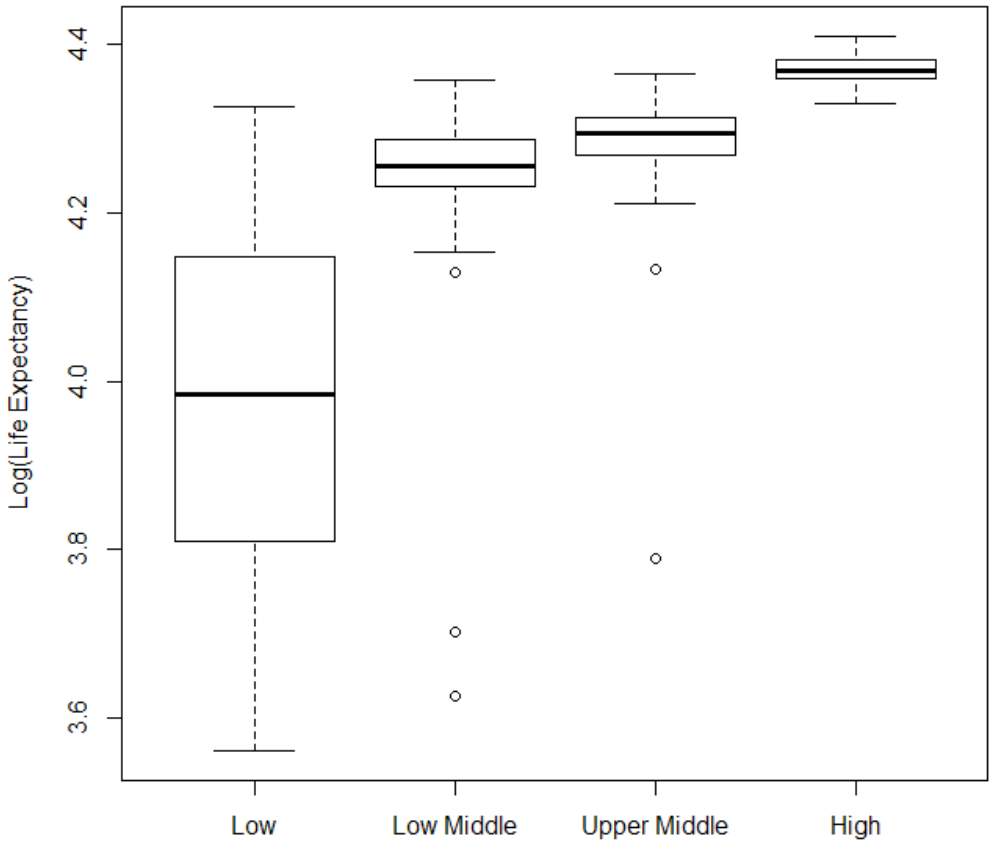
Appendix A.18 Scatter plot between lung cancer mortality rate (ASDR100000) and BaPeq.
Significance of estimates: * p-value < 0.05; **p-value<0.01, *** p-value <0.001.



Appendix A.19 Scatter plot between smoking prevalence and cigarette price.
Significance of estimates: * p-value<0.05; **p-value<0.01, *** p-value <0.001.



Appendix A.20 Box plot of BMI across four income levels.
The bold line represents the median, the box represents 25th to 75th percentile, the whiskers represent the lowest datum still within 1.5 interquartile range of the lower quartile, and the highest datum still within 1.5 interquartile range of the upper quartile.



Appendix A.21 Box plot of life expectancy across four income levels.
The bold line represents the median, the box represents 25th to 75th percentile, the whiskers represent the lowest datum still within 1.5 interquartile range of the lower quartile, and the highest datum still within 1.5 interquartile range of the upper quartile**.**

APPENDIX B

Appendix B.1:

B.1.1 Enzymatic hydrolysis with β-glucuronidase/arylsulfatase

The amount of β-glucuronidase/arylsulfatase needed for enzymatic hydrolysis of OH-PAHs was investigated. Three urine samples (3 mL each) were mixed with 5 mL of acetate buffer and spiked with 0 µl, 10 µl, 100 µl, and 500 µl of β-glucuronidase/arylsulfatase. The samples were extracted, analyzed, and concentrations of OH-PAHs were compared.

Appendix B.15 shows the OH-PAH concentrations in urine after enzymatic hydrolysis with different volumes of β-glucuronidase/arylsulfatase. The results show that 10 µl of β-glucuronidase/arylsulfatase were enough to hydrolyze the OH-PAH adducts. Larger volumes of β-glucuronidase/arylsulfatase caused the SPE cartridge to clog, resulted in greater noise in the ion chromatograms and the OH-PAH concentrations did not increase significantly (Appendix B.15). Although previous studies have used β-glucuronidase/arylsulfatase volumes in the range of 5µl to 25 µl[50, 55, 56, 164]. 10 µl of β-glucuronidase/arylsulfatase has been shown to be adequate for the enzymatic hydrolysis of OH-PAHs and this is what we chose to use[37, 49, 53, 54, 131].

B.1.2 SPE column selection

Different SPE phases, including Focus, Plexa, Isolute 101, and a combination of Plexa (for OH-PAHs) and C18 (for PAHs) (in series), were tested in order to extract both PAHs and OH-PAHs from urine. Dichloromethane was used with all SPE phases as a elution solvent. Initially, Focus, Plexa, and Isolute 101 were evaluated for the extraction

of OH-PAHs from urine. Later, PAHs were included in the method and the experiment was repeated in order to test Plexa vs Plexa-C18 to extract both PAH and OH-PAH. Three milliliters of UTAK urine (blank urine was analyzed before analysis and concentrations were subtracted before calculations) was spiked with 50 µl of OH-PAHs (1 µg/ml, to evaluate Focus vs Plexa vs Isolute 101 SPE cartridges), or PAHs (1 µg/ml, to evaluate Plexa vs Plexa+C18 SPE cartridges), or a mixture of PAH and OH-PAH, followed by derivatization (Figure 1). The extracts were analyzed and the concentrations of PAH and OH-PAH were compared.

Figure S3 shows the OH-PAH recoveries from urine using Focus, Plexa, and Isolute 101 stationary phases and DCM as elution solvent. This experiment showed that the Plexa SPE cartridge had higher overall recoveries for the dihydroxy-PAHs, such as 2,3-, 1,5-, 1,6-, 2,7, and 2,6-dihydroxynaphthalenes, with a mean recovery of 105%. The Focus SPE cartridge had higher overall recoveries for monohydroxy-PAHs, such as hydroxynaphthalenes, hydroxyphenathrenes, some hydroxybenzo[a]pyrenes and others, with a mean recovery of 128% and were comparable to a previous study[131]. This difference in the two stationary phases may be due to differences in their polarity.

For the extraction of PAHs from urine, Plexa only and Plexa and C18 (combined in series) were tested (with DCM and EA used as elution solvents and combined) and the PAH recoveries are shown in Figure S4. The use of Plexa and C18 in combination had higher overall recoveries of PAHs, mean recovery of 71%, compared to Plexa alone, mean recovery of 56%. PAH are non-polar and the use of a non-polar octadecyl stationary phase (C18) improved PAH recoveries, especially for the more nonpolar, higher molecular weight PAH (Figure S4).

B.1.3 SPE elution solvent composition

Several elution solvents were tested in order to optimize the OH-PAHs recoveries. Four aliquots of three milliliters of UTAK urine, each, were spiked with OH-PAHs (30 µl, 1 ng/µl), extracted with Focus SPE, and eluted separately with 3 mL of methanol, 3 ml of DCM + 3 ml of EA, and the mixture of DCM and EA 1:1 (v:v, 3 ml) and 6:4 (v:v, 3 ml). The extracts were analyzed and concentrations of OH-PAHs were compared.

Appendix B.16 shows the OH-PAH recoveries for the Focus SPE cartridge using four different elution solvents: DCM : EA (1 : 1,v/v), DCM : EA (6 : 4, v/v), DCM followed by EA, and methanol. The elution of OH-PAH with methanol resulted in the statistically significantly lower recoveries compared to other elution solvents (DCM and EA, or the mixture of DCM and EA) and ranged from 0% to 152%. No statistically significant difference in recoveries were measured among the other elution solvents (p-value>0.05). In the end, we decided to use 3 ml of 100% DCM and 3 ml of 100% EA, in series (fractions were combined before next step), to elute the mixture of OHPAHs and PAHs from the combination of Plexa and C18 stationary phases.

B.1.4 Choice of derivatizing agent

Three different derivatizing agents, BSTFA (+1%TMCS), BSA/TMCS/TMSI, and MTBSTFA, were tested to investigate which derivatized the largest number of OH-PAH. BSTFA showed poor efficiency for the dihydroxy-PAHs, especially those with hindered OH- groups. BSA worked better than MTBSTFA for hindered groups, but the latter reacts with lower temperature and takes less time to react, forms more stable adducts and shifts the molecular weight more into the higher m/z. For these reasons, it was decided to use MTBSTFA for derivatization.

Appendix B.2 The list of OH-PAH, LMW PAH, HMW PAH, and oxy-PAHs and their labeled surrogates, internal standards, their abbreviations and estimated detection limits.

Class	#	Analyte	Abbreviation	Surrogate	Internal Standard	EDL	
						pg/ml in urine	pg/m^3 of air
OH-PAHs	1	1-Hydroxynaphthalene	1-OH-Nap	[$^2H_7$]-1-OH-Nap	[$^2H_{10}$]-Ace	14.6	12.6
	2	2-Hydroxynaphthalene	2-OH-Nap	[$^2H_7$]-1-OH-Nap	[$^2H_{10}$]-Ace	18.0	15.4
	3	2,3-Dihydroxynaphthalene	2,3-OH-Nap	[$^2H_7$]-1-OH-Nap	[$^2H_{10}$]-Ace	181.4	155.5
	4	1,3-Dihydroxynaphthalene	1,3-OH-Nap	[$^2H_7$]-1-OH-Nap	[$^2H_{10}$]-Ace	141.7	121.4
	5	1,5-Dihydroxynaphthalene	1,5-OH-Nap	[$^2H_7$]-1-OH-Nap	[$^2H_{10}$]-Ace	48.7	41.8
	6	1,6-Dihydroxynaphthalene	1,6-OH-Nap	[$^2H_7$]-1-OH-Nap	[$^2H_{10}$]-Ace	25.1	21.5
	7	2,7-Dihydroxynaphthalene	2,7-OH-Nap	[$^2H_7$]-1-OH-Nap	[$^2H_{10}$]-Ace	19.9	17.0
	8	2,6-Dihydroxynaphthalene	2,6-OH-Nap	[$^2H_7$]-1-OH-Nap	[$^2H_{10}$]-Ace	37.5	32.1
	9	4-Hydroxyphenanthrene	4-OH-Phen	[$^{13}C_6$]-4-OH-Phen	[$^2H_{10}$]-Flt	6.0	5.2
	10	3-Hydroxyphenanthrene	3-OH-Phen	[$^{13}C_6$]-4-OH-Phen	[$^2H_{10}$]-Flt	30.1	25.8
	11	1-Hydroxyphenanthrene	1-OH-Phen	[$^{13}C_6$]-4-OH-Phen	[$^2H_{10}$]-Flt	31.8	27.2
	12	2-Hydroxyphenanthrene	2-OH-Phen	[$^{13}C_6$]-4-OH-Phen	[$^2H_{10}$]-Flt	24.9	21.3
	13	2-Hydroxyanthraquinone	2-OH-AntQn	[$^{13}C_6$]-4-OH-Phen	[$^2H_{10}$]-Flt	93.4	80.0
	14	9-Hydroxyfluorene	9-OH-Flo	[$^2H_9$]-2-OH-Flo	[$^2H_{10}$]-Flt	160.8	137.8
	15	3-Hydroxyfluorene	3-OH-Flo	[$^2H_9$]-2-OH-Flo	[$^2H_{10}$]-Flt	39.4	33.7
	16	2-Hydroxyfluorene	2-OH-Flo	[$^2H_9$]-2-OH-Flo	[$^2H_{10}$]-Flt	41.4	35.5
	17	1-Hydroxy-9-fluorenone	1-OH-Flon	[$^2H_9$]-2-OH-Flo	[$^2H_{10}$]-Flt	26.2	22.4
	18	2-Hydroxy-9-fluorenone	2-OH-Flon	[$^2H_9$]-2-OH-Flo	[$^2H_{12}$]-BkFlt	82.1	70.4
	19	3-Hydroxyfluoranthene	3-OH-Flt	[$^{13}C_6$]-3-OH-Flt	[$^2H_{10}$]-Flt	15.9	13.7

Appendix B.2 (Continued)

Class	#	Analyte	Abbreviation	Surrogate	Internal Standard	EDL	
						pg/ml in urine	pg/m^3 of air
OH-PAHs	20	1-Hydroxypyrene	1-OH-Pyr	[$^{13}C_6$]1-OH-Pyr	[$^{2}H_{12}$]-BkFlt	19.4	16.6
	21+ 22	2-OH-B(a)anthracen+ 3-OH-B(c)pnenanthrene	2-OH-BaA+ 3-OH-BcPh	[$^{13}C_6$]-1-OH-BaA	[$^{2}H_{12}$]-BkFlt	19.3	16.5
	23	10-Hydroxybenzo(a)pyrene	10-OH-BaP	[$^{13}C_6$]-3-OH-BcPh	[$^{2}H_{12}$]-BkFlt	44.3	38.0
	24	12-Hydroxybenzo(a)pyrene	12-OH-BaP	[$^{13}C_6$]-3-OH-BcPh	[$^{2}H_{12}$]-BkFlt	52.1	44.6
	25	7-Hydroxybenzo(a)pyrene	7-OH-BaP	[$^{13}C_6$]-3-OH-BcPh	[$^{2}H_{12}$]-BkFlt	43.7	37.4
	26	9-Hydroxybenzo(a)pyrene	9-OH-BaP	[$^{13}C_6$]-3-OH-BcPh	[$^{2}H_{12}$]-BkFlt	29.6	25.4
	27	3-Hydroxybenzo(a)pyrene	3-OH-BaP	[$^{13}C_6$]-3-OH-BcPh	[$^{2}H_{12}$]-BkFlt	36.5	31.3
	28	4-Hydroxychrysene	4-OH-Chr	[$^{13}C_6$]-3-OH-Chr	[$^{2}H_{12}$]-BkFlt	13.9	11.9
	29	6-Hydroxychrysene	6-OH-Chr	[$^{13}C_6$]-3-OH-Chr	[$^{2}H_{12}$]-BkFlt	43.9	37.7
	30	3-Hydroxychrysene	3-OH-Chr	[$^{13}C_6$]-3-OH-Chr	[$^{2}H_{12}$]-BkFlt	35.6	30.5
	31	2-Hydroxychrysene	2-OH-Chr	[$^{13}C_6$]-3-OH-Chr	[$^{2}H_{12}$]-BkFlt	20.3	17.4
	32	1-Hydroxychrysene	1-OH-Chr	[$^{13}C_6$]-3-OH-Chr	[$^{2}H_{12}$]-BkFlt	9.5	8.1
	33	2,6-Hydroxyanthraquinone	2.6-OH-AntQn	[$^{13}C_6$]-3-OH-Chr	[$^{2}H_{12}$]-BkFlt	6.8	5.8
	34	11-Hydroxybenzo(b)fluoranthene	11-OH-BbFlt	[$^{13}C_6$]-3-OH-Chr	[$^{2}H_{12}$]-BkFlt	38.6	33.1
LMW PAHs	35	Naphthalene	Nap	[$^{2}H_{10}$]-Flo	[$^{2}H_{10}$]-Ace	9.2	7.9
	36	Acenaphtylene	Acy	[$^{2}H_{10}$]-Flo	[$^{2}H_{10}$]-Ace	31.6	27.1
	37	Acenaphthene	Ace	[$^{2}H_{10}$]-Flo	[$^{2}H_{10}$]-Ace	18.5	15.8
	38	Fluorene	Flo	[$^{2}H_{10}$]-Flo	[$^{2}H_{10}$]-Ace	23.1	19.8
	39	Phenanthrene	Phen	[$^{2}H_{10}$]-Phen	[$^{2}H_{10}$]-Ace	3.0	2.5
	40	Anthracene	Ant	[$^{2}H_{10}$]-Phen	[$^{2}H_{10}$]-Ace	19.6	16.8
	41	Fluoranthene	Flt	[$^{2}H_{10}$]-Pyr	[$^{2}H_{10}$]-Flt	15.2	13.0

Appendix B.2 (Continued)

Class	#	Analyte	Abbreviation	Surrogate	Internal Standard	EDL	
						pg/ml in urine	pg/m^3 of air
LMW PAHs	42	Pyrene	Pyr	[$^2H_{10}$]-Pyr	[$^2H_{10}$]-Flt	22.6	19.3
	43	Retene	Ret	[$^2H_{10}$]-Pyr	[$^2H_{10}$]-Flt	60.5	51.8
	44	Benzo(a)anthracene	BaA	[$^2H_{12}$]-TriPh	[$^2H_{10}$]-Flt	27.9	23.9
	45	Chrysene	Chr	[$^2H_{12}$]-TriPh	[$^2H_{10}$]-Flt	19.7	16.9
	46	Triphenylene	TriPh	[$^2H_{12}$]-TriPh	[$^2H_{10}$]-Flt	19.7	16.9
	47	Benzo(b)fluoranthene	BbFlt	[$^2H_{12}$]-BaP	[$^2H_{12}$]-BkFlt	52.1	44.6
	48	Benzo(k)fluoranthene	BkFlt	[$^2H_{12}$]-BaP	[$^2H_{12}$]-BkFlt	56.9	48.8
	49	Benzo(e)pyrene	BeP	[$^2H_{12}$]-BaP	[$^2H_{12}$]-BkFlt	72.5	62.2
	50	Benzo(a)pyrene	BaP	[$^2H_{12}$]-BaP	[$^2H_{12}$]-BkFlt	89.7	76.9
	51	Indeno(1,2,3-cd)pyrene	I(1,2,3-cd)Pyr	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt	36.1	30.9
	52	Dibenz(a,h)anthracene	BahA	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt	43.7	37.5
	53	Benzo(ghi)perylene	BghiPer	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt	29.7	25.5
HMW PAHs	54	Picene	Pic	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt		142.9
	55	Naphtho[1,2-b]fluoranthene	N12bF	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt		142.9
	56	Naphtho[2,3-j]fluoranthene	N23jF	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt		285.7
	57	Naphtho[2,3-b]fluoranthene	N23bF	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt		285.7
	58+ 59	Dibenzo[a,e]fluoranthene+ Dibenzo[b,k]fluoranthene	DBaeF+ DBbkF	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt		142.9
	60	Dibenzo[j,l]fluoranthene	DBjlF	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt		142.9
	61	Dibenzo[a,l]pyrene	DBalP	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt		142.9
	62	Naphtho[2,3-e]pyrene	N23eP	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt		142.9
	63	Dibenzo[a,e]pyrene	DBaeP	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt		285.7

Appendix B.2 (Continued)

Class	#	Analyte	Abbreviation	Surrogate	Internal Standard	EDL	
						pg/ml in urine	pg/m^3 of air
HMW PAHs	64	Coronene	Cor	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt		142.9
	65	Dibenzo[e,l]pyrene	DBelP	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt		285.7
	66	Benzo[b]perylene	BbPer	[$^2H_{12}$]- BghiPer	[$^2H_{12}$]-BkFlt		285.7
Oxy-PAHs	67	9-fluorenone		[$^2H_8$]- 1,4-NQn	[$^2H_8$]- 9-Flon		0.33
	68	9,10-anthraquinone		[$^2H_8$]- 9,10-AntQn	[$^2H_8$]- 9-Flon		0.42
	69	2-methyl-9.10-anthraquinone		[$^2H_8$]- 9,10-AntQn	[$^2H_8$]- 9-Flon		1.4
	70	benzanthrone		[$^2H_8$]- 9,10-AntQn	[$^2H_8$]- 9-Flon		0.56
	71	benz[a]anthracene-7,12-dione		[$^2H_8$]- 9,10-AntQn	[$^2H_8$]- 9-Flon		0.15

Appendix B.3 Selected Ion Monitoring (SIM) windows and m/z ions monitored.
The surrogates for OH-PAHs and PAHs are in bold and IS indicates internal standards

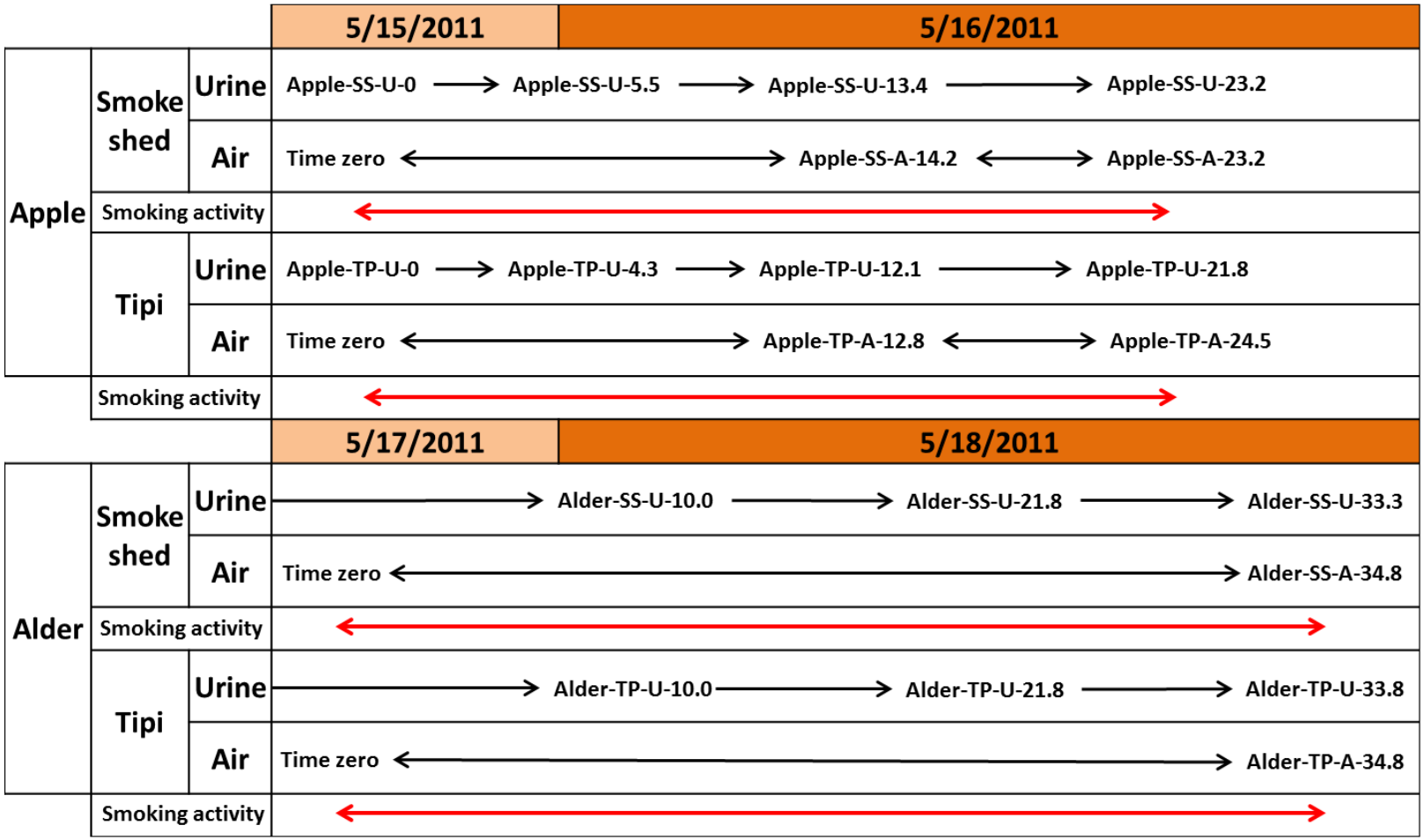
Window	Retention times, min	#	OH-PAH or PAH	MW	m/z 1	m/z 2	m/z 3	m/z 4
1	9.8-11.0	1	Naphthalene	128	128	127		
3	16.0-24.0	2	Acenaphthylene	152	152	151	76	
		3	**Acenaphthene-d10-IS**	164	164	162		
		4	Acenaphthene	154	154	153	152	
		5	**Fluorene-d10**	176	176	174		
		6	Fluorene	166	166	165	163	
4	24.0-26.50	7	1-Hydroxynaphthalene	144	258	201	185	
		8	**1-Hydroxy[2H7]naphthalene**	151	265	208	192	
		9	2-Hydroxynaphthalene	144	258	201	185	
		10	**2-Hydroxy[2H7]naphthalene**	151	265	208	192	
		10	**Phenanthrene-d10**	188	188	189		
		11	Phenanthrene	178	178	176	179	
		12	Anthracene	178	178	176	179	
6	31.0-35.5	13	9-Hydroxyfluorene	182	296	239		165
		14	**Fluoranthene-d10-IS**	212	212	213		
		15	Fluoranthene	202	202	200	203	
		16	**Pyrene-d10**	212	212	213		
		17	Pyrene	202	202	203	200	
7	35.5-37.5	18	3-Hydroxyfluorene	182	296	239		165
		19	Retene	234	219	234	204	
		20	**2-hydroxy[2H9]fluorene**	191	305	248		174
		21	2-hydroxyfluorene	182	296	239		165
8	37.5-39.7	22	2,3-Dihydroxynaphthalene	160	388	331		273
		23	1,3-Dihydroxynaphthalene	160	388	331		273
		24	4-Hydroxyphenanthrene	194	308	251	235	
		25	**4-hydroxy[13C6]phenanthrene**	200	255	239	312	
		26	1-hydroxy-9-fluorenone	196		253		223

Appendix B.3 (Continued)

Window	Retention times, min	#	OH-PAH or PAH	MW	m/z 1	m/z 2	m/z 3	m/z 4
9	39.7-41.5	27	1,5-Dihydroxynaphthalene	160	388	331		273
		28	1,6-Dihydroxynaphthalene	160	388	331		275
		29	2-hydroxy-9-fluorenone	196	310	253		
		30	3-Hydroxyphenanthrene	194	308	251	235	
		31	2,7-Dihydroxynaphthalene	160	388	331		275
		32	2,6-Dihydroxynaphthalene	160	388	331		275
		33	1-Hydroxyphenanthrene	194	308	251	235	
		34	2-Hydroxyphenanthrene	194	308	251	235	
10	41.5-44.5	35	**Triphenylene-d12**	240	240	241		
		36	Benzo(a)anthracene	228	228	226	229	
		37	Chrysene+triphenylene	228	228	226	229	
11	44.5-48.5	38	2-Hydroxyanthraquinone	224	338	281		253
		39	**3-hydroxy[13C6]flouranthene**	224	338	281	265	221
		40	3-Hydroxyfluoranthene	218	332	275	259	189
		41	**1-hydroxy[13C6]pyrene**	224	338	281	265	250
		42	1-Hydroxypyrene	218	332	275	259	
12	48.5-52.5	43	Benzo(b)fluoranthene	252	252	250	253	
		44	**Benzo(k)fluoranthene-d12-IS**	264	264	265		
		45	Benzo(k)fluoranthene	252	252	250	253	
		46	Benz(e)pyrene	252	252	250	253	
		47	**Benzo(a)pyrene-d12**	264	264	265	263	
		48	Benzo(a)pyrene	252	252	250	253	
13	52.5-58.00	49	**1-hydroxy[13C6]benzo(a)anthracene**	250	364	307	291	276
		50	4-Hydroxychrysene	244	358	301	285	270
		51	6-Hydroxychrysene	244	358	301	285	270
		52	2-hydroxybenzo(a)anthracene	244	358	301	285	
		53	3-Hydroxybenzo(c)phenanthrene	244	358	301	285	
		54	**3-Hydroxy[13C6]benzo(c)phenanthrene**	250	364	307	291	

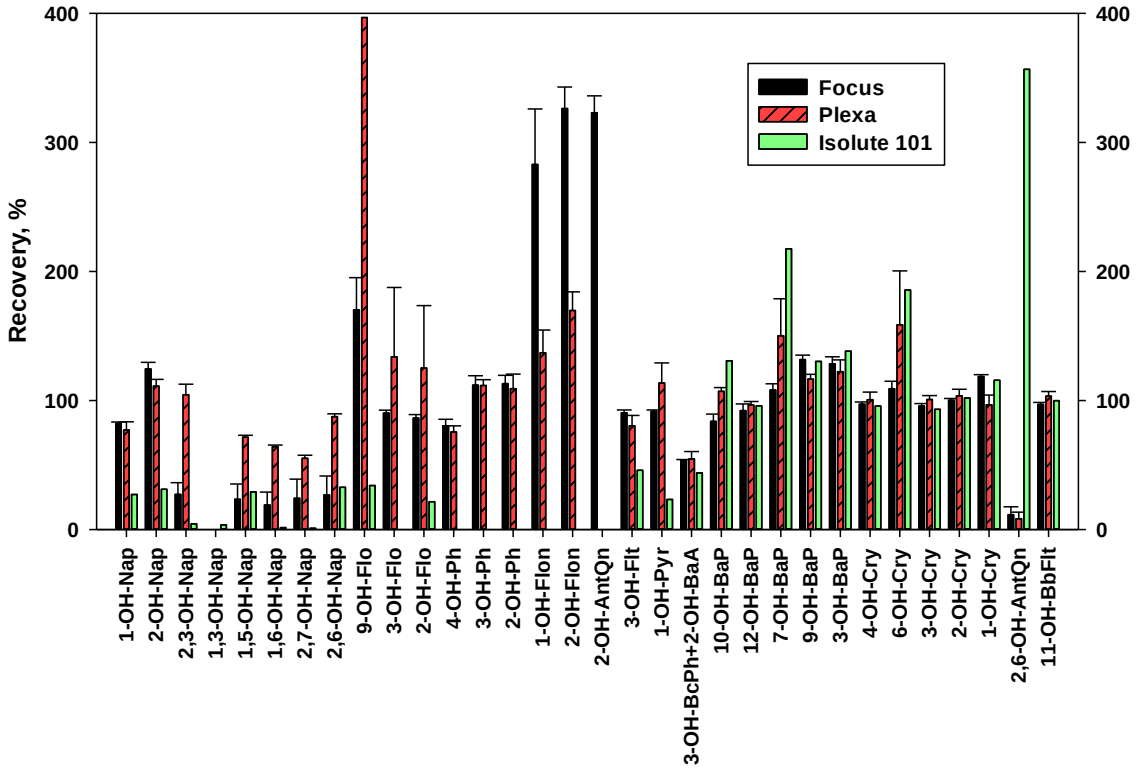
Appendix B.3 (Continued)

Window	Retention times, min	#	OH-PAH or PAH	MW	m/z 1	m/z 2	m/z 3	m/z 4
13	52.5-58.00	55	3-Hydroxychrysene	244	358	301	285	
		56	**3-hydroxy[13C6]chrysene**	250	364	307	291	
		57	1-Hydroxychrysene	244	358	301	285	
		58	2-Hydroxychrysene	244	358	301	285	
14	58.0-61.5	59	Indeno(1,2,3-cd)pyrene	276	276	274	277	
		60	Dibenz(a,h)anthracene	278	278	276	279	
		61	**Benzo(ghi)perylene-d12**	288	288	289		
		62	Benzo(ghi)perylene	276	276	274	277	
15	61.5-67.2	63	2,6-Dihydroxyanthraquinone	240	468	411	395	
		64	10-hydroxybenzo(a)pyrene	268	382	325	309	
		65	12-hydroxybenzo(a)pyrene	268	382	325	309	
		66	11-hydroxybenzo(b)fluoranthrene	268	382	325	309	
		67	7-Hydroxybenzo(a)pyrene	268	382	325	309	
		68	9-Hydroxybenzo(a)pyrene	268	382	325	309	
		69	3-Hydroxybenzo(a)pyrene	268	382	325	309	

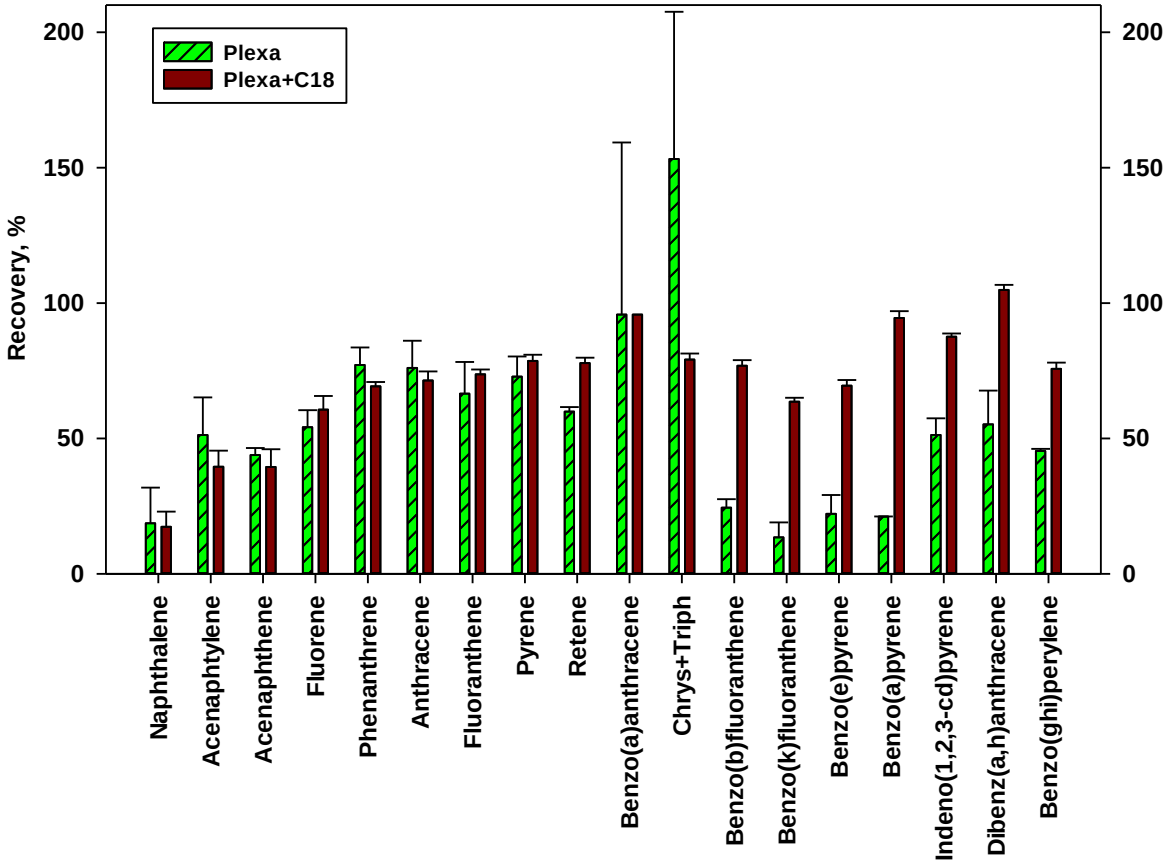


Appendix B.4 Experimental timeline for the collection of urine and personal $PM_{2.5}$ samples.
SS – smoke shed; TP – tipi; U – urine sample; A – air sample; the number at the end of sample name represents the length of exposure, in hours; red arrow shows the duration of fish smoking activity.

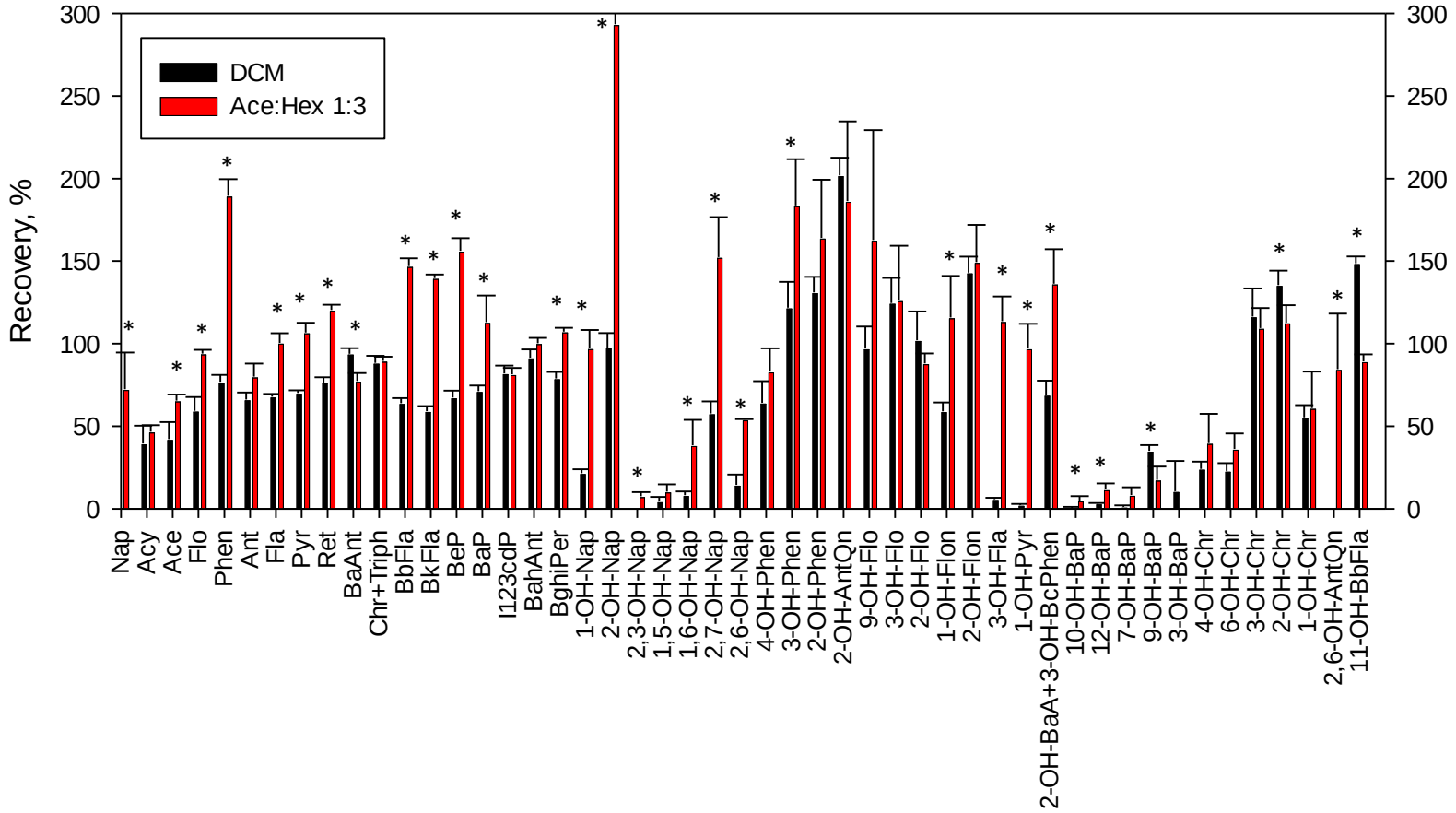
Appendix B.5 Tipi (upper left) and smoke shed (upper right), and fish positioning inside (lower left and lower right, respectively)



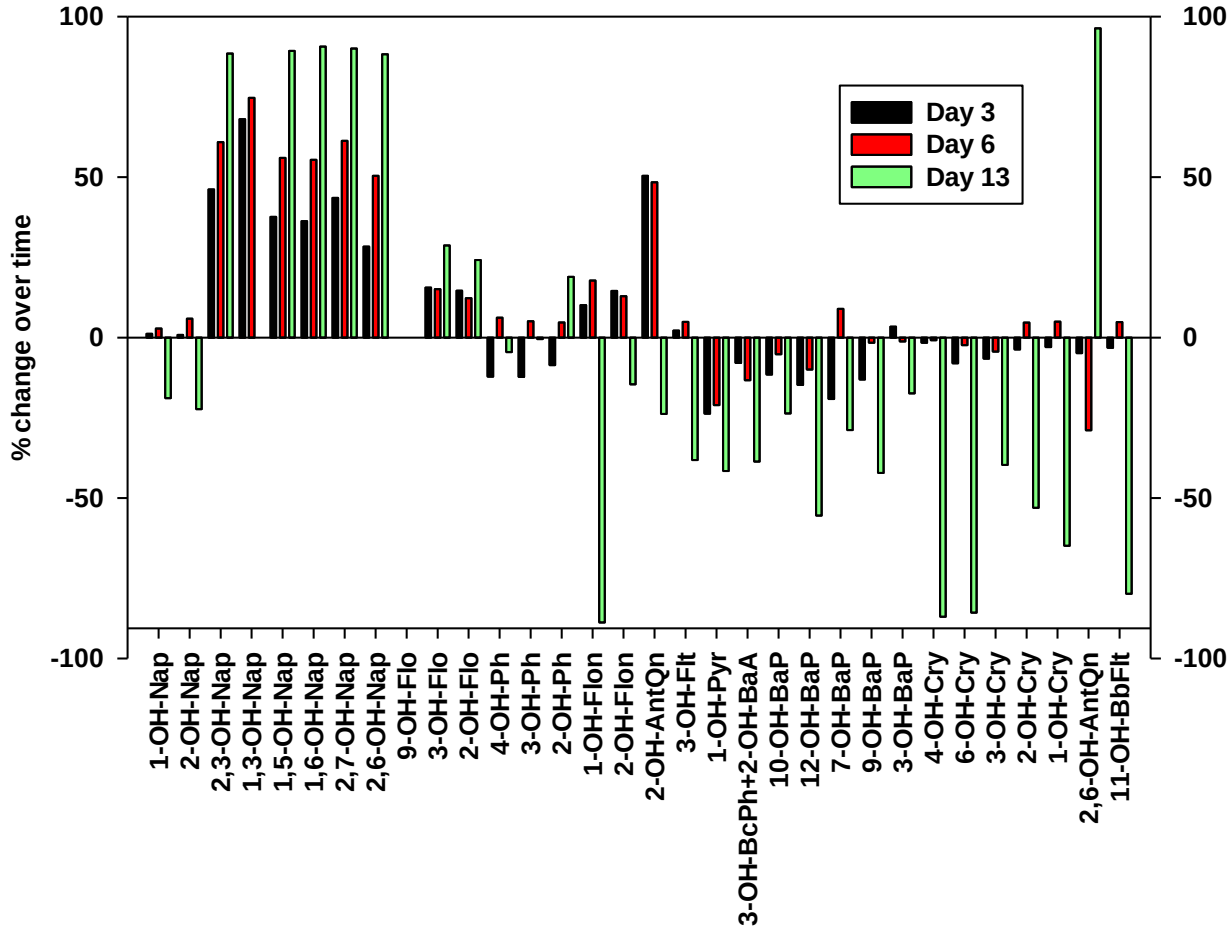
Appendix B.6 Mean OH-PAH recoveries from spiked UTAK urine using different SPE. Three independent extractions, error bars represent standard deviation.



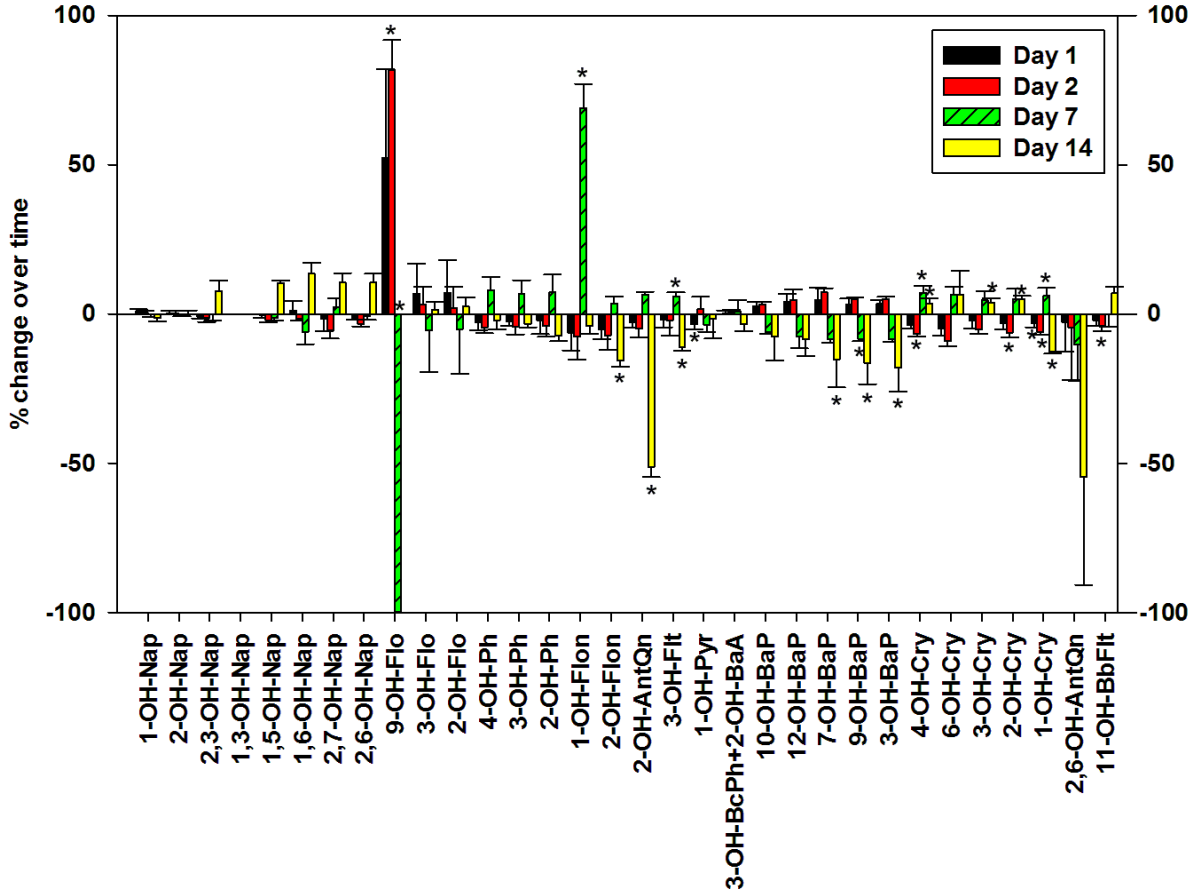
Appendix B.7 Mean PAH recoveries for the entire analytical method using spiked UTAK urine and different SPE phases.
Three independent extractions, error bars represent standard deviation.



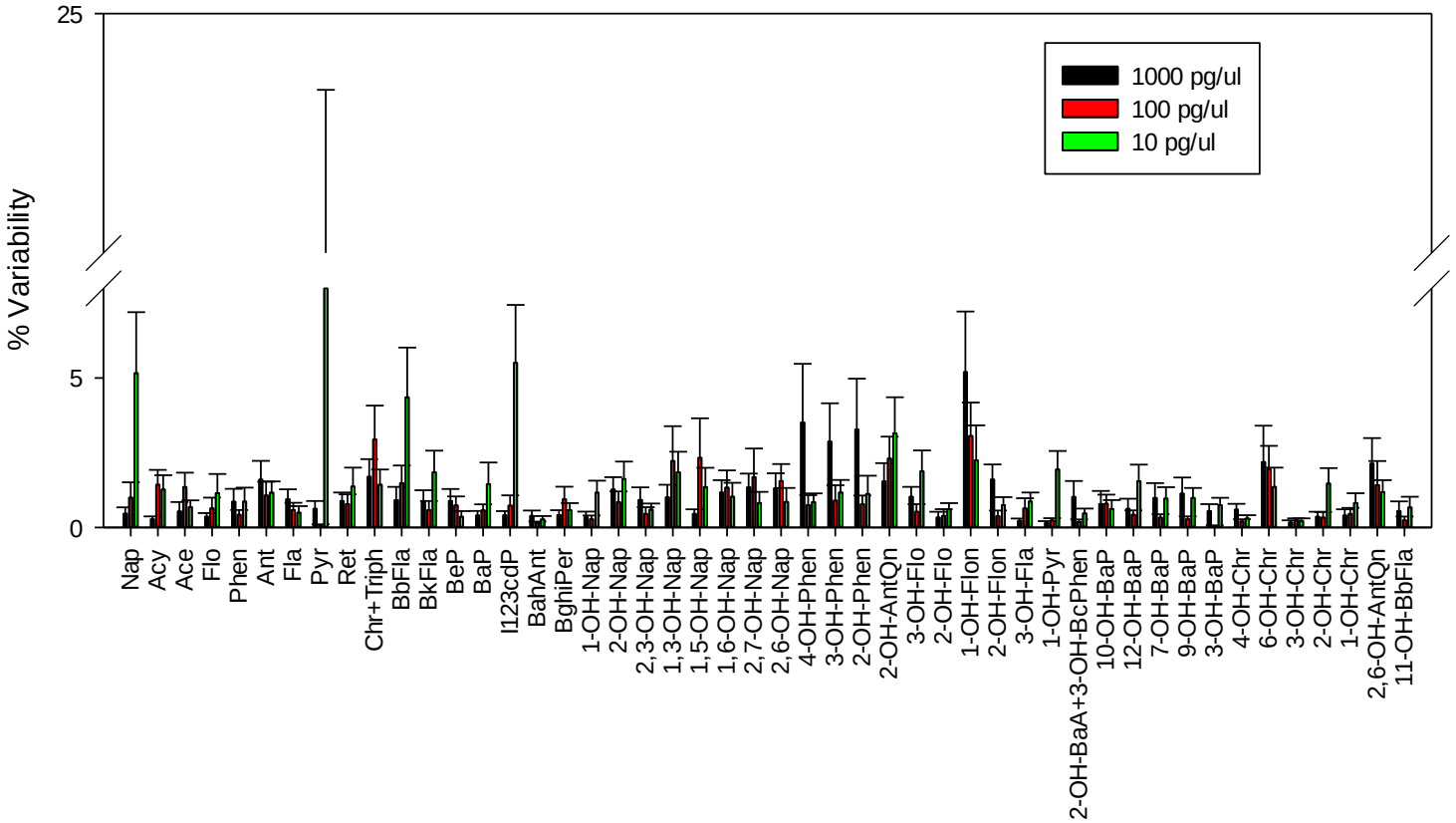
Appendix B.8 Mean PLE recoveries for the extraction of PAH and OH-PAH from $PM_{2.5}$.
Three independent extractions, error bars represent standard deviation. * - statistically different (p-value<0.05)



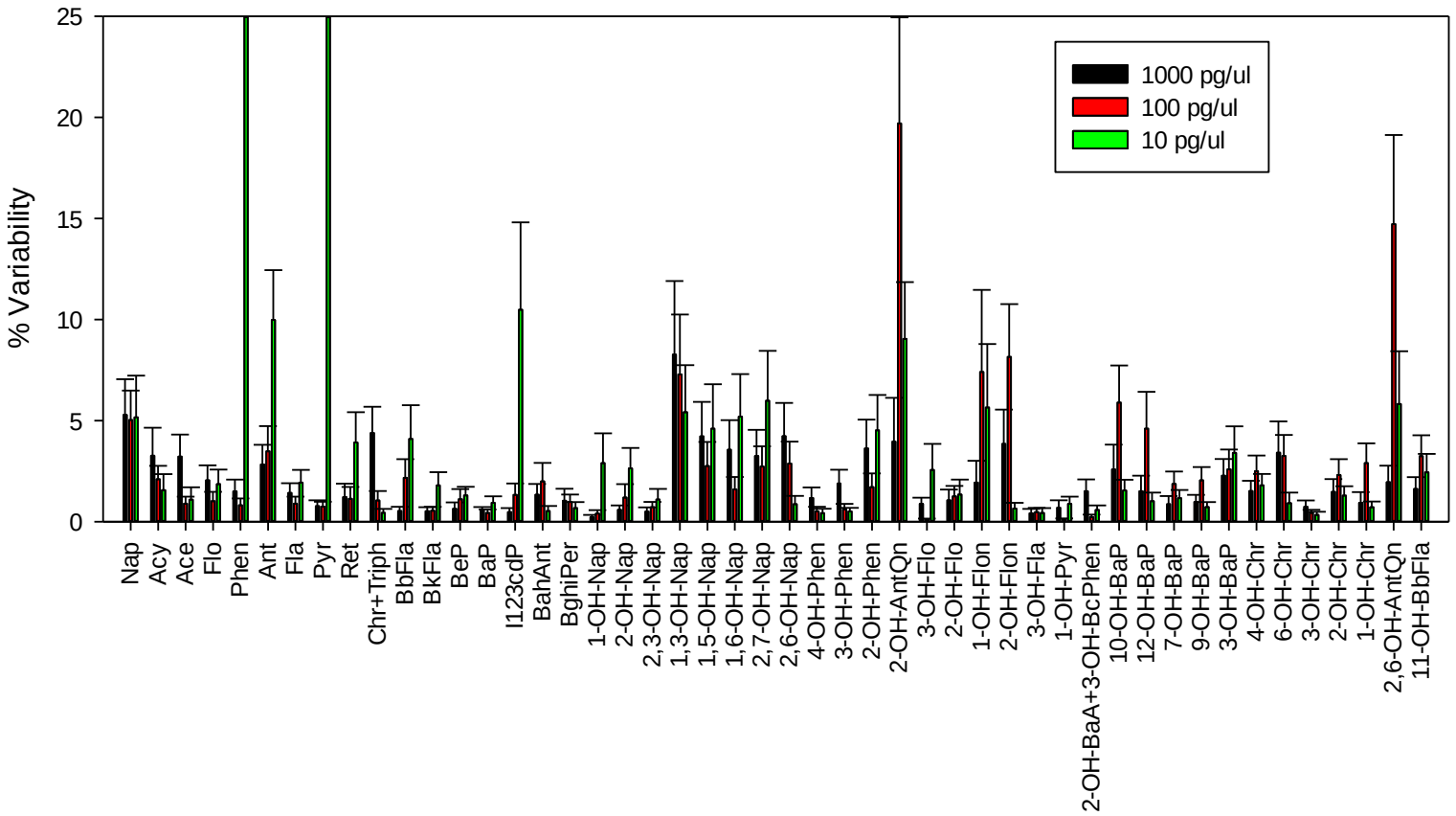
Appendix B.9 Stability of spiked OH-PAHs in frozen UTAK urine.
One extraction, storage temperature -20ºC.



Appendix B.10 Mean stability of the products of OH-PAH and MTBSTFA derivatization in the extract (frozen at -20ºC).
Three independent extractions, error bars represent standard deviation. * - statistically (p-value<0.05) different from Day 1.



Appendix B.11 Mean intra-day variability of the Parent and OH-PAH method for urine.
Three independent extractions, error bars represent standard deviation. The solutions of three concentrations (10, 100, and 1000 pg/ul) were prepared and analyzed three times during 24 hours.

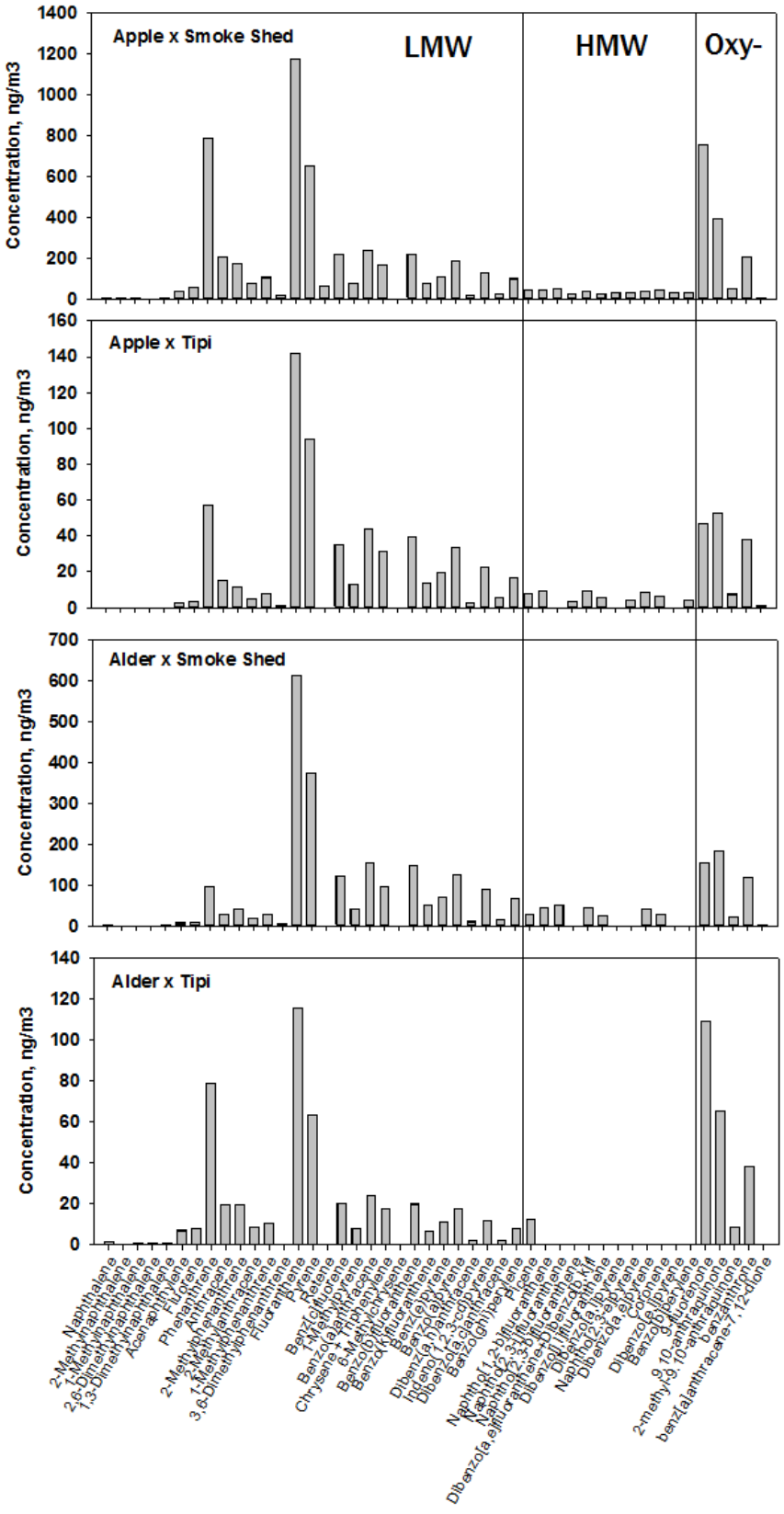


Appendix B.12 Mean inter-day variability of the OH-PAH method for urine.
Three independent extractions, error bars represent standard deviation. The solutions of three concentrations (10, 100, and 1000 pg/ul) were prepared and analyzed three times over three consecutive days.

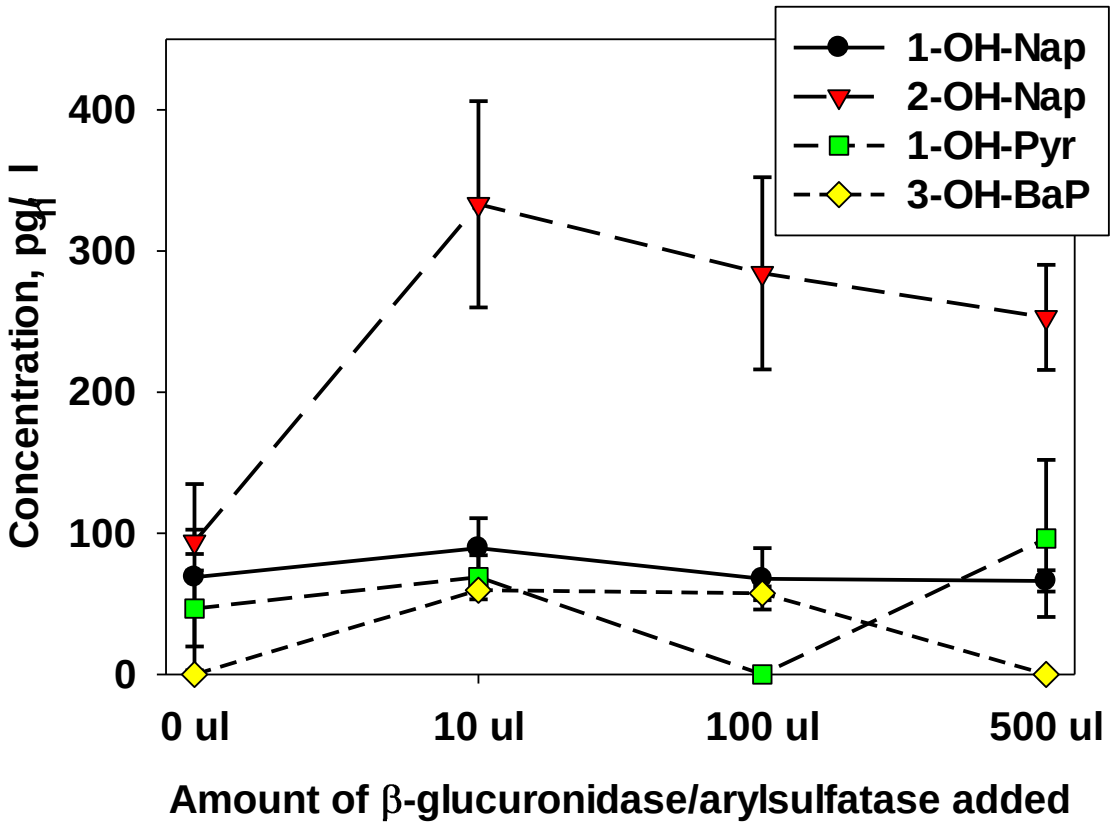
Appendix B.13 Analysis of NIST SRM samples.
NM – not measured; NA – not available; * - Surrogate was available for this compound

SRM 3672. Organic contaminants in smoker's urine	Our Results, ng/L		NIST Results, ng/L		
	Concentration	Standard Deviation	Concentration	Standard Deviation	% diff
1-Hydroxynaphthalene*	25630	949	34442	4382	-26%
2-Hydroxynaphthalene	11949	1127	8733	163	37%
1,5-Dihydroxynaphthalene	668	367	NM	NM	NA
1,6-Dihydroxynaphthalene	302	15	NM	NM	NA
9-Hydroxyphenanthrene	NM	NM	977	62	NA
4-Hydroxyphenanthrene*	82	68	49	5	67%
3-Hydroxyphenanthrene	195	59	125	7	56%
2-Hydroxyphenanthrene	252	40	84	1	76%
1-Hydroxyphenanthrene	148	24	136	14	86%
9-Hydroxyfluorene	2384	1102	337	78	607%
3-Hydroxyfluorene	530	61	428	18	24%
2-Hydroxyfluorene*	892	67	870	15	3%
1-Hydroxypyrene*	211	36	173	10	22%
Phenanthrene	987	128			NA
Fluoranthene	76	33	NM	NM	NA
Pyrene*	111	25	NM	NM	NA
Retene	444	67			NA

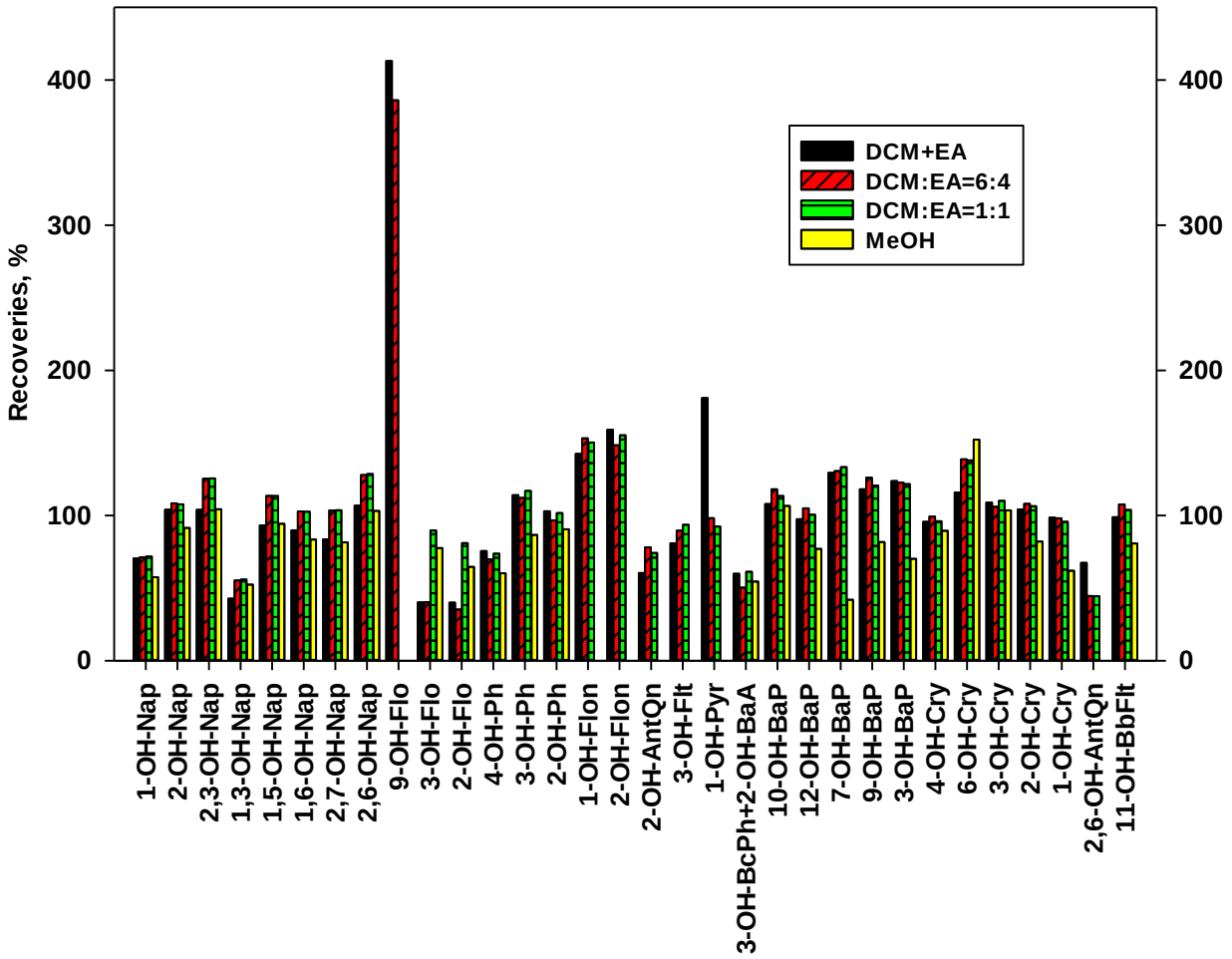
SRM 3673. Organic contaminants in non-smoker's urine	Our Results, ng/L		NIST Results, ng/L		
	Concentration	Standard Deviation	Concentration	Standard Deviation	% diff
1-Hydroxynaphthalene*	150943	10925	210933	33627	-28%
2-Hydroxynaphthalene	2151	305	1345	31	60%
1,5-Dihydroxynaphthalene	1727	231			NA
9-Hydroxyphenanthrene	NM		12	1	NA
4-Hydroxyphenanthrene*	74	62	10	1	611%
3-Hydroxyphenanthrene	74	45	28	1	169%
2-Hydroxyphenanthrene	68	18	25	4	177%
1-Hydroxyphenanthrene	112	7	49	8	129%
9-Hydroxyfluorene	965	865	110	26	776%
3-Hydroxyfluorene	142	43	39	4	264%
2-Hydroxyfluorene*	163	38	107	7	52%
1-Hydroxypyrene*	44	24	30	2	43%
Phenanthrene	851	21			NA
Fluoranthene	99	39	NM	NM	NA
Pyrene*	120	24	NM	NM	NA
Retene	339	64			NA



Appendix B.14 PAH profile from Personal Air Samples

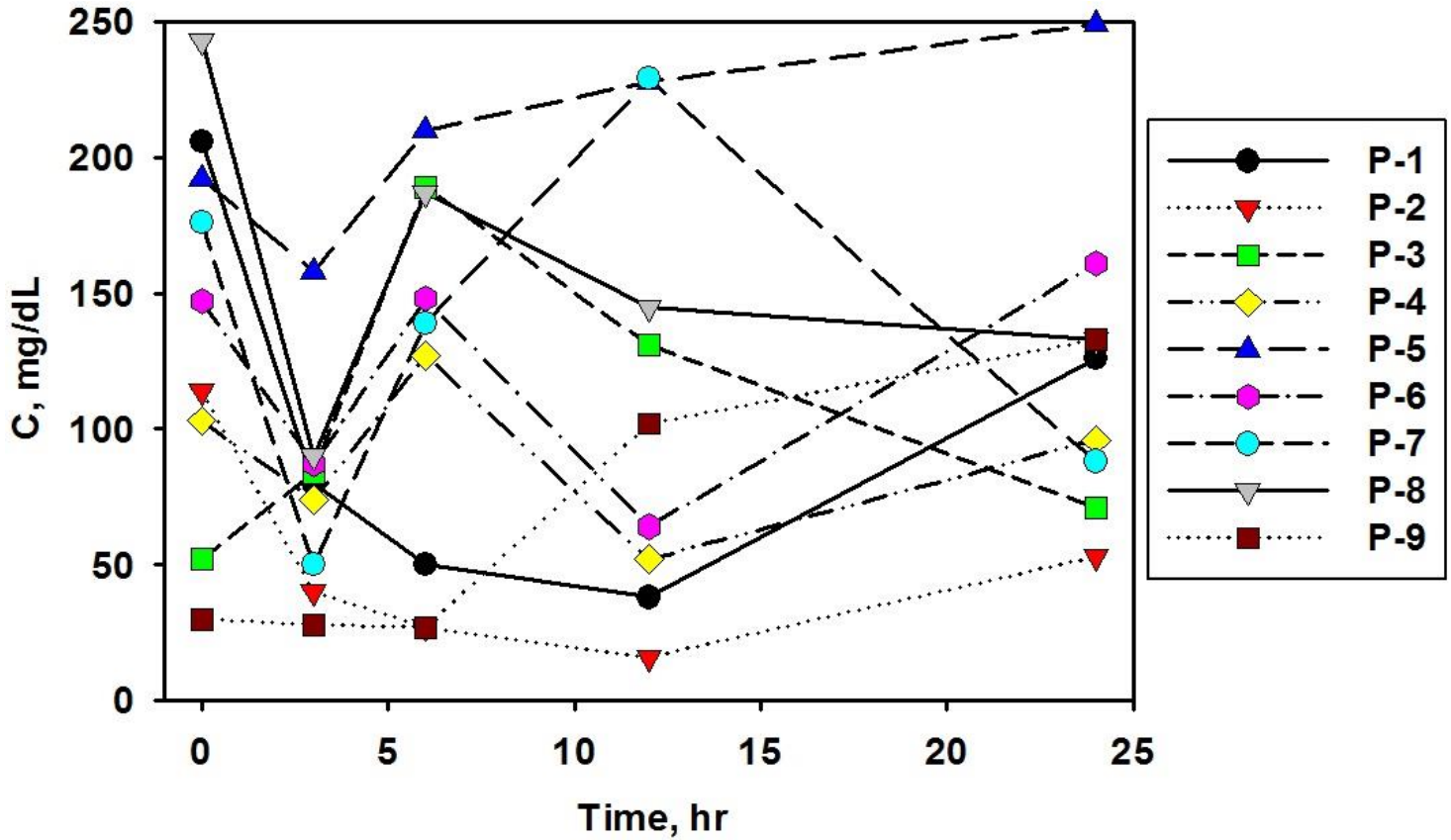


Appendix B.15 Mean concentrations of selected OH-PAHs in urine extracts after addition of different volumes of β-glucuronidase/arylsulfatase.
Three independent extractions, points represent average values, whiskers – standard deviation.

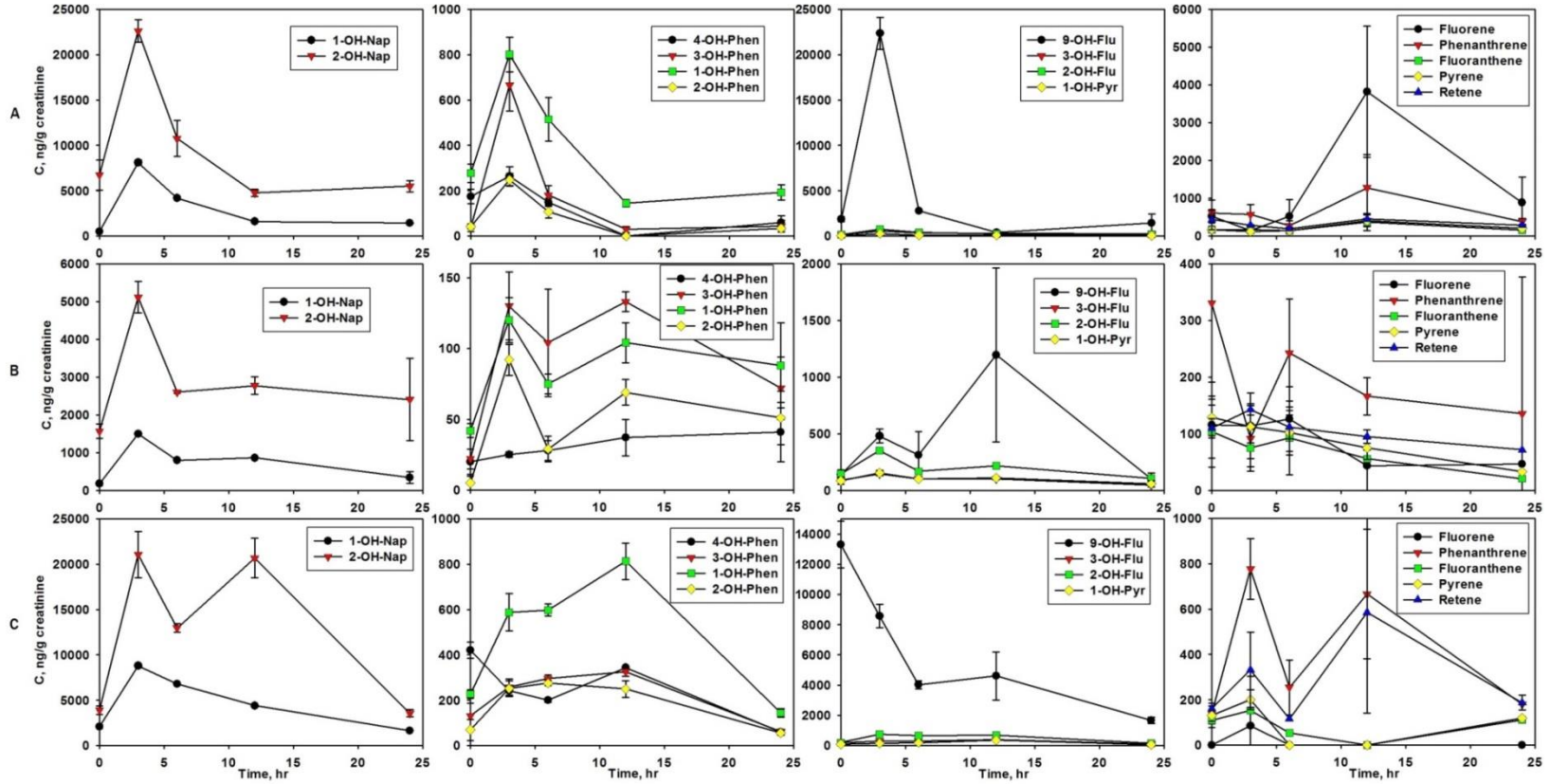


Appendix B.16 OH-PAHs recoveries from standard solution using Focus SPE with different eluent compositions.
One extraction.

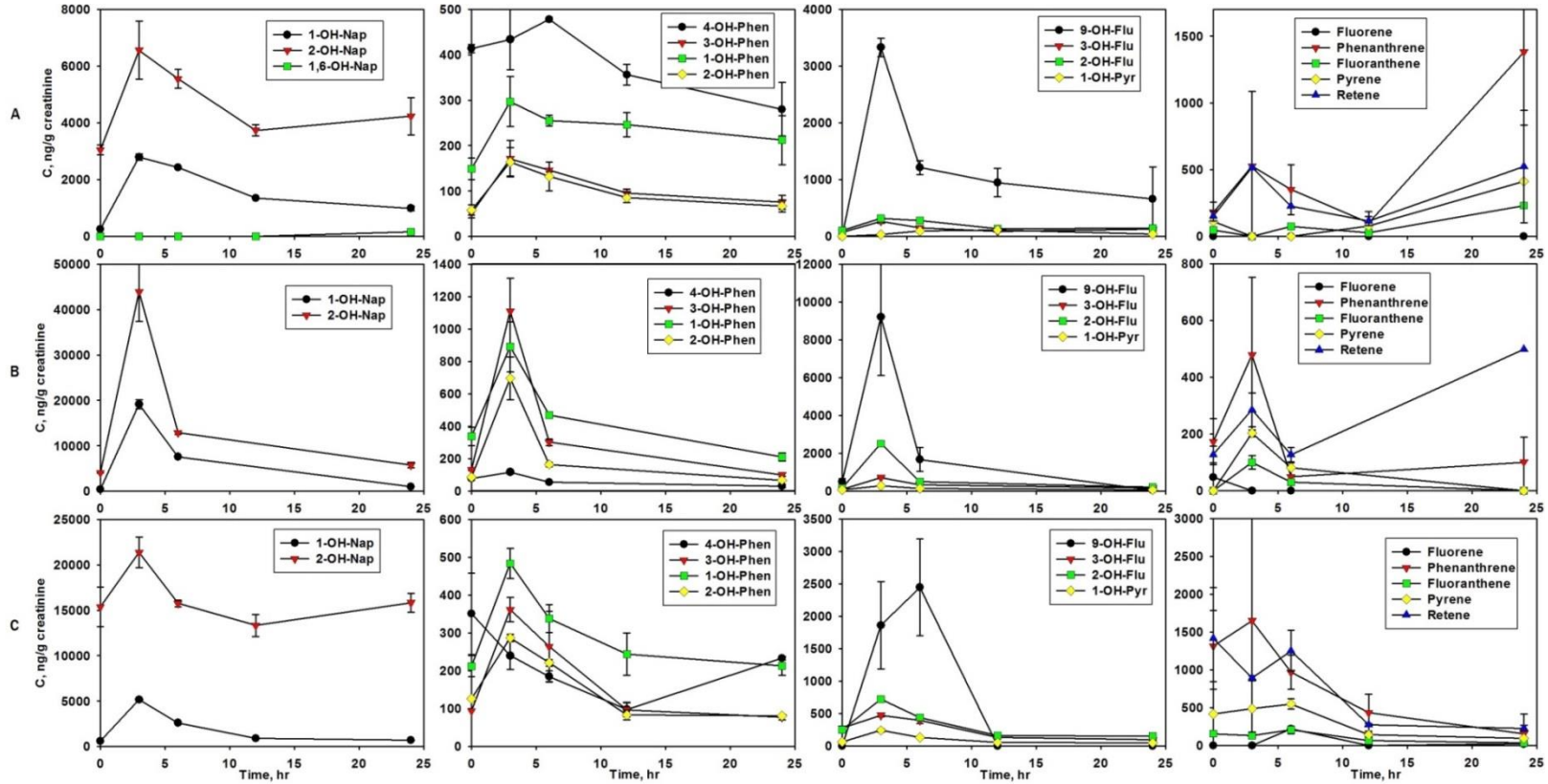
APPENDIX C



Appendix C.1 Creatinine concentration for all participants.
Fish consumption occurred at time zero



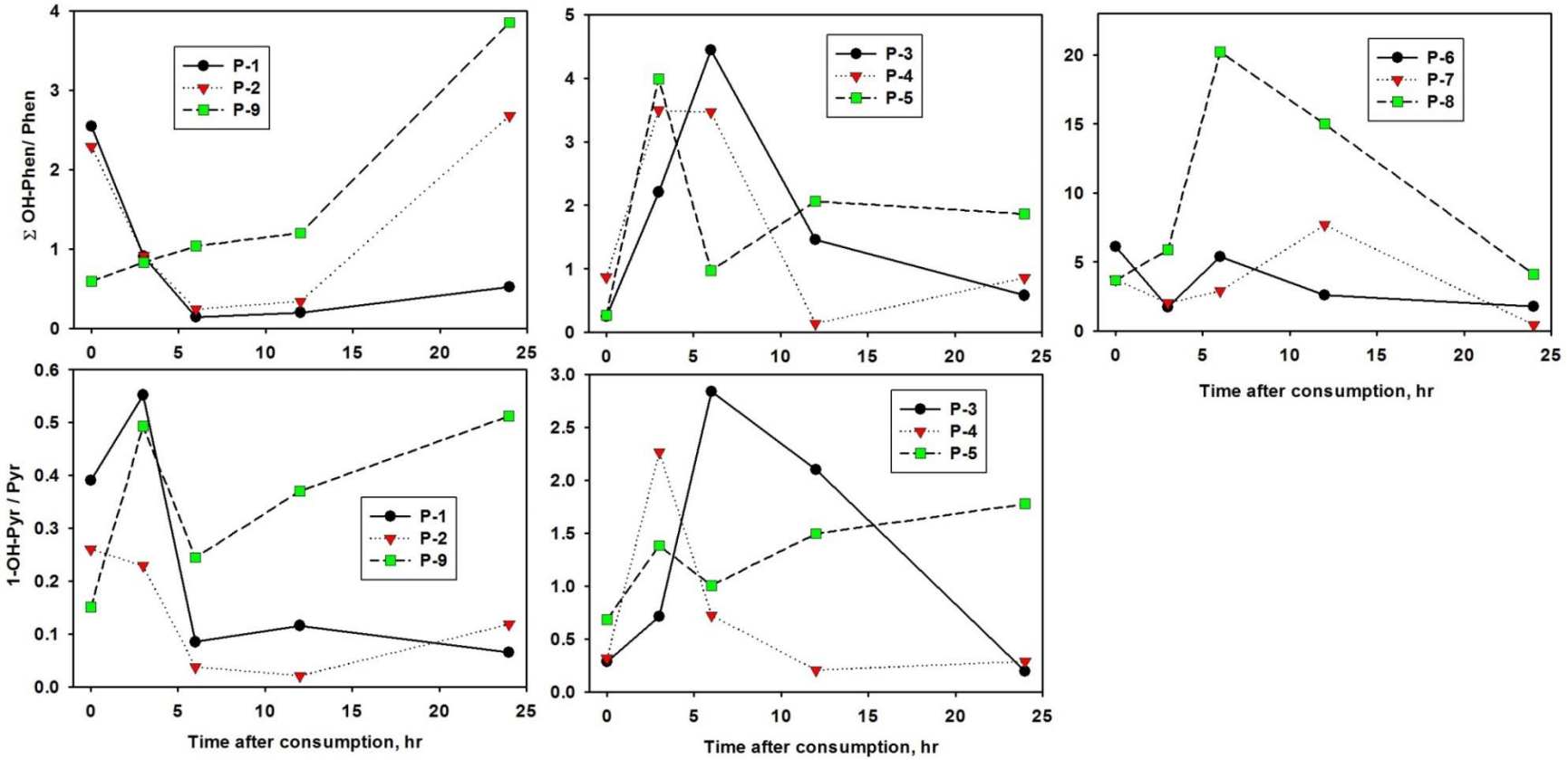
Appendix C.2 The mean creatinine adjusted PAH and OH-PAH concentrations over 24 hr time period.
Three independent extractions, the error bars represent standard deviation. The consumption of fish occurred right at time zero. A) P-4, B) P-5, C) P-6.



Appendix C.3 The mean creatinine adjusted PAH and OH-PAH concentrations over 24 hr time period.
Three independent extractions, the error bars represent standard deviation. The consumption of fish occurred right at time zero. A) P-7, B) P-8, C) P-9.

Appendix C.4 Median (minimum and maximum) urinary concentrations of PAHs and OH-PAHs
N=5 for all participants.

	Urinary concentration, ng/g creatine								
	P-1	P-2	P-3	P-4	P-5	P-6	P-7	P-8	P-9
1-OH-Nap	2859 (1457-7257)	2620 (415-3934)	1839 (417-4119)	1613 (483-8106)	801 (182-1495)	4391 (1623-8793)	1345 (247-2790)	4282 (399-19113)	898 (595-5150)
2-OH-Nap	8492 (4676-19638)	12709 (12192-19435)	10828 (6045-21597)	6724 (4777-22604)	2606 (1572-5116)	12976 (3560-21068)	4231 (3044-6563)	9309 (4077-43952)	15768 (13324-21381)
4-OH-Phen	112 (44-212)	360 (111-584)	13 (2-24)	162 (60-264)	28 (20-41)	242 (59-420)	414 (280-478)	67 (32-118)	233 (98-351)
3-OH-Phen	109 (26-289)	56 (17-171)	105 (24-407)	43 (29-667)	104 (22-133)	258 (62-326)	95 (51-171)	217 (101-1110)	97 (77-362)
1-OH-Phen	237 (153-423)	297 (164-485)	198 (117-378)	277 (145-801)	88 (42-120)	588 (144-813)	246 (149-297)	404 (211-891)	244 (212-484)
2-OH-Phen	33 (16-175)	38 (24-95)	124 (95-236)	74 (34-248)	51 (5-92)	249 (56-275)	85 (58-164)	125 (68-696)	126 (81-287)
9-OH-Flu	188 (2-3643)	904 (522-2332)	1508 (22-3401)	1852 (386-22356)	314 (103-1197)	4608 (1672-13311)	1079 (658-3334)	1089 (57-9208)	2155 (1863-2447)
3-OH-Flu	357 (97-401)	312 (125-618)	190 (165-221)	305 (152-533)	102 (47-147)	298 (96-380)	124 (75-265)	250 (89-719)	279 (96-474)
2-OH-Flu	302 (107-678)	393 (178-547)	236 (207-441)	240 (141-727)	169 (109-354)	663 (143-742)	142 (104-321)	364 (176-2511)	253 (152-724)
1-OH-Pyr	137 (42-190)	48 (13-72)	107 (40-205)	83 (54-270)	102 (58-156)	146 (61-349)	67 (31-109)	105 (65-291)	63 (48-240)
Flu	704 (74-1309)	1179 (318-2082)	493 (409-649)	524 (138-3823)	114 (44-126)	85 (85-85)	ND	48 (48-48)	119 (21-218)
Phen	2045 (503-6145)	2795 (1020-5985)	974 (588-1849)	1258 (799-2558)	514 (403-678)	705 (592-1708)	829 (393-2139)	525 (406-1220)	3439 (658-4031)
Flt	719 (99-3233)	358 (215-1036)	152 (73-306)	162 (139-366)	75 (21-104)	110 (54-152)	61 (28-231)	65 (30-100)	128 (35-206)
Pyr	965 (108-1758)	314 (212-1258)	150 (69-262)	170 (119-402)	102 (33-129)	131 (119-200)	112 (76-412)	142 (81-203)	416 (94-550)
Ret	719 (147-2366)	1248 (510-3583)	332 (153-496)	290 (192-446)	110 (72-143)	187 (116-585)	226 (115-523)	207 (127-499)	892 (228-1418)



Appendix C.5 The ratio of the sum of four hydroxy-phenanthrenes to phenanthrene concentrations (first row) for 9 participants and the ratio of 1-hydroxypyrene to pyrene concentrations (second row) for 6 participants.
The fish consumption occurred at time zero.