

Evaluation of Exposures to Diesel Particulate Matter Utilizing Ambient
Air Monitoring and Urinary Biomarkers Among Pedestrian Commuters
who Cross the U.S.-Mexico Border at San Ysidro, CA.

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Abstract

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Background: Walk-in-line pedestrians crossing the U.S.-Mexico border northbound at the San Ysidro Port of Entry (SYPOE), referred to as “Border Commuters,” may be at an increased risk of experiencing elevated traffic-related air pollution, including diesel exhaust (DE). DE exposure has been associated with numerous adverse health effects, particularly cardiovascular and respiratory problems, including lung cancer. “Border Commuters” wait in line for extended periods and stand within 10 feet of highly concentrated traffic, particularly to diesel buses. Understanding the magnitude of traffic-related exposures is important for this vulnerable population. It was hypothesized that “Border Commuters” who reside in Tijuana, Baja California, Mexico and cross SYPOE northbound as a pedestrian will experience higher exposure to traffic-related pollutants than “Non-Border Commuters” defined as those who live

and work or go to school in or near San Ysidro, California, U.S.A. and do not cross into Mexico.

Methods: Ninety-one participants were enrolled for this study; 80% were “Border Commuters” and 20% were “Non-Border Commuters.” Questionnaires, time activity diaries, and urine samples were collected from all participants. Of the “Border Commuters”, 56 personal 24-hour $PM_{2.5}$, 1-nitropyrene (1-NP) - a marker for diesel exhaust – and carbon monoxide (CO) samples were collected. There were 22 at-home indoor and 14 at-home outdoor 1-NP samples collected. Additionally, area samples collected at the border included 35 days of 1-NP, black carbon (BC), CO, fine particulate matter ($PM_{2.5}$) and ultrafine particulate matter (UFP). Of the “Non-Border Commuters”, 15 personal 24-hour $PM_{2.5}$, 1-NP, and CO samples were collected. Additionally, 3 at-home indoor and outdoor 24-hour 1-NP samples were collected. **Results:** Personal exposure to $PM_{2.5}$ was nearly 2-fold higher among “Border Commuters” compared to “Non-Border Commuters” (39 ± 30 vs 21 ± 11 $\mu\text{g}/\text{m}^3$, $p < 0.01$ Mann-Whitney), while personal exposure to 1-NP was more than 8-fold higher among the “Border Commuters” (1.7 ± 2.3 vs 0.22 ± 0.21 pg/m^3 , $p < 0.01$ Mann-Whitney). “Border Commuters” had a 3-fold increase exposure to CO than “Non-Border Commuters” (2.8 ± 1.8 vs 0.22 ± 0.21 ppm, $p < 0.01$ Mann-Whitney). Two metabolites of 1-NP were readily detected in urine samples, the most abundant of which was 8-hydroxy-1-nitropyrene (8-OHNP) followed by 8-hydroxy-N-acetyl-1-aminopyrene (8-OHNAAP). “Border Commuters” had greater than a 2-fold higher concentration of 8-OHNP (0.071 ± 0.066 vs 0.032 ± 0.021 pg/mL , $p = 0.05$ Mann-Whitney) and a 3-fold higher concentration of 8-OHNAAP (0.063 ± 0.11 vs 0.021 ± 0.013 pg/mL , $p = 0.11$ Mann-Whitney) as compared to “Non-Border Commuters”. Home indoor concentrations of 1-NP were 30-60% of home outdoor concentrations with “Border Commuters” having higher concentrations both

indoors (0.64 ± 0.81 vs 0.078 ± 0.075 pg/m^3 , $p=0.04$ Mann-Whitney) and outdoors (1.0 ± 0.93 vs 0.27 ± 0.24 pg/m^3 , $p=0.11$ Mann-Whitney) compared to “Non-Border Commuters”. Border concentrations of 1-NP weighted by the time spent at the border, total travel given season, and season were all predictors of personal exposure to 1-NP among “Border Commuters”. However, when placed in a multivariate linear regression model total travel given season was the only predictor variable to remain significant. Season was the only predictor for personal exposure to $\text{PM}_{2.5}$ while total travel was the only predictor for 8-OHNP among “Border Commuters.”

Median values (interquartile range; IQR) of daily averages for fixed-site measurements made at the border were as follows: 40,000 (24,000-52,000) UFP/cm^3 , 5 (3-6) ppm CO, 1.3 (0.5-2.6) pg/m^3 1-NP, 4 (3-11) $\mu\text{g}/\text{m}^3$ BC, 41 (23-57) $\mu\text{g}/\text{m}^3$ real-time $\text{PM}_{2.5}$, and 15 (13-22) $\mu\text{g}/\text{m}^3$ gravimetric $\text{PM}_{2.5}$. Wind speed was a predictor of gravimetric $\text{PM}_{2.5}$ at the border explaining 22% of the variance. Relative humidity and vehicle delay were both predictors of UFP measured at the border, explaining 13% and 21% of the variance, respectively. However, when modeled together none remained significant. There were no predictors for 1-NP measurements at the border. **Conclusions:** This is the first quantitative study characterizing traffic-related exposure to a vulnerable population, indicating that this vulnerable population is indeed at high risk for exposure. “Border Commuters” experience higher exposure to 1-NP and $\text{PM}_{2.5}$ as compared to “Non-Border Commuters”, as determined by both personal and at-home measurements. In addition, traffic-related air pollution exposure among “Border Commuters” within 10 feet of highly concentrated traffic is of great public health concern as concentrations at the border are similar to near-roadway studies that link exposure to adverse health effects. Interventions to reduce border wait times would significantly reduce traffic pollutant exposures in this vulnerable

population. However, further work needs to be done to understand the spatial heterogeneity of at-home exposures between the two study groups.

TABLE OF CONTENTS

Chapter 1: Introduction and Literature Review	4
1.1 U.S.-Mexico Binational Border Environmental Collaboration	4
1.2 U.S. and Mexico Air Pollutant Particulate Matter Standards	5
1.3 Sources and Health Effects of Particulate Matter	6
1.4 Diesel Exhaust	7
1.5 Health Effects of Diesel Exhaust	8
1.6 Previous and Current Markers for Diesel Particulate Matter.....	9
1.7 1-Nitropyrene as a Marker for Diesel Particulate Matter	10
1.8 Metabolism and Toxicological Mechanism of 1-Nitropyrene.....	15
1.9 Conclusion	16
1.10 Specific Aims.....	19
1.11 Research Design and Methods.....	21
1.11.1 Statement of Purpose.....	21
1.11.2 Population.....	21
1.11.3 Community Collaboration.....	22
1.11.4 Subject Characteristics and Recruitment.....	22
1.11.5 Informed Consent Process.....	23
1.11.6 Ambient and Personal Monitoring & Biological Sampling	23
1.11.7 Human Subjects.....	25
Chapter 2: Traffic Pollutant Exposures Experienced by Pedestrians Waiting to Enter the U.S. at a Major U.S.-Mexico Border Crossing	26

2.1 Abstract	26
2.2 Introduction.....	27
2.3 Material and Methods	29
2.3.1 Personal Sampling	29
2.3.2 Fixed Site Monitoring at the U.S.-Mexico Border	31
2.3.3 Sample Collection	31
2.3.4 Statistical Analysis	36
2.4 Results.....	38
2.5 Discussion	40
Chapter 3: Determinants of Personal Exposure to 1-Nitropyrene and PM _{2.5}	
in U.S.-Mexico Border Residents	53
3.1 Abstract	53
3.2 Introduction.....	54
3.3 Material and Methods	56
3.3.1 Personal and Fixed Site Sampling.....	56
3.3.2 Statistical Analysis	57
3.4 Results.....	58
3.5 Conclusions.....	59
3.5 Discussion	61
Chapter 4: Urinary Metabolites of 1-Nitropyrene in U.S.-Mexico Border	
Residents Who Frequently Cross the San Ysidro Port of Entry	70
4.1 Abstract	70

4.2 Introduction.....	71
4.3 Material and Methods	73
4.3.1 Study Population	73
4.3.2 Sample Collection	74
4.3.3 Statistical Analysis	75
4.4 Results.....	76
4.5 Discussion.....	79
Chapter 5: Summary	90
References.....	93
Appendixes	103
A. Pictures of Recruitment Ad in Spanish Only	103
B. Recruitment Flyer in English	106
C. Recruitment Flyer in Spanish.....	106
D. Eligibility Script for “Border Commuters” in English	107
E. Eligibility Script for “Border Commuters” in Spanish.....	108
F. Eligibility Script for “Non-Border Commuters” in English.....	109
G. Eligibility Script for “Non-Border Commuters” in Spanish.....	110
H. Questionnaire in English.....	111
I. Questionnaire in Spanish.....	115
J. Time Activity Diary in English.....	119
K. Time Activity Diary in Spanish	120
L. Equipment Information Sheet.....	121

M. “Border Commuters” Crossing Information Sheet	122
N. At Home Area Monitoring Information Sheet.....	123
O. “Border Commuters” Information Sheet About Crossing the Border in English	124
P. “Border Commuters” Information Sheet About Crossing the Border in Spanish.....	125
Q. U.S.-Mexico Border at San Ysidro Area Monitoring Information Sheet.....	126
R. Check-Off List.....	127
S. Instructions for Equipment if “Border Commuters” are Inspected by a U.S. Customs Border Officer.....	129
T. U.S. Customs and Border Protection Letter of Support	130
U. Procedure for Collection of Gravimetric PM2.5.....	131
V. Standard Operating Procedure for Gravimetric Analysis	134
W. Standard Operating Procedure for 1-NP Filter Extraction and Analysis.....	137
X. Template for 1-NP Filter Bench Sheet.....	148
Y. Standard Operating Procedure for Urinary Metabolite Extraction and Analysis	149
Z. Urine Data Sheet.....	165

LIST OF FIGURES

Figure 1.1	U.S.-Mexico Border	4
Figure 1.2	1-Nitropyrene	11
Figure 1.3	Metabolic Pathway of 1-Nitropyrene	14
Figure 1.4	U.S.-Mexico Border at San Ysidro, CA.....	17
Figure 1.5	Pedestrian Commute Northbound at the U.S.-Mexico Border at San Ysidro, CA	18
Figure 2.1	U.S.-Mexico Border at San Ysidro and Study Participants	45
Figure 2.2	Border Monitoring Equipment	46
Figure 2.3	Windrose Data.....	47
Figure 2.4	Relationship between Personal and Border 1-NP	47
Figure 4.1	Relationship Between Unadjusted Urinary Metabolites and Personal 1-Nitropyrene Among all Study Participants	82
Figure 4.2	Relationship Between Unadjusted Urinary Metabolites and Personal 1-Nitropyrene Among all Study Participants Excluding Values Below Limit of Detection	82
Figure 4.3	Relationship Between Unadjusted Urinary Metabolites and “Border Exposure” Among “Border Commuters”	83
Figure 4.4	Relationship Between Unadjusted Urinary Metabolites and “Border Exposure” Among “Border Commuters” Excluding Values Below Limit of Detection	83

Figure 4.5	Relationship Between Unadjusted Urinary Metabolites and “Home Exposure” Among all Study Participants.....	84
Figure 4.6	Relationship Between Unadjusted Urinary Metabolites and “Home Exposure” Among all Study Participants Excluding Values Below Limit of Detection	84
Figure 4.7	Relationship Between Unadjusted Urinary Metabolites for all Study Participants	85
Figure 4.8	Relationship Between Unadjusted Urinary Metabolites Normalized to Personal 1-Nitropyrene Among all Study Participants Excluding Values Below Limit of Detection.....	85
Figure 4.9	3-Dimensional Relationship Between Unadjusted Urinary Metabolites and Personal 1-Nitropyrene Among all Study Participants.....	86
Figure 4.10	3-Dimensional Relationship Between Unadjusted Urinary Metabolites and Personal 1-Nitropyrene Among all Study Participants Excluding Values Below the Limit of Detection.....	86

LIST OF TABLES

Table 1.1	1-Nitropyrene Concentrations Found in the Literature.....	12
Table 2.1	Study Participant Characteristics	48
Table 2.2	Levels of Traffic-Related Pollutants at the U.S.-Mexico Border Crossing at San Ysidro, CA and Estimated Exposure to “Border Commuters” During their Northbound Wait in the Pedestrian Lane.....	49
Table 2.3	Comparison Between 24-hour Personal Exposure Among “Border Commuters” and “Non-Border Commuters”	50
Table 2.4	Univariate Regression Predictors of Traffic Pollutant, 1-Nitropyrene, Measured at the U.S.-Mexico Port of Entry at San Ysidro.....	51
Table 2.5	Univariate Regression Predictors of Traffic Pollutant, Gravimetric PM _{2.5} , Measured at the U.S.-Mexico Port of Entry at San Ysidro.....	51
Table 2.6	Univariate Regression Predictors of Traffic Pollutant, Ultrafine Particulate Matter, Measured at the U.S.-Mexico Port of Entry at San Ysidro.....	52
Table 2.6	Multivariate Regression Predictors of Traffic Pollutant, Ultrafine Particulate Matter, Measured at the U.S.-Mexico Port of Entry at San Ysidro.....	52
Table 3.1	PM _{2.5} Concentrations for Personal and Fixed Site Samples	64
Table 3.2	1-Nitropyrene Concentrations for Personal and Fixed Site Samples	65
Table 3.3	Seasonal Differences for Personal Concentrations to PM _{2.5} and 1-Nitropyrene	66

Table 3.4	Univariate Regression Predictors of Personal Measurements to 1-Nitropyrene among “Border Commuters”	67
Table 3.5	Univariate Regression Predictors of Personal Measurements to PM _{2.5} among “Border Commuters”	68
Table 3.6	Multivariate Regression Predictors of Personal Measurements to 1-Nitropyrene among “Border Commuters”	69
Table 4.1	Comparison Between 8-OHNP Before and After Adjusting for Creatinine	87
Table 4.2	Comparison Between 8-OHNAAP Before and After Adjusting for Creatinine	88
Table 4.3	Univariate Regression Predictors of Unadjusted 8-OHNP (pg/mL) Among “Border Commuters”	89
Table 4.4	Univariate Regression Predictors of Personal 1-Nitropyrene (pg/m ³) Among all Study Participants with and without Values Below Limit of Detection.....	89

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DEDICATION

To the people dearest to my heart:

My amazing parents, Jerry and Rose Galaviz.

My adorable grandparents, Alejandro and Maria Galaviz.

My sweet-hearted little brothers, Michael and Jerry Galaviz.

Words cannot describe the love I have for all of you. You are my world.

And of course to my love and best friend, Travis Cook.

Chapter 1

Introduction and Literature Review

1.1 U.S.-Mexico Binational Border Environmental Collaboration

The U.S.-Mexico border is defined as the area situated 100 km (62.5 miles) north and south of the inland boundaries and extends into the sea boundaries to the east and the west (Figure 1.1).

The U.S. and Mexico have developed a binational collaborative program called Border 2020

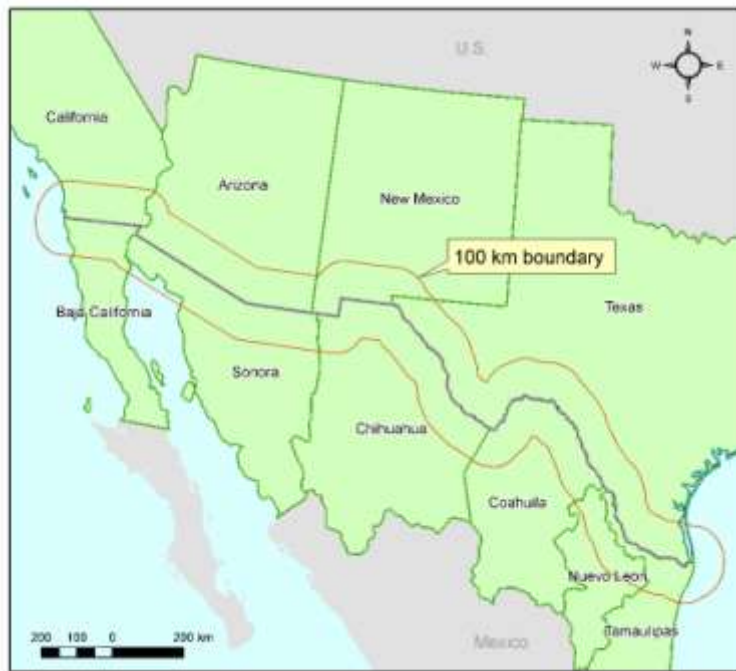


Figure 1.1 U.S.-Mexico Border.

<http://water.epa.gov/infrastructure/wastewater/mexican/>

which is detailed in the Border 2020: U.S.-Mexico Environmental Program (1). Border 2020 builds on the La Paz Agreement of 1983, which marked the first binational effort to address environmental and health concerns along the 2000-mile border between the U.S. and Mexico in hopes of maximizing economic and social well-being.

The program takes a “bottom-up” approach with issues and projects

identified and implemented at the local level through partnerships with state, federal, and international organizations. Development, implementation, and continued progression of Border 2020 results from direct administration by the ten border states, the U.S., the tribal governments,

the U.S. Environmental Protection Agency (USEPA), Mexico's Secretaría de Medio Ambiente Y Recursos Naturales (SEMARNAT), and in partnership with the U.S. Department of Health and Human Services (HHS), Mexican Secretariat of Health (SS), and other federal agencies.

The eight-year cooperative Border 2020 program has the following stated mission, "To protect the environment and public health in the U.S.-Mexico border region, consistent with the principles of sustainable development." Five goals have been developed by Border 2020, which include, reducing air pollution, improving access to clean and safe water, promoting materials management and waste management and clean sites, enhancing joint preparedness for environmental response, and enhancing compliance assurance and environmental stewardship. Of the five goals, reduction of air pollution is of greatest interest to this study considering the goal of this dissertation is quantifying personal exposure to traffic-related air pollution at the U.S.-Mexico Border San Ysidro Port of Entry (SYPOE).

1.2 U.S. and Mexico Air Pollutant Particulate Matter Standards

Six criteria air pollutants have been established by the United States and Mexico. The USEPA refers to them as the six primary National Ambient Air Quality Standards, which were enacted by the Clean Air Act of 1990 (2). Mexico's SEMARNAT, created in 2000, refers to them as *Normas Ambientales para Aire*. The six criteria air pollutants include: carbon monoxide (CO), ozone (O₃), nitrogen dioxide (NO₂), sulfur dioxide (SO₂), particulate matter (PM), and lead (Pb). Due to its chemical complexity, PM is categorized and regulated by aerodynamic diameter. Coarse particulate matter (PM₁₀) has an aerodynamic diameter of <10 μm and fine particulate matter (PM_{2.5}) <2.5 μm. Both the U.S. and Mexico have an annual average limit for

PM₁₀ of 50 µg/m³ and a 24-hour average limit of 150 µg/m³. Mexico does not have a PM_{2.5} standard but the U.S. has an annual average limit of 15 µg/m³ and a 24-hour average limit of 65 µg/m³.

1.3 Sources and Health Effects of Particulate Matter

A variety of anthropogenic sources are causes for ambient air PM emissions, including but not limited to, industry, transportation, construction, and agriculture. Biophysical sources are also a contributor to ambient air PM, including brush fires, volcanoes, dust storms, and pollen. PM composition can include a variety of acids, organics, mold, spores, pollen, inorganics, smoke, dirt, soil and dust particles thus allowing for the potential of thousands of chemicals and constituents to cause biological harm following inhalation exposure. Of particular concern is PM₁₀ and PM_{2.5} due to their small size and as such is the reason for their regulation. PM_{2.5} is thought to possess more combustion derived toxic compounds compared to PM₁₀ (3). There is no regulatory standard for ultrafine particles (UFPs) which are particles with an aerodynamic diameter <0.1µm, however recent research suggests large contribution to health risks as a result of: 1) their large surface area which allows them to transport toxic materials to the alveoli as a result of their minute size (4) and 2) their capability of translocating into the systemic circulation thereby directly interacting with multiple organ systems within minutes of exposure (5, 6). Short- and long-term exposure to PM_{2.5} is associated with a wide range of cardiovascular, respiratory, immunological, CNS, and reproductive health effects (3, 7-9) . Of potential concern is exposure to short-term high levels, as peaks have been shown to trigger asthma attacks and cardiovascular

events (4, 10). PM₁₀ does not normally penetrate beyond the larynx, therefore, symptoms generally include irritation to the upper respiratory system.

1.4 Diesel Exhaust

Diesel exhaust (DE) is comprised of both gases (gaseous diesel exhaust; GDE) and particulates (diesel particulate matter; DPM) of which both play a role in contributing to health risk following exposure. GDE consists of benzene, acetaldehyde, and formaldehyde to name just a few. DPM contributes to PM and as with PM is a mixture of a variety of different types of particles; however, research indicates DPM to be of even greater health concern than PM (11, 12). Reasons for such concern result from its complex composition, which includes organic compounds, elemental carbon, metals, and high concentrations of ultrafine, fine, and nano-particulate matter (13). Some chemical substances in atmospheric DE that are of special concern due to their toxicity are polycyclic aromatic hydrocarbons (PAHs) and nitro-polycyclic aromatic hydrocarbons (NPAHs). PAHs are widespread pollutants commonly found in ambient air. They are one of the most hazardous, widespread, and best characterized airborne toxic compounds (22). NPAHs are derivatives of PAHs and are generated by nitration during combustion processes and atmospheric formation from PAHs by either gas-phase reactions or heterogeneous gas-particle interaction of parent PAH adsorbed onto particles with nitrating agents. The main contributors of NPAHs in urban air are automobiles, domestic heating systems such as furnaces and kerosene heaters, power incinerators, steel, aluminum, and iron factories, coke oven emissions, and coal burning for industrial and domestic purposes (23). Studies have indicated most NPAHs to be found in PM_{2.5} compared to PM₁₀ under ambient conditions (23-26).

Sources of DE include on-road diesel engines such as trucks and buses and off-road diesel engines such as locomotives, marine vessels, and heavy duty equipment. Composition of DE varies considerably and depends on diesel engine type, grade of diesel used, operating conditions, lubricating oil, and whether an emissions controls system is present. Long-range transmission of DE is important but DE pollution is generally more dangerous when emitted near people residing or walking near roadways. Although there is no ambient air quality standard for DE, a reference concentration (RFC) does exist. The RFC is used as a health benchmark and estimates a safe daily exposure level during a person's lifetime (including sensitive populations). The USEPA has set an inhalation RFC for DE of $5\mu\text{g}/\text{m}^3$.

1.5 Health Effects of Diesel Exhaust

Epidemiological and toxicological studies have reported that exposure to DE is associated with a variety of adverse health effects, including a wide range of acute and chronic effects. Both carcinogenicity and mutagenicity have been implicated in numerous studies (14-16). Acute exposure can cause irritation to the eyes, nose, throat, and lungs; neurological symptoms such as lightheadedness and nausea; and respiratory effects such as coughing and exacerbation of asthma (17). Chronic exposures in experimental animal inhalation studies have shown both immunological effects and a range of dose-dependent inflammation and cellular changes in the lung (17). In 2012, the International Agency for Research on Cancer (IARC) classified DE as carcinogenic to humans (Group 1) based on sufficient evidence that exposure is associated with an increased risk for lung cancer (16). This classification was based on a large U.S. National Cancer Institute/National Institute for Occupational Safety and Health study

published in 2012 that conducted a nested case-control study in a cohort of 12,315 underground miners, which resulted in an increased risk of death from lung cancer due to DE exposure (18).

Other human studies have also shown an association between exposure and lung cancer. Lipsett conducted a meta-analysis of 30 epidemiological studies to investigate the relationship between lung cancer rates and occupational exposure to DE (19). Pooled risk estimates were done by using a random effects model allowing for heterogeneity. Subset analysis and linear metaregressions were done to evaluate and control for heterogeneity. The study found that neither confounding by smoking nor publication bias could explain the consistently increased relative risks for lung cancer observed in diesel-exposed populations, and smoking-adjusted studies suggested evidence of an exposure-response relationship. Overall, the meta-analysis provided evidence that a casual relationship between occupational exposure to diesel exhaust and lung cancer exists, which is consistent with other review papers (16, 20). DE has also been indicated to contribute to premature death from heart and lung diseases (21).

1.6. Previous and Current Markers for Diesel Particulate Matter

As a result of the highly complex mixture of DPM, contribution of other combustion sources that produce the same chemical components, and its small contribution to total ambient air pollution assessing exposure becomes a challenge. Although there is no unique constituent of DPM that serves as a surrogate for exposure, elemental carbon (EC) is considered a superior exposure marker in that EC concentrations are much higher in DPM emissions than in other combustion products. EC has and continues to be used in environmental studies as a marker for DPM (26, 27) Although there are no occupational sampling methods for DE used by the

Occupational and Safety Health Administration (OSHA), they do suggest the use of EC as published by the National Institute for Occupational Safety and Health (NIOSH) (NIOSH method 5040). However, there are issues when using EC as an exposure marker for DE, most notably sources other than diesel combustion contribute to its total concentration and the ratio of EC to DE can vary depending on factors such as engine operating conditions (27, 28). EC is considered acceptable when being used for occupational related exposure assessments but only if there are relatively few known EC interferences. In occupational settings these EC interferences can be easily minimized. However, in non-occupational environments there can be a variety of DE sources not easily controlled for such as fuel oil combustion from home heating, industry, or power plants and gasoline engine exhaust.

Other exposure markers have been suggested as promising surrogate markers for DE including the use of PAHs or PAH ratios, such as naphthalene, phenanthrene, benzo[*a*]pyrene, pyrene, benzo[*e*]pyrene, and anthracene (29, 30). However, PAHs are widespread pollutants with other major sources, including incomplete combustion of organic material, such as fossil fuels and biomass. Overall, having a highly sensitive, specific, and easily measured exposure marker to track the contribution of DPM to PM and to assess associated health effects is valuable

1.7 1-Nitropyrene as a Marker for Diesel Particulate Matter

1-Nitropyrene (1-NP) is a four ring NPAH (Figure 1.2). It is a by-product of combustion and is the predominant NPAH emitted in diesel engine exhaust (31, 32). Due to its low

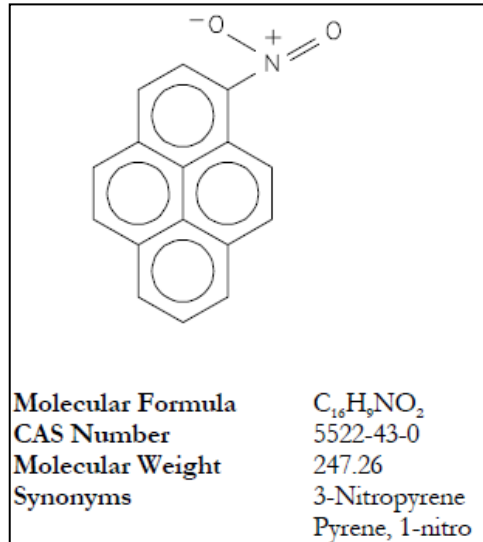


Figure 1.2 1-Nitropyrene
 Source: http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox034.pdf

molecular weight it condenses onto particulate matter as a result of low vapor pressure. The principal degradation pathway of 1-NP appears to be photodecomposition (23).

Empirical evidence has shown 1-NP to be a specific constituent of DPM (24, 33-35) and much less abundant in PM derived sources (30) which makes it a promising marker for DPM compared to previous and current markers that are used such as EC. For example, in one study Kakimoto (36) reported that >99% of 1-NP in ambient PM was from DPM in 3 different cities in Japan.

In another study, emission factor per gram of PM was at least 40-fold higher for DPM than for other combustion sources (33, 37). To date, there have been many studies using 1-NP as an exposure marker for DPM due to its specificity (34, 38-40). Quantification of 1-NP collected on air filters using HPLC-MS/MS has been validated (40-43). Table 1.1 shows 1-NP concentrations found in the literature, including both occupational and ambient levels.

Table 1.1 1-NP Concentrations Found in the Literature

Sampling Location (city)	Sampling Location (type)	[1-NP] pg/m ³	Reference Number
Ho Chi Minh City, Vietnam	Urban	8.1 ± 3.9	(44)
	Urban	9.1 ± 3.9	
	Traffic site	73 ± 40	
Denmark	Urban/high traffic	127 ± 44	(45)
	Rural/open-land	30 ± 15	
Torrence, CA	Urban	30	(46)
Los Angeles (LA) and Riverside (Riv), CA	Traffic (LA)	6.5	(47)
	Traffic (LA)	21	
	Downwind (Riv)	6.5	
	Downwind (Riv)	12.2	
Baltimore (Balt) and Fort Mead (FM), MD	Urban (Balt)	27	(48)
	Urban (Balt)	8.1	
	Rural (FM)	20	
	Rural (FM)	1.4	
Kanazawa, Japan	Urban/downtown	32	(37)
	Suburban	1.7	
Shenyang, China	Work-shift	80 ± 26	(34)
	In-home	22 ± 11	
Seattle, WA	Duwamish fixed site	4.6 ± 9.5	(39)
Seattle, WA	Urban/Residents	0.3 ± 0.2	(40)

Albinet (49) characterized 17 NPAHs in both the gas and particulate phase using three sampling locations in Southern France: an urban sampling site, a site downtown, and a rural site. The highest NPAH concentrations, including 1-NP, were measured at the sampling stations closest to engine emissions with diesel sources as the primary emitter. Miller-Schulze (34) found 1-NP concentrations to be dramatically higher than other NPAHs for the traffic-based samples compared to samples removed from traffic in Shenyang, China. Hayakawa (50) found that concentrations peaked during the morning (9-11am) and evening (5-7pm) in urban air. Hayakawa and Kakimoto (36, 50) found high correlation coefficients ($r = 0.931$) between atmospheric concentrations of NPAHs and traffic volume. These studies consistently report the

similar finding that 1-NP is present exclusively in primary emissions, specifically diesel. Overall, 1-NP has demonstrated to accurately reflect exposure to DPM in environmental and occupational settings thus supporting its utility as a marker across a wide range of exposures.

In addition, the applicability and utilization of 1-NP as an exposure marker for DPM is strengthened in that specific biomarkers can be collected and quantified in urine (51-53). Hydroxy-N-acetyl-1-aminopyrenes (6- and 8-OHNAAP) and hydroxy-1-nitropyrenes (6-, and 8-OHNP) have been shown to be the most abundant isomers in human urine (42) (Figure 1.3). Toriba found that urinary 1-NP metabolite concentrations among taxi drivers in Shenyang, China increased with increasing 1-NP concentrations in ambient air (42). In conclusion, 1-NP and its metabolites have consistently demonstrated to be valid and useful markers of exposure for DE.

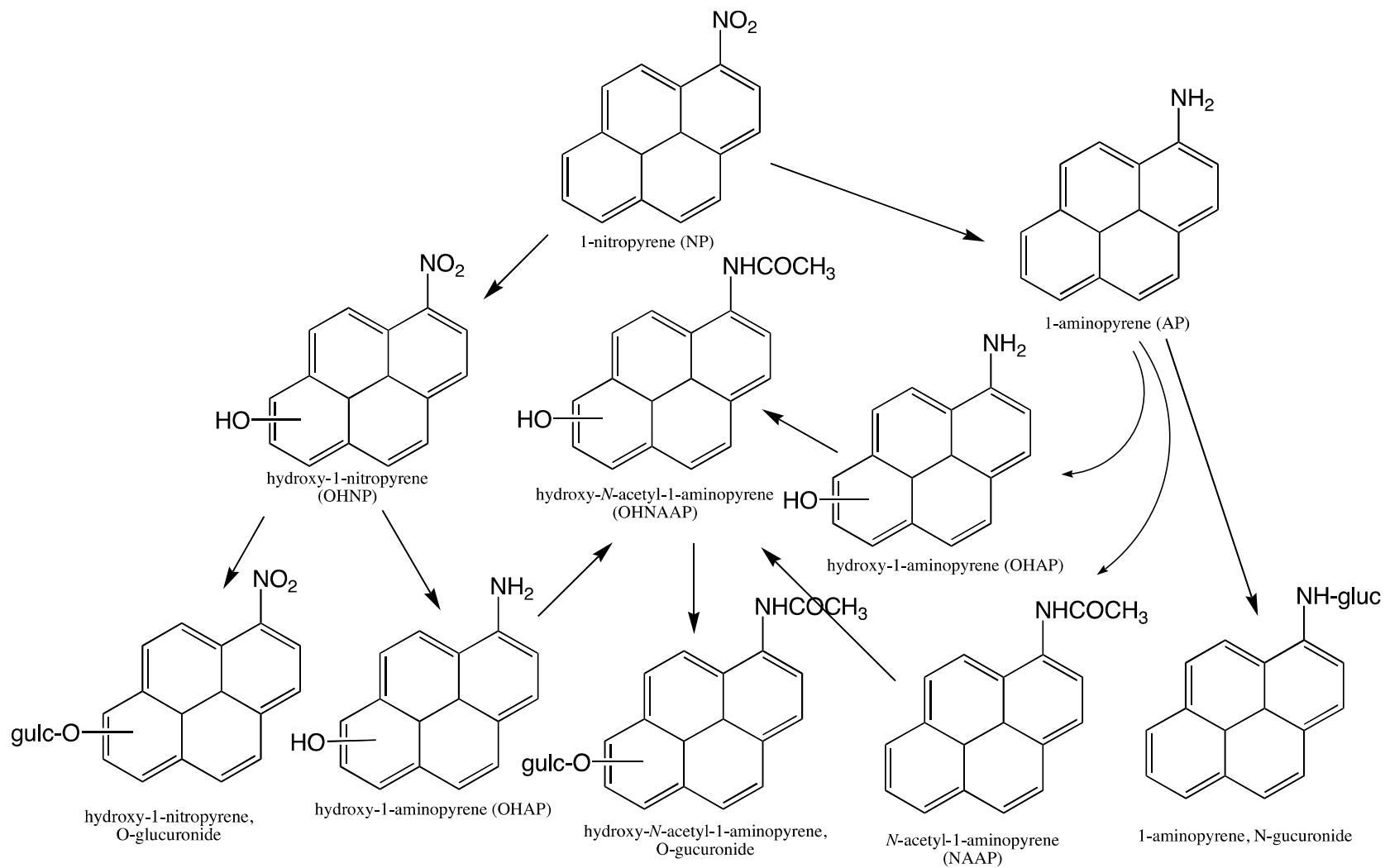


Figure 1.3 Metabolic Pathways of 1-Nitropyrene

1.8 Metabolism and Toxicological Mechanism of 1-Nitropyrene

Studies have shown that metabolism of 1-NP occurs through cytochrome P450 (CYP450)-dependent oxidation, acetylation, and nitro reduction (54-59). As can be seen in Figure 3, 1-NP can be detoxified to 1-aminopyrene by nitro reduction or can be activated by CYP450 enzymes to hydroxyl-1-nitropyrene. 1-NP can also undergo ring oxidation, depending on the concentration of oxygen which can then react with DNA, potentially forming DNA adducts (31). Arimochi found that metabolism of 1-NP by nitroreductase increased DNA adduct levels and mutagenicity (56). Xanthine oxidase and NAD(P)H:quinone oxidoreductase, which are expressed in the vascular wall, have been associated with nitroreduction of 1-NP (60, 61). Cytochrome P450 family 1, subfamily A, polypeptide 1 (CYP1A1), CYP1B1, and CYP3A4 have been demonstrated *in vitro* to catalyze oxidative metabolism of 1-NP in human cell lines (55, 57, 58). Raunio reported that CYP1A1 and CYP3A4 are not constitutively expressed in the vascular wall, leading to the idea that xanthine oxidase and NAD(P)H:quinone oxidoreductase play a primary role of 1-NP metabolism in the vascular wall (62). CYPs are usually membrane-bound and localized to the inner mitochondrial or endoplasmic reticular membrane with high expression in the liver and thus would play a major role in metabolism of 1-NP in the liver. *In vitro* and *in vivo* studies in various tissues and species using cell culture, urine, or fecal samples have found the most abundant isomers to be hydroxyl-1-nitropyrene (3-, 6-, and 8-OHNP), hydroxy-N-acetyl-1-aminopyrenes (3-, 6-, and 8-OHNAAP), trans-4,5-dihydro-4,5-dihydroxy-1-nitropyrene, N-acetyl-1-aminopyrene (NAAP), and 1-aminopyrene (1-AP) (63-67).

The complete underlying toxicological mechanisms of 1-NP exposure are not explicitly known, however there is some insight into pathological mechanisms. Studies have also shown marked changes in cellular morphology, decreased proliferation, and different forms of cell death, including apoptosis and paraptosis due to 1-NP exposure (68, 69). Exposure to 1-NP elicits a pro-inflammatory response, inducing expression of the following cytokines and chemokines in bronchial epithelial cells: CCL20, CXCL1/-3/-8, TNF- α , and IL-6 (70). Zhang also demonstrated an association between inflammation and 1-NP (71). Research shows that chronic systemic inflammation is associated with cardiovascular disease (3, 72), thus making the suggestion that chronic exposure to 1-NP may be associated with cardiovascular disease. In addition to inducing inflammation, Andersson demonstrated that treatment with 1-NP induced DNA damage, increased reactive oxygen species (ROS), decreased cell viability, and increased expression of the endoplasmic reticulum (ER) stress chaperone GRP78 (73). ER stress can promote mitochondrial production of ROS, and elevated levels of ROS can contribute to activation of the ER stress pathway due to formation of peroxynitrite (ONOO-) (74, 75). ER stress has been described as one of the key events behind cardiovascular disease (73).

1.9 Conclusions

In conclusion, exposure to traffic-related air pollutants, such as DE, is a particularly insidious problem, causing both subtle and non-subtle health effects at various levels of exposure. The pedestrians who cross the U.S.-Mexico border at San Ysidro, CA (Figure 1.4) appear to be particularly vulnerable to this threat due to their close proximity to highly concentrated traffic, specifically diesel buses, while crossing on foot northbound (Figure 1.5).

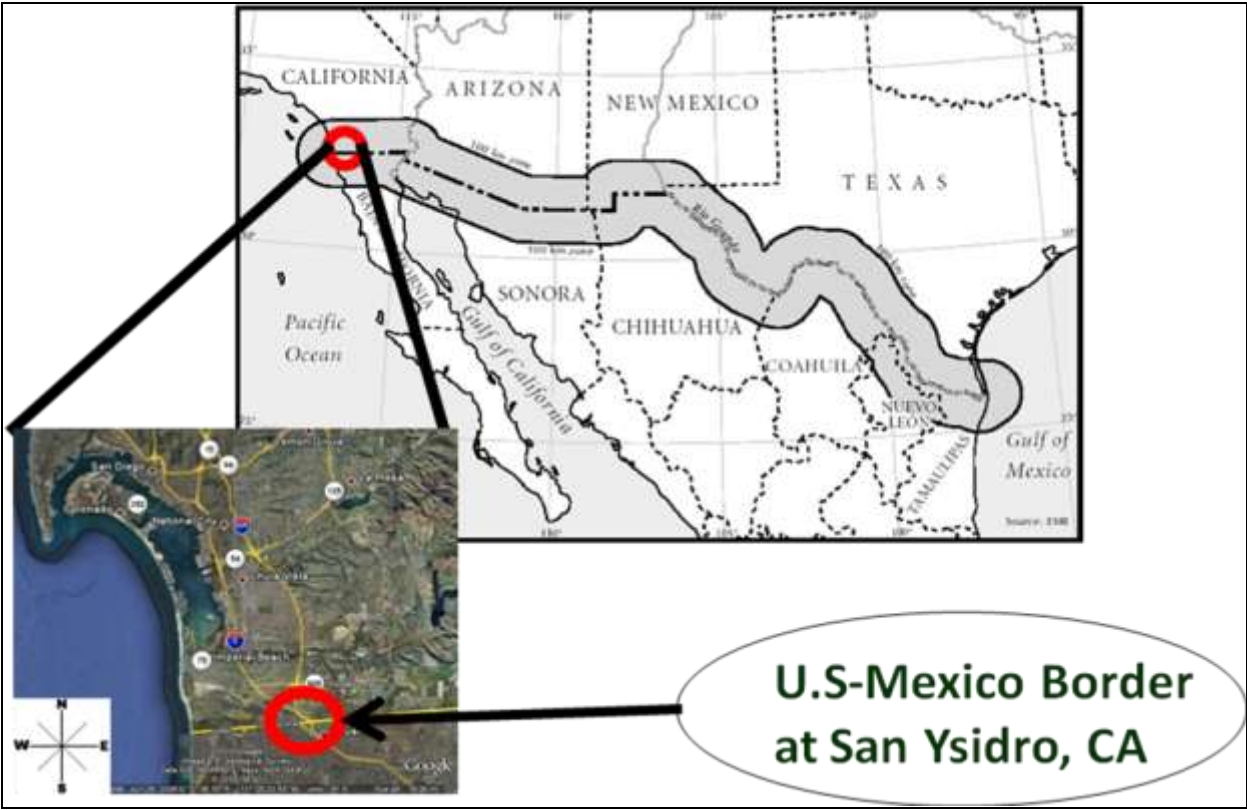


Figure 1.4 U.S.-Mexico Border at San Ysidro, CA.



Figure 1.5. Pedestrian Commute Northbound at the U.S.-Mexico Border at San Ysidro, CA. Photo used with permission from Dr. Albert S. Fu (Kutztown University).

Surveys have been complete on those who cross the border to evaluate the frequency and purpose of their crossings (76, 77). However, an objective assessment quantifying traffic-related air pollutants, particularly DE, that pedestrians experience during their northbound border commute has not been previously conducted.

The U.S. federal government has begun a *San Ysidro Border Station Project*¹.

¹A new San Ysidro port of entry will replace the existing port and will be completed by 2014. The new port will incorporate the latest in security and antiterrorism enhancements to improve pedestrian and vehicular processing, increase operational efficiency, provide greater officer and public safety, decrease operational and maintenance costs, and improve the traveler's experience.

This project consists of the demolition and new construction of the U.S.-Mexico border at San Ysidro (78). Quantitative information on traffic-related air pollutants, particularly DE, is vital in identifying risk factors for elevated exposure, and in disseminating the results and recommendations to both the border crossing design committee and the community in hopes of eliciting structural and/or behavioral changes to reduce exposure. Considering DE is of greater health concern than PM, characterizing personal exposure to DE and human uptake using 1-NP and its urinary metabolites will be one of the main objectives. Overall, it is of public health integrity to understand and characterize risk factors for elevated exposure to traffic-related air pollutants among pedestrians and thus is the overall focus of this dissertation.

1.10 Specific Aims

The emission of PM and DE from idling stop-and-go vehicles, particularly buses, waiting in line to cross the San Ysidro Port of Entry (SYPOE) northbound at the U.S.-Mexico border has the potential to cause significant elevated exposure and uptake for northbound pedestrian commuters. Northbound wait times can range between 1 – 4 hours. The hypothesis is that people who live in Tijuana, Baja California, Mexico and cross the SYPOE by foot to go to work or school in the community of San Ysidro, CA or neighboring communities (referred to as “Border Commuters”) will have higher concentrations of personal PM_{2.5} and 1-NP in addition to higher urinary 1-NP metabolites than people who live and work or go to school in San Ysidro, CA or neighboring communities (referred to as “Non-Border Commuters”).

Specific Aims:

1. Measure PM_{2.5}, 1-NP, and 1-NP urinary metabolites among “Border Commuters” and compare to measurements among “Non-Border Commuters.”
2. For “Border Commuters”
 - a. To characterize border concentrations of various pollutants, and to estimate exposures during the border commute.
 - b. To correlate urinary biomarkers of 1-NP with estimates of their exposure to airborne 1-NP obtained through
 - i. Personal 1-NP measurements
 - ii. Concentrations of 1-NP measured at the border given the time spent at the border
 - iii. At-home concentrations of 1-NP measured indoors given the time spent indoors
3. Utilize time activity diary data to investigate variables that contribute to between subject variation for personal exposure to 1-NP, PM_{2.5}, and urinary metabolites. The goal is to develop a preliminary indication of the most important factors influencing exposure.

1.11 Research Design and Methods

1.11.1 Statement of Purpose

A comprehensive exposure assessment with the inclusion of personal and area measurements of traffic-related air pollutants and biological markers was conducted to better understand personal exposure and uptake for a susceptible population.

1.11.2 Population

San Ysidro, CA is a community located at the U.S. – Mexico border. San Ysidro’s population is overwhelmingly comprised of ethnic minorities; Hispanics, African American, American Indian and Alaska Native, and Asian account for 95% of the total population (79). It is a “young” community with a median age of 25.9 years (79). The median household income of \$27,943 is 33% less than the U.S. average of \$41,994 (79). Two-thirds of the housing stock is multi-family, and 35% of the San Diego’s county subsidized housing is located here, with more than 8,000 families on the waiting list (79). Of the 4,411 students in the San Ysidro School District, 82% are classified as low socio-economic status as compared to 45% in San Diego County, and 29% nationally (79). Tijuana, Baja California, Mexico is also located at the U.S.-Mexico border. It is a developing industrialized city with a rapidly growing population of 1,559,683 (80). It is considered a “young” community with 65% of its population below the age of 34 years with a median age of 25 years (80).

1.11.3 Community Collaboration

Casa Familiar, a 501(c)-3 non-profit agency incorporated in the State of California in 1972, has focused on sustainable border community development by looking to the border as a place of opportunity. It was organized to serve Spanish-speaking monolingual clients in the community of San Ysidro. Over the years, the services and target population have expanded to include all of South San Diego's population. Casa Familiar is a widely recognized authority when it comes to understanding the unique challenges faced by border communities. They offer over fifty programs spanning the program areas of Human Services, Community Development, Recreation Services, Technology, Arts and Culture, and Education. Casa has worked in the community of San Ysidro for over 30 years and as a result is highly respected and utilized by the surrounding community members, and civic and governmental groups. Casa has been assisting the federal government's designers and planners to address community issues and concerns and provide feedback on the *San Ysidro Border Station Project*. They assisted in the recruitment of study participants and bringing the findings of this study to the table during discussions of future border crossing design plans and for advocating the benefits of reduced border delays. They assisted in dissemination of our findings and providing environmental education to the community.

1.11.4 Subject Characteristics and Recruitment

Participants who live in Tijuana and frequently commute across the SYPOE for work or school in or near San Ysidro were recruited (referred to as "Border Commuters"). A comparison

group was also recruited who live and work or go to school in or near San Ysidro and do not commute into Mexico (referred to as “Non-Border Commuters”). All study participants were recruited through word of mouth, vending machine advertisements, and flyers with support from Casa Familiar (Appendix A-B). Eligibility was assessed during the recruitment process with the use of an eligibility script to minimize bias and confounding (Appendix D-G).

1.11.5 Informed Consent Process

Participant written names and signatures were obtained to document the informed consent process. The consent forms were the only document to contain this personal information and the remaining documents were identified by a numerical code. Once completed, consent forms were stored in a locked filing cabinet. Consent forms were retained for three years and have since been destroyed. When the results of this study are reported and/or published in the scientific literature, no information on any participants’ names or identifying codes will appear.

1.11.6 Ambient and Personal Air Monitoring and Biological Sampling

Testing the hypothesis was accomplished by quantifying personal exposure to ambient levels of PM_{2.5} and 1-NP. In addition, collection of urine allowed for quantification of actual body uptake, and estimation of body burden through detection of 1-NP metabolites. To further understand the contribution of microenvironments to exposure, concentrations of PM_{2.5}, and 1-NP at the border were collected during the “Border Commuters” walk-in-line commute, in addition to at-home indoor and outdoor sampling. To further characterize concentrations of various traffic-related air pollutants at the border, additional measurements were made,

including, UFPs, carbon monoxide (CO), and black carbon (BC). Collection of border ambient air samples established exposure concentrations that walk-in-line commuters experienced.

All study participants were met at a designated location of their choice in or near San Ysidro. They were given sampling equipment and study materials to participate in the study. They were given a backpack that contained the sampling equipment in which they wore for 24-hours. They were given a 24-hour time activity diary to complete which they were asked to fill out every 2-hours (Appendix J-K). All participants' equipment and sample information was kept on an *Equipment Information Sheet* (Appendix L). Participants whose at-home measurements were taken were given an *At-Home Monitoring Information Sheet* to record exact time spent at home and total running time of equipment (Appendix N). "Border Commuters" were given a *Border Crossing Information Sheet* to collect the exact start and stop time while at the border as a pedestrian crossing northbound (Appendix M). They were also given three other documents, an *Information Sheet about Border Crossing* (Appendix O-P) and *A letter of Support from the U.S. Customs and Border Protection* (Appendix T) to help alleviate any concerns and an *Instruction Sheet for the Monitoring Equipment* that they would give to the U.S. Customs and Border Protection Officer if taken to secondary inspection (which fortunately never occurred) (Appendix S). "Border Commuters" then estimated the time they would cross the border northbound on the following day so that area monitoring at the border could be conducted. An example of the *Area Monitoring Information Sheet is attached* (Appendix Q). Border instruments were set-up 1-2 hours prior to the "Border Commuters" estimated commute time and were located near U.S. Customs and Border personnel to ensure safety of the equipment. Following 24-hours of personal monitoring the participants were met again at a location of their choice in or near San

Ysidro. They provided a spot urine sample and were given a questionnaire of approximately 20 minutes in length (Appendix H-I). To assist in keeping track of materials and equipment needed for the study a *Check-Off List* was always referred to (Appendix R).

1.11.7 Human Subjects

All human subject data collection procedures were compliant with the University of Washington (UW) and San Diego State University (SDSU) Institutional Review Board (IRB) requirements (UW IRB#38672, SDSU vIRB#381041).

Chapter 2

Traffic Pollutant Exposures Experienced by Pedestrians Waiting to Enter the U.S. at a Major U.S.-Mexico Border Crossing

2.1 Abstract

Background: Traffic-related air pollution is of great public health importance as a result of morbidity and mortality risks associated with both acute and chronic exposures. This is the first exposure assessment conducted to quantify northbound pedestrian commuter exposure to traffic-related air pollutants at the U.S.-Mexico border, performed at the San Ysidro, CA, Port of Entry (SYPOE). The northbound pedestrian pathway is located within 10 feet (3 meters) of 24 lanes of idling stop-and-go traffic, including diesel buses. **Methods:** Seventy-three persons who regularly crossed the SYPOE in the pedestrian line and 18 persons who did not cross were recruited to wear personal air monitors for 24-hours to measure traffic pollutants PM_{2.5}, 1-nitropyrene (1-NP) – a marker for diesel exhaust – and carbon monoxide (CO). Fixed site concentrations were collected at the crossing and occurred during the time subjects were crossing northbound to approximate their exposure to 1-NP, ultrafine particles (UFP), gravimetric PM_{2.5}, real time PM_{2.5}, carbon monoxide (CO), and black carbon (BC) while standing in line during their border wait northbound. **Results:** Subjects who crossed the border in pedestrian lanes had an 8-fold increase exposure to 1-NP (1.7 ± 2.3 vs 0.22 ± 0.21 pg/m³, $p < 0.01$ Mann-Whitney), a 3-fold increase exposure to CO (2.8 ± 1.8 vs 1.0 ± 0.79 ppm, $p < 0.01$ Mann-Whitney), and a 2-fold increase exposure to gravimetric PM_{2.5} (39 ± 30 vs 21 ± 11 µg/m³, $p < 0.01$ Mann-Whitney), vs non-border commuters. Median values (interquartile range; IQR) of daily averages for fixed site

measurements made at the border were as follows: UFP, 40,000 (24,000-52,000) #/cm³; CO, 5 (3-6) ppm; 1-NP, 1.3 (0.5-2.6) pg/m³; BC, 4 (3-11) µg/m³; real time PM_{2.5}, 41 (23-57) µg/m³; and gravimetric PM_{2.5}, 15 (13-22) µg/m³. In regression analysis for border measurements, there were no significant predictor variables for 1-NP. Wind speed explained 22% variability for gravimetric PM_{2.5}. Univariate regression analysis for UFP found that border wait time for vehicles explained 21% of variability and relative humidity 13%, but when modeled together none remained significant. **Conclusions:** Personal exposure to traffic-related pollutants was higher for persons who routinely waited in line to cross the US-Mexico border at the SYPE than for subjects who did not cross. Concentrations at the border of UFP, PM_{2.5}, CO, and BC are similar to those in other near-roadway studies that show associations with acute and chronic adverse health effects. These observations warrant concern for adverse health effects experienced by pedestrian commuters waiting in long line at SYPOE.

2.2 Introduction

The U.S.-Mexico border is 2000 miles in length and has 43 land entry vehicle ports. Seven of the ports are located in California and account for 37.8% of the cross-border vehicle traffic. The San Ysidro Port of Entry (SYPOE) is the western most port (Figure 2.1A) and bounded by the community of San Ysidro, California, U.S.A. to the North and the city of Tijuana, Baja California, Mexico to the south. In 2011, SYPOE had the greatest number of northbound crossings with 12.4 million personal vehicles and 8.5 million pedestrians (81) and has been referred to as the busiest land border crossing in the world. Currently, there are 24 vehicle and 14 pedestrian lanes for northbound crossers. SYPOE operates 24 hours a day for 365

days a year. Delays crossing the border northbound into the U.S. are much longer than those southbound into Mexico with average wait times often more than an hour for vehicles using the regular inspection lanes and at least an hour for walk-in-line pedestrians, with peak times averaging 2-4 hours (78). The pedestrian pathway (Figure 2.1B and 2.1C) is located within 10 feet (3 meters) of idling traffic, and when coupled with long border wait-times pedestrians may have high exposures to traffic-related air pollutants. Acute exposure to traffic related air pollution has been shown to trigger cardiovascular events (82). Chronic exposure to traffic exhaust consists of a wide range adverse health effects including cardiovascular, respiratory, cancer, and reproductive effects (83-97). Of particular concern is the proximity of the bus lane to the pedestrian pathway (Figure 2.1C). Diesel exhaust (DE) is now considered a carcinogen by IARC (16) and previous studies have linked DE exposure to 80% of total carcinogenic risk in the South Coast Air Basin in California (98). Air pollutants of concern at SYPOE include both gases, such as carbon monoxide (CO), particulates, such as fine particulate matter (PM_{2.5}) and ultrafine particulates (UFP), and DE. Surveys have been conducted on pedestrians who cross the border to evaluate the frequency and purpose of their crossings (76, 77). However, quantitative assessment of air pollutant exposures that walk-in-line commuters experience during the border crossing has not been previously investigated.

There are 12 continuous air monitoring stations located in San Diego and 7 located in Baja California Norte, however, none are located at or near SYPOE. Walk-in-line pedestrians likely experience much greater levels of air pollution that would be predicted from regional air quality monitoring stations, which are typically located in areas relatively unaffected by local sources such as traffic. Traffic related air pollution is often highly localized; concentrations of

ultrafine particles (UFPs) and CO have been shown to decrease exponentially with distance from freeways, reaching background concentrations within approximately 150 meters of the roadway (99). Similarly, measurement data for black carbon (BC) – a marker of diesel exhaust particle (DEP) emissions – indicate strong spatial concentration gradients with peaks near high volume traffic corridors (100). A recent study synthesized 41 roadside monitoring studies encompassing more than 700 air pollutant concentrations of which almost all pollutants were highly elevated near busy roads, decaying to background within approximately 115 meters (492 feet) from the edge of the road (101).

The objective of this study was to characterize traffic-related air pollutant exposures experienced by pedestrians who frequently cross SYPOE and stand in long lines during the northbound commute, as compared to exposures experienced by people who live in San Ysidro or nearby but do not cross SYPOE. Personal measurements included 1-NP, gravimetric PM_{2.5}, and CO. In addition, fixed site measurements were made at the SYPOE and included 1-NP, gravimetric PM_{2.5}, real time PM_{2.5}, UFPs, CO, and BC.

2.3 Materials and Methods

2.3.1 Personal Sampling

Participants were recruited through an advertisement located at the SYPE and with the help of Casa Familiar (501c-3 non-profit community agency in San Ysidro, CA) through various mechanisms, including word-of-mouth and information booths with sign-up sheets located at community colleges and Casa Familiar ‘Sin Limites’ community meetings. “Border Crosser”

eligibility criteria included: 1) 18 years of age or older, 2) non-smokers residing in a non-smoking home, 3) free of any chronic lung, liver, or heart condition, 4) occupationally not exposed to DE, 5) crossed the border as a pedestrian more than 4 days per month. “Non-Border Crossers” served as the comparison group. They had the same eligibility criteria except that they had to live and work or go to school in San Ysidro or nearby and did not cross into Mexico in the previous 4 months. To minimize exposure variability between groups, the comparison group had to live in areas of similar population density as Tijuana and included area codes: 91910, 91911, 92154, 92173, and 91950. Study participants were given the choice of one of two options for participation. Option 1 included carrying a backpack for 24 hours containing a CO and RH/Temp monitor, completing a 24-hour time activity diary (TAD), filling out a questionnaire, and giving a spot urine sample. Option 2 included the above plus the requirement to carry a pump attached to an impactor to collect personal measurements of 1-NP and gravimetric PM_{2.5} (Figure 2.1D). Results from the TAD and urine samples are reported elsewhere (102, 103). Sixteen potential study participants reported that they smoked or lived in a smoking household, and hence were ineligible to participate. Of the 91 study participants, 76 participants (56/73 “Border Commuters” and 15/18 “Non-Border Commuters”) agreed to carry the 1-NP sampling device (option 2). Age and sex of “Border Commuters” were matched to “Non-Border Commuters” to the extent possible. All participants were met at a location and time of their choice in South San Diego and given sampling equipment for one 24 hour period that included a border crossing, if applicable. All human subject data collection procedures were compliant with University of Washington (UW) and San Diego State University (SDSU) IRB requirements (UW IRB#38672, SDSU vIRB#381041). To minimize risks to participants, U.S. Customs and Border Protection (CBP)

officers were given pictures of the air monitoring pumps and equipment, and were informed when participants were crossing.

2.3.2 Fixed Site Monitoring at the U.S.-Mexico Border

Prior to data collection a letter of support from CBP was obtained. This allowed access into SYPOE, always accompanied by a CBP officer to minimize disruption and expedite data collection in a safe manner. Measurements were collected immediately north of the primary inspection area (Figure 2.2A and 2.2B). This fixed sampling site was selected based primarily on security criteria (close to CBP officers) while trying to maximize proximity to pedestrian pathway to estimate exposure as accurately as possible. However, due to security reasons fixed site sampling location was located 2.4 meters (8 feet) further east than the pedestrian pathway. Sampling pump inlets were placed at an estimated average breathing height of 4 feet (1.2 meters) with limitations preventing a higher placement. Start and stop times of fixed site equipment was 1-2 hours before and after the northbound SYPOE commute of “Border Commuters.”

2.3.3 Sample Collection

Both personal and fixed site 1-NP was sampled with a PM_{2.5} impactor (BGI HPEM, Waltham, MA and SKC PEM, Eighty Four, PA) outfitted with a 37 mm PTFE filter (SKC #224-1709, Eighty Four, PA) and drain disc (Whatman230800, Tisch Scientific), and connected to a SKC personal air sampling pump (SKC AirChek Pump XR5000, Eighty Four, PA) operated at 4 liters per minute. Air sampling pumps were calibrated before and after use (Defender 510, SKC Inc., Eighty Four, PA). Greater than 10% deviation in flow rate resulted in sample exclusion.

There was no sample exclusion of 1-NP measurements due to flow rate deviating beyond 10% between pre- and post- flow rate measurements. Filters were conditioned for 24 hours before and after sampling in a temperature- and humidity controlled room (ambient temperature, 22 ± 1 °C, relative humidity, $35 \pm 5\%$). Pre- and post-weight measurements were obtained from an ultramicrobalance (UMX-2, Mettler-Toledo, Inc., Columbus, OH). Assembly and disassembly of PM_{2.5} impactors and filters occurred in a supplied air glove box (Aldrich AtmosBag Z530220, Sigma-Aldrich Co., St. Louis, MO) with a HEPA filter attached to the air inlet (HEPA Capsule 12144, Pall Corporation, Port Washington, NY). Three of the 34 sampling days were set up to collect simultaneously co-located 1-NP samples; between-filter average coefficient of variability was <6%. Filter samples and spot urine samples were immediately placed on ice following sample collection and transported to the SDSU School of Public Health Laboratory and stored in a -20°C freezer. Air filters and urine samples were shipped to the UW Department of Environmental and Occupational Health Sciences (DEOHS) prior to analysis. Air filters and urinary samples were analyzed for 1-NP and 1-NP metabolites using HPLC-MS/MS from the methodology described by Miller-Schulze (41, 43). Data analysis for urinary 1-NP metabolites are reported elsewhere (103).

The limit of detection (LOD) for gravimetric PM_{2.5} was calculated by taking the pooled variance from a one-sided test from pre-weight blank measurements, which were stratified by day (3 days total) to control for between-day variability, resulting in a mass value of 11 µg. All post-weights were measured on one day so there was no need to account for between-day variability. To substitute for values <LOD, the mass value of 11µg was then divided by the square root of 2 resulting in a final mass value of 7.8 µg. For filters with a net weight gain less

than 7.8 μg , a minimum detectable concentration was calculated by substituting a mass gain of 7.8 μg and dividing by the sample specific air volume.

1-NP LOD was calculated from the HPLC/MS/MS chromatograms, calculating the average 1-NP peak area of the field blanks and adding 2 times the standard deviation. This minimum detectable peak area was then divided by the average area of the internal standard, and then input into the calibration equation to obtain a LOD in mass units. This mass was then divided by the average air volume of the samples collected resulting in a LOD of 0.05 pg/m^3 for personal 24-hour samples and 0.24 pg/m^3 for fixed site samples. Eighteen of the 34 (53%) gravimetric $\text{PM}_{2.5}$ samples were below the LOD while six of the 18 filter samples (18%) were below the 1-NP LOD.

All fixed site measurements had the same start and stop time for each sampling day. The real-time instruments logged data at 1 minute intervals and could provide estimates of concentrations during time “Border Commuters” were in line, except for 1-NP and gravimetric $\text{PM}_{2.5}$. If more than one “Border Commuter” crossed SYPOE northbound in one sampling day separate pumps that collected 1-NP and gravimetric $\text{PM}_{2.5}$ had to be set up with pre-set start and stop times for each “Border Commuter.” This increased reliability of border exposure estimates for 1-NP and gravimetric $\text{PM}_{2.5}$ for each “Border commuter.” There was never an overlap of measurements collected for 1-NP and gravimetric $\text{PM}_{2.5}$ in one sampling day; This was to insure that if more than one measurement was taken it was set-up to cover the entire sampling period so that one value of 1-NP and gravimetric $\text{PM}_{2.5}$ could be obtained for each sampling day.

For fixed site border measurements, real time $\text{PM}_{2.5}$ was logged in one minute intervals using the Thermo-MIE personal DataRAM (pDR) 1200 light scattering instrument (Thermo-

electron Corp., Waltham, MA) connected to a metal particle size-selective inlet cyclone (BGI model GK 2.05, Thermo Electron Corp., Waltham, MA) upstream and to a filter holder for 37 mm filters and a SKC air sampling pump (AirChek Pump XR5000, SKC Inc., Eighty Four, PA) downstream of the photometric sensing chamber. The external pump was operated at 4 liters per minute in order to achieve a particle size cut off of 2.5 μm . Before each use and as recommended by the manufacturer, the pDR was zeroed using particle-free air provided by a HEPA filter. The use of a light-scattering monitor is subject to concern regarding the potential for inaccurate readings resulting from condensational growth of the particles due to water uptake by hygroscopic particle components. Therefore, the following empirical correction factor (CF) was used when relative humidity was greater than 60%: $CF = ((1 + 0.25 (RH)) / (1 - RH))$ (104). Ten of the 12 real time $\text{PM}_{2.5}$ samples at the border were above 60% RH and thus corrected for accordingly.

UFPs were logged in 5 minute average concentrations with a hand-held Condensation Particle Counter (CPC, TSI Model 3007, Inc., Shoreview, MN) which operates by condensing isopropyl alcohol onto ambient particles which are then detected optically and counted by light scattering detection. The applicability and reliability of using TSI model CPC 2007 in ambient air measurements are supported by previous work (105). Prior to field measurements, comparison with co-located CPCs, measuring simultaneously at 1-minute intervals were tested and revealed high correlation coefficients obtained by the least-square regression method ($R^2 = 0.96 - 0.99$).

BC was sampled every minute with a black carbon aerosol monitor (microAeth AE51, AethLabs, San Francisco, CA). Instruments were not available until the last 5 sampling days.

Three of the 5 samples had 2 co-located instruments and these showed high correlations in 1 minute values ($R^2=0.90-0.99$).

Carbon monoxide, RH, and temperature were measured using HOBO dataloggers (HOBO Pro CO Data Logger and HOBO Pro RH/Temp Data Logger, Onset Corporation, Bourne, MA & EasyLog EL-USB- CO Data Logger, DATAQ Instruments, Inc., Akron, OH) which collected logged measurements every minute. Pollutant measurements were corrected for temperature and pressure although differences between pre- and post-adjusted concentrations were never less than 0.05%. Field and lab blanks accounted for 10% of collected samples. Forty-two of the 56 personal CO measurements were invalid due mainly to instrument unavailability and less due to failure as a result of being dropped or mishandled incorrectly.

Other variables of interest that were collected included wind speed (mph), wind direction (degrees), vehicle border wait times (minutes) and number of buses. Wind speed, wind direction, and vehicle border wait times were summarized by taking the median values for each sampling day during hours sampling was taking place. Wind speed and wind direction were collected from the website www.wunderground.com using the closest station to SYPOE, *Proteccion Civil Tijuana Station* (Latitude 32.5°N, Longitude 117.0°W, Weather Underground, Inc., Atlanta, GA). Wind rose plots for meteorological data were generated using WRPLOT View Version 7 (Lakes Environmental, Waterloo, Ontario).

Border wait times for vehicles to reach the primary inspection booth are estimated by CBP and posted on their website hourly (<http://apps.cbp.gov/bwt/>). An estimation of border delay is determined by south-facing cameras and the use of pre-determined geographical fixes in relationship to how many vehicle lanes are open, and estimates by officers. CBP does not

provide a historical database of vehicle border wait times to the public, therefore, a software program was developed by the UW DEOHS Systems Administrator to collect the real-time vehicle border wait data each hour as it was posted, and archived this data. In regards to collecting bus information CBP has no formal method of collecting border wait time as they do with vehicle wait time. The best estimate of bus volume was to record the total number of buses passing northbound per hour which was done myself on days when possible.

2.3.4 Statistical Analysis

STATAIC ver11 (STATA Corp LP, College Station, Texas) was used to perform all statistical analysis. An alpha level of 0.05 was used to determine statistical significance. Summary statistics for personal and fixed site measurements of 1-NP and gravimetric PM_{2.5} were based on averaged daily values whereas UFP, real time PM_{2.5}, BC, CO, vehicle delay, temperature, and relative humidity were calculated based on the daily mean values of continuous data.

Non-parametric comparison tests (Wilcoxon-Mann-Whitney U-test) were used to compare personal exposure to gravimetric PM_{2.5}, 1-NP, and CO between “Border Commuters” and “Non-Border Commuters.”

Three multilinear regression models were created to determine if there were any significant meteorological, season, and traffic predictor variables for fixed site measurements of 1-NP, gravimetric PM_{2.5}, and UFP. There were not enough sampling days for real time PM_{2.5}, BC, and CO to perform multivariate regression analysis due to concerns of overfitting the data. Prior to modeling the following assumptions were tested and passed: independence of errors,

linearity, homoscedasticity of residuals, multicollinearity, influential outliers, and normality. Continuous variables were not normally distributed and thus were log transformed. Continuous variables included temperature (°F), RH (%), wind speed (mph), bus count, and vehicle delay (min). Categorical variables included season and wind direction. Season was dichotomized and classified into “Autumn and Winter” vs “Spring and Summer” with “Spring and Summer” being the reference category based on photodecomposition being the primary pathway for degradation (106). Autumn and Winter was defined as the time period between September 1st and February 28th. Wind direction was dichotomized to look at the effect of border concentrations during periods when winds came from the coast (West and Northwest) based on previous data that reported decreased concentrations of traffic-related pollutants in the community of San Ysidro when winds came from the coast (81), and thus was classified into “W and NW” vs “Other” where “W and NW” served as the reference category. The final linear regression model of interest took the form:

$$\text{Log}(Y_i) = \beta_0 + \beta_1 \text{Log}(\text{temperature}) + \beta_2 \text{Log}(\text{RH}) + \beta_3(\text{season}) + \beta_4 \text{Log}(\text{wind speed}) + \beta_5(\text{wind direction}) + \beta_6 \text{Log}(\text{bus count}) + \beta_7 \text{Log}(\text{vehicle delay}) + \varepsilon_i$$

Where Y_i is the average, log-transformed concentration of 1-NP, gravimetric $\text{PM}_{2.5}$, or UFP at the fixed site, β_1 - β_7 is the slope estimates for the corresponding covariates, and ε_i is the error term.

2.4 Results

Data collection occurred between March 30th, 2010 and December 17th, 2010. Ninety-one healthy participants (73 “Border Commuters” and 18 “Non-Border Commuters”) enrolled in the study; all self-classified themselves as Hispanic (Table 2.1). “Border commuters” (n = 73) cited the major reason for crossing was for work or school with studenting made up the majority of “Border Commuters” but a minority of “Non-Border Commuters”(Table 2.1).

“Border Commuters” crossed the border on 35 separate dates. Usually, 2 persons crossed the border on one day (range 1 – 6), but usually at separate times, for example the first subject might be waiting in line from 7 am – 8:30 am and the second from 8:15 am -9:45am. Fixed site border sampling took place from before the first subject arrived at the line to after the last subject had passed. Table 2.2 presents the concentrations of measured pollutants and delay variables at the border fixed site in two ways: first, for the overall values for the 35 days (called ‘Fixed-Site Overall’), then as concentrations and variables measured during only those times that the “Border Commuters” were standing in line waiting to cross (called ‘Border Commuter Exposure’).

During the sampled days, the median (interquartile range; IQR) value for temperature was 64°F (57-69) and 72% (63-78) for relative humidity. Of the sampled pollutants, UFPs had a median concentration overall of 40,000 particles/cc, with some days being as high as 91,000 particles/cc (Table 2.2). For DE markers, 1-NP had a median of 1.3 pg/m³, with a range of 0.2 – 9.5 pg/m³, and BC a median of 4 µg/m³, with a range of 3-13. CO had a median of 5 ppm (range 3 – 6), gravimetric PM_{2.5} a median of 15 µg/m³ (range 8 – 167), and real time PM_{2.5} a median of 41 µg/m³ (range 14 – 81). During the time border commuters were in line to cross northbound,

the median reported northbound vehicle delay time (an indicator of the amount of idling traffic near pedestrian line) was 83 minutes with a range of 32 – 137. The “Border Commuters” spent a median of 60 minutes and up to 200 minutes waiting in the pedestrian line to cross northbound.

Meteorological data show winds coming predominantly from the West which is typical for this part of the South Coast (Figure 2.3). Eight of the 35 sampling days were affected by rain, ranging from 0.02-0.3 inches during data collection days. A decrease in average concentration for fixed site measurements during days with rain was only seen for 1-NP NP (median: 0.70 ± 0.66 vs 2.4 ± 2.5 $\mu\text{g}/\text{m}^3$), real time $\text{PM}_{2.5}$ (median: 20 ± 15 vs 47 ± 20 $\mu\text{g}/\text{m}^3$), gravimetric $\text{PM}_{2.5}$ (average: 16 ± 8 vs 26 ± 31 $\mu\text{g}/\text{m}^3$), and BC (2 vs 7 ± 4 $\mu\text{g}/\text{m}^3$); however, this was only significant for 1-NP ($p=0.02$ Mann-Whitney). There was only one measurement day for BC on rainy days.

Table 2.3 compares the 24-hour personal traffic-related air pollution measurements between “Border Commuters” and “Non-Border Commuters.” Among all personal exposures to pollutants, “Border Commuters” experienced significantly increased 24-hour personal exposures. For 1-NP, a marker for DE and a potent mutagen, levels were more than 8 times higher for those crossing the border compared to non-border crossers (mean 1.7 ± 2.6 vs 0.22 ± 0.21 $\mu\text{g}/\text{m}^3$, p -value <0.01 Mann-Whitney). Personal 1-NP measurements were positively associated with border measurements of 1-NP ($r=0.34$, $p=0.01$, $n=56$), indicating that the border explained 12 % of variance (Figure 2.4). Personal $\text{PM}_{2.5}$ and CO did not show any associations with border measurements of $\text{PM}_{2.5}$ and CO, respectively.

To investigate predictor variables of fixed site SYPOE border concentrations, univariate and multivariate analysis was applied. There were no predictor variables for 1-NP (Table 2.4).

The concentration of gravimetric PM_{2.5} decreased with increasing wind speed but no other significant predictor variables were noted (Table 2.5). Increased Vehicle Delay times and RH were significant predictors of UFP concentrations and explained 21% (adjusted R²=0.17) and 13% (adjusted R²=10%) of the variance, respectively (Table 2.6). However, when modeled together the significance was lost for both predictor variables with slight decreases in beta coefficients from the univariate to multivariate analysis (Table 2.7).

2.5 Discussion

This study is the first to measure exposures to pedestrians crossing at a US-Mexico border Port of Entry. We found similar levels of fixed site measurements for PM_{2.5}, UFP, BC, and CO as other near roadway traffic studies. For example, UFP concentrations were similar to those reported in a recent study conducted at the U.S.-Mexico Port of Entry at El Paso-Ciudad Juarez (107). Unlike SYPOE, heavy trucks are allowed to cross at El Paso-Ciudad Juarez and usually emit more UFP per vehicle than passenger vehicles. However, the very large number of passenger vehicles combined with long delay times probably accounts for this discrepancy and explains the high levels of UFPs at the SYPOE. Another example includes a study conducted near a major freeway with heavy-duty diesel traffic in Los Angeles, CA (samplers located 49 feet from the end of the 710 freeway). It found median real time PM_{2.5} concentrations to be 12.1 μg/m³ in the winter and 14.2 μg/m³ in the summer, BC concentrations to be 3.99 μg/m³ in the winter and 2.07 μg/m³ in the summer, UFP concentrations to be 33,235/cm³ in the winter and 32,490/cm³ in the summer, and CO concentrations to be 0.39 ppm in the winter and 0.25 ppm in the summer (108).

In regards to 1-NP, measurements were lower than other studies that quantified 1-NP near heavy traffic roadways. A summary of 1-NP concentrations can be found in table 1.1. For example, Reisen (47) reported average concentrations of 4.3 pg/m³ for 1-NP at a sampling site located near two freeways and a busy street in Los Angeles, CA. One potential reason our study has lower 1-NP levels may be due to the fact that there are limited sources of DE, including the restriction against commercial trucks at SYPOE. However, the fact remains that “Border Commuters” have increased exposure to 1-NP, a potent mutagen found in DE, vs “Non-Border Commuters” as evidenced by the 24-hour personal samples with border concentration explaining 12% of personal exposure to 1-NP ($r=0.34$). Although “Border Commuters” had higher 24-hour personal exposure to CO and gravimetric PM_{2.5} vs “Non-Border Commuters” there was no positive linear association between personal samples and the border fixed site samples of CO and gravimetric PM_{2.5}. The sample size for CO was limited and restricted the power of analysis for this pollutant. Further research would be required to further characterize contribution of microenvironments, such as at-home exposure, to understand what predictor variables should be accounted for when assessing exposure.

BC has been used in many studies as a marker for DE; however, recent research has shown that 1-NP is a promising marker for DE due to numerous factors, including: 1) analytical sensitivity, 2) specificity to DE, 3) lack of secondary compound formation, and 4) its ability to be detected across a wide range of exposures. Due to small sample size for BC measurements, associations between BC and 1-NP were not calculated.

This was a pilot study and as a result there were limitations, in particular sample size was small for fixed site measurements. This reduced the ability to carry out more complex regression

modeling, including the effect of interaction terms such as wind speed, wind direction, and season. In addition, fixed site sampling occurred only during hours in which supervision by a CBP officer could be arranged, which was limited to 6am-5pm, Monday-Friday. Vehicle data for buses was collected using a visual inspection as the primary source of estimation. Ideally, a more precise method would be preferred with the added inclusion of counts per type of vehicle in the entire border line (gas vs diesel) to look at contribution of type of vehicle to pollutant concentrations. A major limitation was the location of the fixed site equipment, which was limited to an area located near Customs and Border Protection (CBP) officers for security reasons. It would have been preferred to set up the equipment south of the primary inspection gate and within the same distance that pedestrians experience to vehicle traffic to get a more precise measurement of pedestrian exposure to traffic related pollutants. Our sampling location likely underestimated pedestrian exposure to traffic-related pollution while standing in line. For personal sampling, a limitation was that we did not give more monitoring equipment to the study participants. Concerns that the “Border Commuters” might be mistaken for a security threat at the border limited the amount of equipment we gave them to carry across the SYPOE.

It should be noted that air concentrations reported here should not be compared directly to national and state regulatory standards as the standards apply to large air basin monitoring (not personal exposures (2)). Of the air pollutants measured in this study, only PM_{2.5} and CO are regulated in the USA. The U.S. has an annual average limit of 12 µg/m³ and a 24-hour average limit of 35 µg/m³ (2). Although there is no regulatory standard for UFPs, research has suggested that UFPs may be important contributors to health risks due to their large surface area and their ability to reach the alveoli (4) and to translocate into the systemic circulation (5, 6). BC is

another air contaminant of health concern with no environmental regulatory standard. It is often used as a marker for diesel exhaust but lacks the specificity of 1-NP.

SYPE is currently undergoing a reconfiguration and expansion due to the 30 year old facility no longer being able to support current and projected future traffic needs. San Diego's Regional Planning Agency (SANDAG) predicts a 70% increase in vehicle traffic by 2030 (76). The new SYPE will have 62 northbound vehicle primary inspection booths (currently 24) with one dedicated bus lane; however, the pedestrian pathway will still be in the same geographic location and still within several feet of idling vehicle and bus traffic with no physical barrier (ie vegetation or wall) between the source (vehicles) and receptor (pedestrians). It is noteworthy to point out that such physical barriers can significantly impact the dynamics of air pollutant dispersion. For example, previous studies demonstrated decreased exposure to air pollutants among receptors where a physical barrier was present between the source and receptor (109, 110). There is also an impaction factor that may further increase pedestrian exposure to traffic-related pollutants. Impaction is defined as a physical barrier immediately downstream of both the source and receptor. Previous work has shown significant increases of concentration levels of air pollutants with the presence of impaction barriers due to the inhibition of pollutant dispersion (111). The pedestrian pathway is located between 24-lanes of vehicle traffic to the west and a wall to the east (that acts as an impaction barrier) as can be seen in Figure 1C. This poses a significant concern considering the predominant wind direction comes from the west (Figure 2.3). Overall, despite the new SYPE configuration, it will remain a high-density traffic corridor for both vehicles and pedestrians and thus will remain a potentially high exposure environment

for pedestrian commuters. Therefore, adding a physical barrier, such as vegetation, that requires little maintenance between the vehicles and pedestrians seems to be a feasible mitigation factor.

The primary method of reducing traffic-related pollutant exposure would be the use of zero to near-zero emission vehicle technologies; however, factors such as enforcement and feasibility can affect the availability and usage of such vehicles. In addition, the variability of emission vehicle technologies between U.S. and Mexico is considerable when accounting for factors such as vehicle engine type and grade of fuel. Therefore, relying on the use of zero to non-zero emission vehicle technologies to mitigate exposure would be a challenge. The most practical mitigation measure would be to reduce the number of minutes that the pedestrians have to wait in line. This would directly decrease their exposure.

In conclusion, this is the first study that has attempted to quantify traffic-related exposure to pedestrians crossing SYPOE. It is of public health priority to focus on implementing mitigating actions to reduce pedestrian exposure to traffic-related air pollutants as the reported herein levels are similar to studies that show associations between exposure and health risk (82-97). A health study to identify types of health risks and quantify risk experienced by pedestrian commuters is recommended.

Tables and Figures

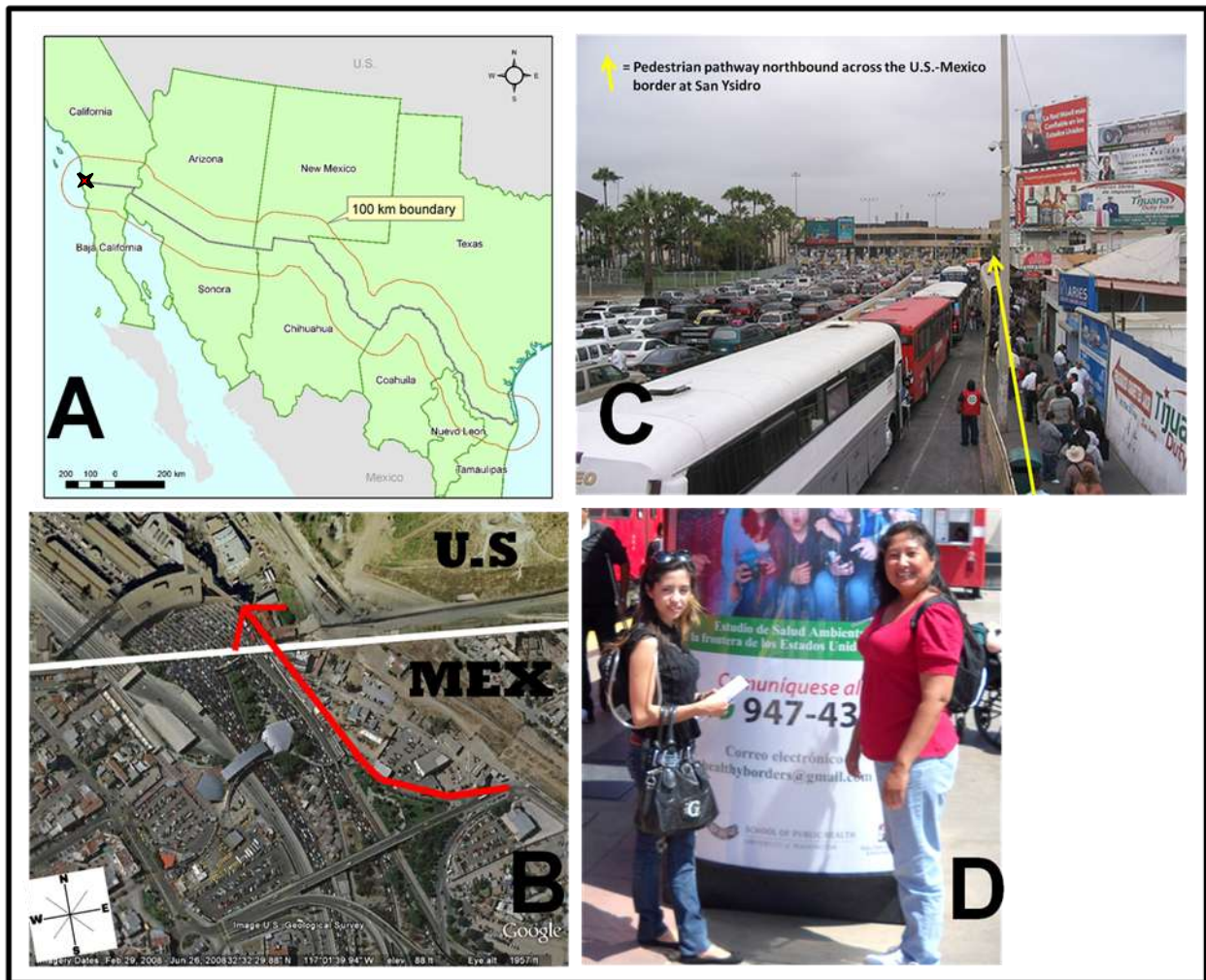


Figure 2.1 U.S.-Mexico Border at San Ysidro and Study Participants 1A) U.S.-Mexico Border with San Ysidro Port of Entry indicated by red “X” Source: EPA page at <http://water.epa.gov/infrastructure/wastewater/mexican/> B) Pedestrian pathway northbound indicated by red arrow C) Proximity of pedestrian pathway to diesel bus lane and 24 vehicle lanes. Photo used with permission from Dr. Albert S. Fu (Kutztown University) D) Study participants wearing personal monitoring equipment (photo used with permission from study participants)

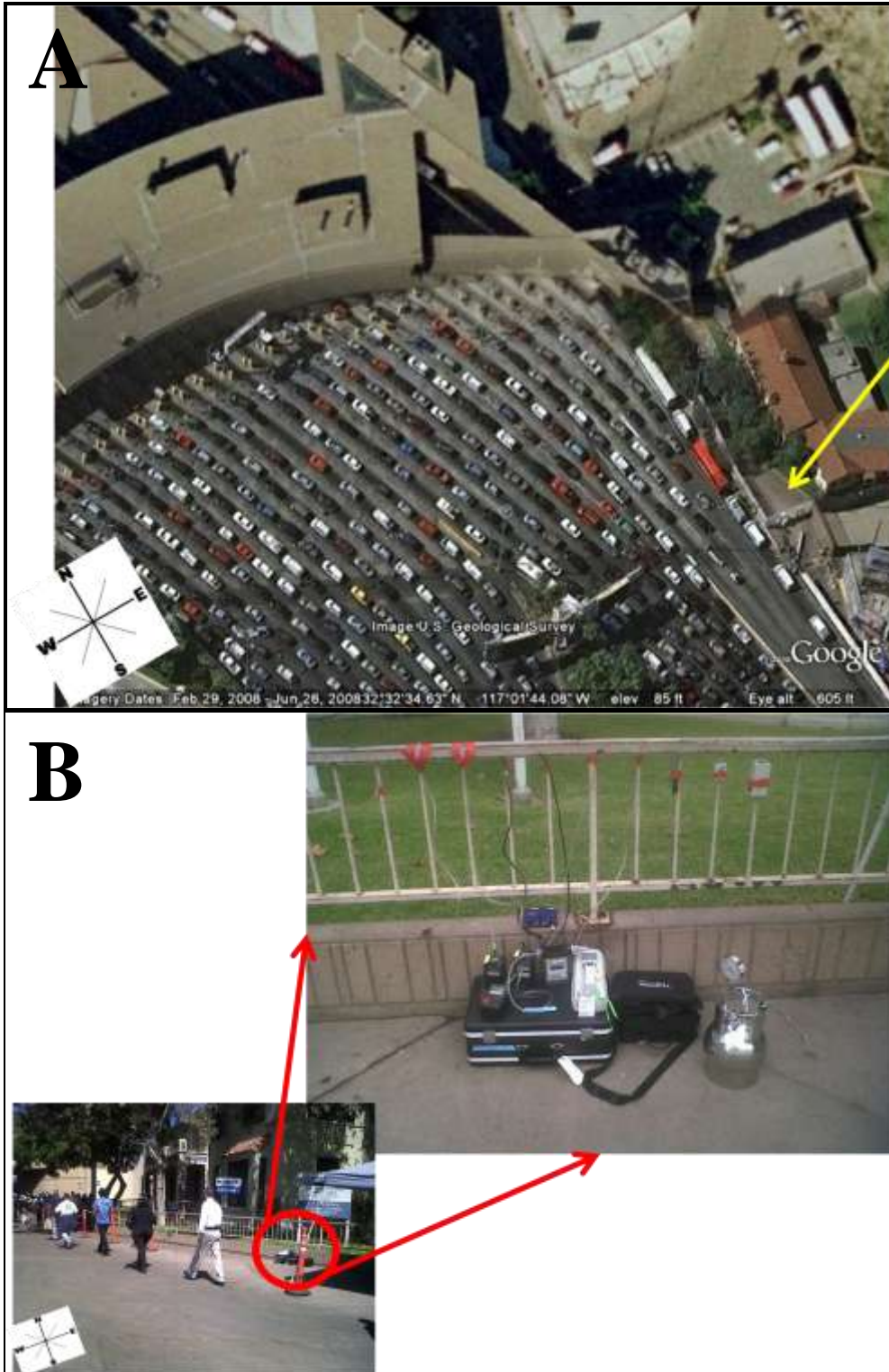


Figure 2.2 Border Monitoring Equipment. 2A) Yellow arrow indicates area where border monitoring data was collected. Figure 3B) Close up of monitoring equipment

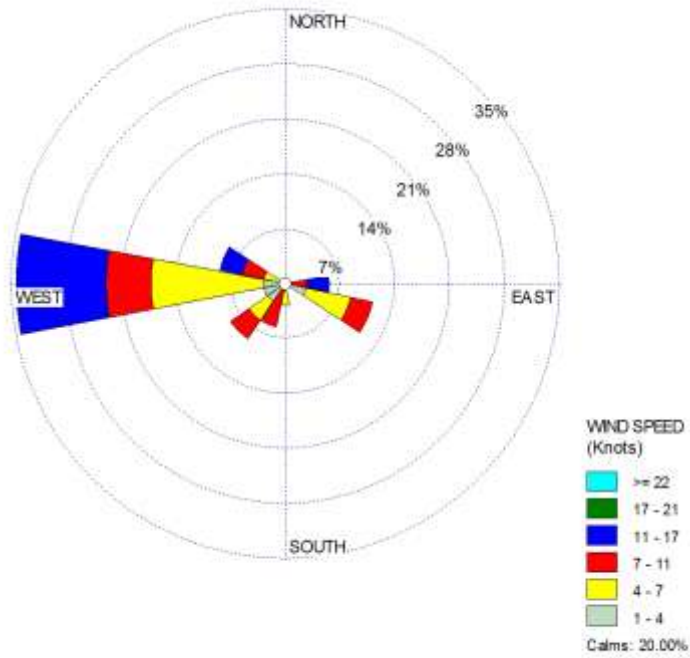


Figure 2.3 Windrose Data

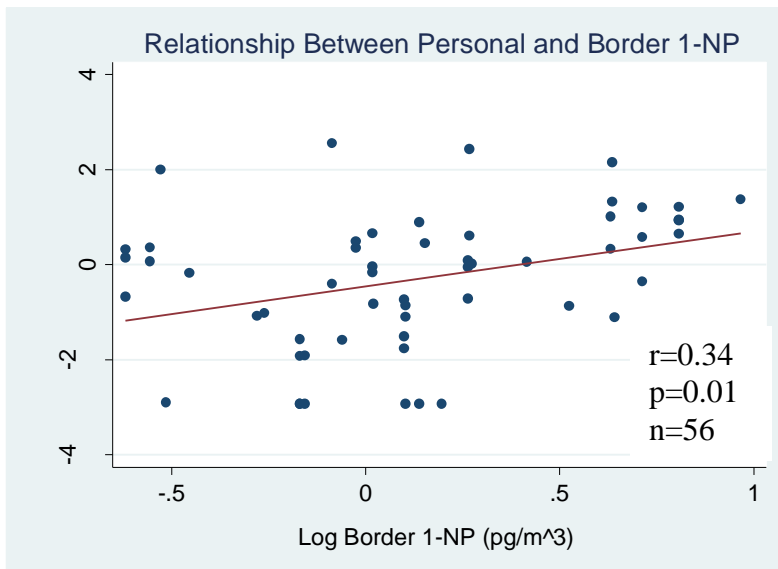


Figure 2.4 Relationship between Personal and Border 1-NP

Table 2.1 Study Participant Characteristics

	Border Crossers			Non-Border Crossers		
	N	Mean	Range	N	Mean	Range
Age	73	26	19-59	18	34	18-76
Sex						
Male	38			5		
Female	35			13		
Education						
≤High School graduate	9			2		
Some post HS education	50			8		
≥College graduate	14			8		
Main Occupation						
Employed	13			11		
Not employed outside home	14			4		
Student	46			3		

Table 2.2 Levels of Traffic-Related Pollutants at the US-Mexico Border Crossing at San Ysidro, CA and Estimated Exposure to Pedestrians During their Northbound Wait in the Pedestrian Line

	n	Mean (SD)	GM	95% CI	RSD%	Median	IQR	Min	Max
Fixed Site Overall									
average 1-NP (pg/m ³)	34	2.0 (2.3)	1.1	[0.78, 1.7]	116	1.3	0.48-2.6	0.24	9.5
gravimetric PM _{2.5} (µg/m ³)	34	24 (28)	18	[15, 23]	118	15	13-22	8	167
UFP (#/cm ³ x 10 ³)	31	40 (17)	36	[30, 43]	43	40	24-52	14	82
real time PM _{2.5} (µg/m ³)	12	41 (21)	35	[24, 52]	37	41	23-57	14	81
BC (µg/m ³)	5	7 (5)	5	[2, 14]	73	4	3-11	3	13
CO (ppm)	12	5 (2)	5	[4, 6]	36	5	3-6	2	8
Vehicle delay (min)	27	79 (23)	75	[66, 85]	29	79	61-93	29	132
# buses passing gate/ hour	29	53 (22)	48	[41, 57]	42	51	39-58	20	105
Border Commuter Exposure									
average 1NP (pg/m ³)	67	2.0 (2.0)	1.3	[1.0, 1.6]	97	1.3	0.68-2.8	0.24	9.3
gravimetric PM _{2.5} (µg/m ³)	67	24 (24)	19	[16, 22]	101	17	12-26	7	147
UFP (#/cm ³ x 10 ³)	50	42 (10)	37	[32, 43]	48	39	28-54	12	91
real time PM _{2.5} (µg/m ³)	17	48 (22)	41	[30, 58]	46	53	30-57	6	94
BC (µg/m ³)	18	7 (4)	6	[4, 8]	57	6	3-11	3	13
CO (ppm)	37	5 (2)	5	[4, 5]	33	6	4-6	2	8
Vehicle delay (min)	58	83 (25)	79	[73, 86]	30	83	65-100	32	137
Border Commute (min)	73	66 (42)	55	[48, 63]	63	60	30-80	20	200

*Sampling times ranged from 53-548 minutes with an average of 198 minutes

Table 2.3 Comparison Between 24-hour Personal Exposure Among Border Commuters and Non-Border Commuters

24-hour Traffic Pollution Measures^a	N	Mean (SD)	GM	95% CI	Median	IQR	Min	Max	p-value
Average 1NP (pg/m ³)									
Border Commuters	56	1.7 (2.3)	0.73	0.50-1.07	0.96	0.33-1.87	0.05	12.8	<0.01*
Non-Border Commuters	15	0.22 (0.21)	0.15	0.09-0.24	0.15	0.05-0.30	0.05	0.86	
PM _{2.5} (µg/m ³)									
Border Commuters	56	39 (30)	31	26-37	26	18-49	13	146	<0.01*
Non-Border Commuters	15	21 (11)	19	15-24	18	13-22	13	48	
CO (ppm)									
Border Commuters	14	2.8 (1.8)	2.3	1.6-3.4	2.2	1.4-4.0	0.78	7.2	<0.01*
Non-Border Commuters	15	1.0 (0.79)	0.63	0.33-1.2	0.80	0.20-1.7	0.10	2.4	

*p-value for comparison between border crossers and non-border crossers using Wilcoxon -Mann-Whitney U-test, significant at the 0.05 level (2-tailed)

^a actual minutes sampled ranged from 964 to 2140 minutes

Table 2.4 Univariate Regression Predictors of Traffic Pollutant, 1-Nitroyprene, Measured at the US-Mexico Port of Entry at San Ysidro.

Model Parameter	B (SE)	r ²	CI	P-value*
Temperature	0.20 (3.5)	<0.01	[-6.8, 7.2]	0.95
Relative Humidity	0.84 (0.82)	0.03	[-0.84, 2.5]	0.32
Season	0.39 (0.41)	0.03	[-0.44, 1.2]	0.34
Wind Speed	-0.28 (0.51)	0.01	[-1.3, 0.76]	0.59
Wind Direction	-0.35 (0.38)	0.03	[-1.1, 0.43]	0.36
Bus Count	-0.59 (1.1)	0.01	[-2.9, 1.7]	0.61
Vehicle Delay	0.66 (0.66)	0.04	[-0.71, 2.0]	0.33

*Significant at the 0.05 level (2-tailed), n = 34 except

Table 2.5 Univariate Regression Predictors of Traffic Pollutant, Gravimetric PM_{2.5}, Measured at the US-Mexico Port of Entry at San Ysidro.

Model Parameter	B (SE)	r ²	CI	P-value*
Temperature	-2.88 (1.85)	0.07	[-6.6, 0.89]	0.13
Relative Humidity	0.58 (0.45)	0.05	[-0.35, 1.5]	0.21
Season	0.29 (0.22)	0.05	[-0.16, 0.75]	0.20
Wind Speed	-0.76 (0.25)*	0.22	[-1.3, -0.25]	0.01
Wind Direction	-0.14 (0.21)	0.01	[-0.57, 0.30]	0.52
Bus Count	0.08 (0.67)	<0.00	[-1.3, 1.5]	0.90
Vehicle Delay	0.11 (0.40)	<0.01	[-0.72, 0.94]	0.79

*Significant at the 0.05 level (2-tailed), n = 34 except

Table 2.6 Univariate Regression Predictors of Traffic Pollutant, Ultrafine Particulate Matter, Measured at the US-Mexico Port of Entry at San Ysidro.

Model Parameter	B (SE)	r ²	CI	P-value*
Temperature	0.86 (1.59)	0.01	[-2.4, 4.1]	0.60
Relative Humidity	-0.72 (0.35)*	0.13	[-1.4, -0.01]	0.05
Season	0.32 (0.18)	0.10	[-0.043, 0.68]	0.08
Wind Speed	0.02 (0.22)	<0.00	[-0.43, 0.46]	0.94
Wind Direction	0.11 (0.17)	0.01	[-0.23, 0.45]	0.52
Bus Count	0.06 (0.56)	<0.00	[-1.1, 1.2]	0.92
Vehicle Delay	0.82 (0.34)*	0.21	[0.12, 1.5]	0.02

*Significant at the 0.05 level (2-tailed), n = 31 for UFP

Table 2.7 Multivariate Regression Predictors of Traffic Pollutant, Ultrafine Particulate Matter, Measured at the US-Mexico Port of Entry at San Ysidro.

Model Parameter	B (SE)	r ²	CI	P-value*
Relative Humidity	-0.43 (0.37)	0.26	[-1.2, 0.34]	0.26
Vehicle Delay	0.67 (0.36)		[-0.083, 1.4]	0.08

*Significant at the 0.05 level (2-tailed), n = 31 for UFP, adjusted r²=0.19

Chapter 3

Determinants of Personal Exposure to 1-Nitroyprene and PM_{2.5} in U.S.-Mexico Border Residents

3.1 Abstract

Background: Factors that predict personal exposure to 1-NP – a marker for diesel exhaust – and PM_{2.5} were identified among a population with high potential for exposure to urban and traffic-related air pollution. **Methods:** The study population included 56 “Border Commuters” defined as Tijuana, Baja California, Mexico residents who commute northbound across the U.S.-Mexico San Ysidro Port of Entry (SYPOE) for work or school in or near San Ysidro, CA, and a comparison group of 15 “Non-Border Commuters” who live in or near San Ysidro and do not cross SYPOE. Home indoor and outdoor and border samples in addition to time activity diaries were collected to estimate the contribution of microenvironments to personal exposure to 1-NP and PM_{2.5}. **Results:** “Border Commuters” had a 3.5-fold higher concentration of PM_{2.5} inside their homes compared to “Non-Border Commuters” ($25 \pm 17 \mu\text{g}/\text{m}^3$ vs $7 \pm 4 \mu\text{g}/\text{m}^3$, $p=0.01$ Mann-Whitney). Their outdoor concentrations were 5-fold higher ($19 \pm 12 \mu\text{g}/\text{m}^3$ vs $4 \pm 2 \mu\text{g}/\text{m}^3$, $p=0.02$ Mann-Whitney). The same trend was seen for 1-NP inside ($0.64 \pm 0.81 \text{ pg}/\text{m}^3$ vs $0.078 \pm 0.075 \text{ pg}/\text{m}^3$, $p=0.04$ Mann-Whitney) and outside ($1.0 \pm 0.93 \text{ pg}/\text{m}^3$ vs $0.27 \pm 0.24 \text{ pg}/\text{m}^3$, $p=0.11$ Mann-Whitney) their homes with “Border Commuters” having 8-fold and 4-fold higher concentrations compared to “Non-Border Commuters”, respectively. Linear regression analysis of personal 1-NP concentrations among border commuters revealed two significant covariates: season and time spent at the border given concentration of 1-NP at the border. Season was the

only predictor of personal $PM_{2.5}$. A seasonal effect was noted with autumn and winter having increased concentrations of 1-NP and $PM_{2.5}$ compared to spring and summer.

3.2 Introduction

Accurate assessment of exposure to ambient air pollution within a population is critical for understanding the extent of exposure. Sheppard mentions that the consequence of exposure measurement error is the possibility of incorrect inferences when the predicted exposure used in an analysis is different from the underlying true exposure (112). To minimize measurement error, this study combines linear regression modeling with time-activity data and personal and microenvironment monitoring, otherwise known as a hybrid model - the preferred method for exposure assessment. However, the feasibility of conducting a hybrid model is constrained by economics, especially when wanting to conduct a large study. Therefore, other practical but least robust models that are used to predict exposure include proximity models, geostatistical models, dispersion models, and land-use regression models. Examples of how these other models are used include studies that rely on outdoor surrogates as a proxy for personal exposure, including ambient concentrations from fixed sampling sites, distance to major roadways, and traffic density (7, 113-115). Using outdoor surrogates to predict exposure and/or health risk can introduce exposure or risk misclassification as a result of inherent uncertainties and assumptions which then impact the magnitude of these estimates. Potentially important contributors to misclassification or confounding include spatial, temporal, and between-person variability. Examples of factors that contribute to spatial and temporal variability include topography (for example inland vs. coast, city vs. urban, valley vs. mountain) and atmospheric conditions

(including rain, wind speed, and humidity). Examples of factors that contribute to between-person variability include socioeconomic status and time activity patterns. Also, people spend a larger percentage of their time indoors, so using an outdoor surrogate to estimate personal exposure may either underestimate or overestimate exposure. Factors that contribute to differences among indoor concentrations include different pollution sources and air exchange rates (which affects penetration efficiency of outdoor air pollution).

The goal of this study is to understand the contribution of microenvironments and time activity patterns to personal exposure to 1-nitropyrene (1-NP) – a marker for diesel exhaust – and PM_{2.5} in a study population with potentially high exposure to urban and traffic-related air pollution. The study population of interest includes residents of Tijuana, Baja California, Mexico who cross the U.S.-Mexico Border San Ysidro Port of Entry (SYPOE) to work or go to school in or near San Ysidro, California, U.S.A (referred to as “Border Commuters”). A comparison group of 15 “Non-Border Commuters” were included and defined as those who live and work or go to school in or near San Ysidro and do not cross into Mexico.

There are well established and reliable methods to collect PM_{2.5} data for personal and area samples. However, the methods that exist for diesel exhaust (DE) exposure lack specificity. Examples of markers that are used for DE exposure include black carbon and elemental carbon. However, recent research has proposed 1-NP to be a more specific marker than conventional markers. The specificity of 1-NP for DE is due to its preferential formation by the specific high temperature combustion processes in diesel engines. In one study of three Japanese cities, greater than 99% of 1-NP in PM was derived from DE (36). 1-NP is also the most abundant nitro-polycyclic aromatic hydrocarbon (NPAH) observed in PM from DE (30, 116). 1-NP is not

formed as a secondary pollutant, because photochemical nitration of airborne pyrene preferentially forms 2-NP and 4-NP, but not 1-NP (106). Thus, 1-NP is highly specific to DE, is readily detectable in urban PM and is expected to be particularly useful as a marker of exposure to DE for use in exposure and health assessment studies. Thus by collecting personal, home indoor and outdoor, and fixed site border measurements of 1-NP and PM_{2.5} this study will assess the exposure impact from different micro-environments and provide insight into important predictor variables that can be used in future studies among this unique study population.

3.3 Materials and Methods

3.3.1 Personal and Fixed Site Sampling

Personal exposure to 1-NP and PM_{2.5} was monitored on seventy-one participants. Fifty-six of the participants (24 female and 32 male) were classified as “Border Commuters.” The remaining 15 participants (10 female and 5 male) were defined as “Non-Border Commuters.” Fixed site measurements of 1-NP and PM_{2.5} were also collected concurrently inside and outside the homes on a subset of participants, during the times participants were at home. For example, if a participant left their home for any particular reason the sampling equipment was turned off and then turned on when they returned home. This was to assure that concentrations were representative of their time spent at home. Fixed site samples were also collected at the San Ysidro Port of Entry (SYPE) during the time “Border Commuters” crossed the border northbound. Fixed site instrument inlets were approximately 1.2 meters (4 feet) above the ground and co-located with a RH/Temp Data Logger (Onset Corporation,

Bourne, MA). Home indoor samples were placed in the living room and outdoor samples were either in the front or back yard depending on the security of the location as to prevent theft. Participant recruitment, eligibility criteria, and sample collection details are described elsewhere (102).

3.3.2 *Statistical Analysis*

All statistical analysis was performed with STATAIC ver11 (STATA Corp LP, College Station, Texas). The exposure data were not normally distributed and thus log transformations were used for all continuous linear regression variables. Nonparametric comparison tests (Wilcoxon-Mann-Whitney U-test) were used to compare differences between “Border Commuters” and “Non-Border Commuters.” Significance was calculated with 95% confidence. Two linear regression models were applied to predict personal “Border Commuter” exposure to 1-NP and PM_{2.5}. Prior to modeling independence of errors, linearity, homoscedasticity of residuals, multicollinearity, and influential outliers were tested. Predictor variables included “Season”, “Total Travel”, “Total Travel Mexico”, “Total Travel U.S.”, “Near Diesel” (only for the 1-NP regression model), $C_{\text{border}} \times T_{\text{border}}$ (henceforward referred to as “Border Exposure), and $C_{\text{home}} \times T_{\text{border}}$ (henceforward referred to as “Home Exposure”). “Season” was dichotomized into Spring and Summer (3/20-9/21) vs. Autumn and Winter (9/22-3/19) with “Spring and Summer” being the reference category based on the primary pathway for degradation being photodecomposition (106); “Total Travel” was the amount of time (in minutes) spent on or near a roadway; “Total Travel Mexico” was the amount of time (in minutes) spent on or near a roadway in Mexico; “Total Travel U.S.” was the amount of time (in minutes) spent on or near a

roadway in the U.S.; “Near Diesel” was the amount of time (in minutes) spent near any diesel powered vehicles or equipment; “Border Exposure” was the concentration of 1-NP (in pg/m^3) or $\text{PM}_{2.5}$ (in $\mu\text{g}/\text{m}^3$) at the border, multiplied by the amount of time (in minutes) spent at the border. “Home Exposure” was the concentration of 1-NP (in $\text{pg}/\text{m}^3 * \text{min}$) or $\text{PM}_{2.5}$ (in $\mu\text{g}/\text{m}^3 * \text{min}$) inside the home multiplied by the amount of time (in minutes) spent inside the home.

The exposure model is defined as follows:

$$\text{Log}(Y_i) = \beta_0 + \beta_1(\text{season}) + \beta_2\text{Log}(\text{total travel}) + \beta_3(\text{total travel Mexico}) + \beta_4\text{Log}(\text{total travel U.S.}) + \beta_5(\text{near diesel}) + \beta_6 \text{Log}(C_{\text{border}} \times T_{\text{border}}) + \beta_7 \text{Log}(C_{\text{home}} \times T_{\text{border}}) + \varepsilon_i$$

where Y_i is the average, log-transformed concentration of 1-NP or $\text{PM}_{2.5}$ for personal exposure among “Border Commuters”, β_1 - β_7 are the slope estimates for the corresponding covariates, and ε_i is the error term.

3.4 Results

“Border Commuters” and “Non-Border Commuters” had an average age of 26 years (range: 19-59) and 34 years (range: 18-53 years), respectively. Personal exposure, home outdoor and indoor, and border exposure to $\text{PM}_{2.5}$ and 1-NP are summarized in Table 3.1 and 3.2. Personal exposure and border concentrations are reported elsewhere (102) and are included in Table 3.1 and 3.2 for comparison reasons. 1-NP concentrations were highest at the border while $\text{PM}_{2.5}$ were highest for “Border Commuters”. “Border Commuters” had significantly higher exposure to personal 1-NP, personal $\text{PM}_{2.5}$, home outdoor $\text{PM}_{2.5}$, home indoor $\text{PM}_{2.5}$, and home

indoor 1-NP levels compared to “Non-Border Commuters”. Ratio of PM_{2.5} inside vs outside the home for both groups is similar, whereas 1-NP is 3-4 fold higher outdoors vs indoors.

A seasonal effect was seen for personal measurements (Table 3.3): personal 1-NP and PM_{2.5} were higher in the autumn and winter compared to the spring and summer, for both groups, although the differences were only statistically significantly for the “Border Commuters.” The small sample size limited our ability to detect any seasonal differences for home indoor and outdoor concentrations.

The final regression model for “Border Commuters” personal exposure to 1-NP and PM_{2.5} was determined by univariate to multivariate analysis. The results from the univariate analysis are displayed in Tables 3.4-3.6. “Season” was an interaction term for all of the predictor variables except for “Near Diesel.” For 1-NP, “Season,” “Total Travel given season,” and “Border Exposure” were all significant predictors of personal exposure and explained 21%, 28%, and 12% of the variance, respectively. However, “Total Travel given season” was the only covariate that remained significant in the multivariate regression analysis (Figure 3.6). “Season” ($R^2=0.28$) was the only predictor variable for personal exposure to PM_{2.5}.

3.5 Conclusions

We used personal and fixed site area measurements to explore the impact of urban and traffic-related pollution among a population with potentially high exposure to vehicle exhaust. People who reside in Tijuana and cross the border experience different exposures to 1-NP and PM_{2.5} than people who live in or near San Ysidro and do not cross into Mexico. There is a fairly substantial difference with respect to personal exposure and home indoor and outdoor exposure

to 1-NP and PM_{2.5} with “Border Commuters” experiencing higher exposure. The Mann-Whitney analysis of personal exposure to 1-NP and PM_{2.5} indicates a seasonal effect but only among “Border Commuters.” However, the “Non-Border Commuter” comparisons were based on a very small sample size; hence, conclusions based on these results are limited. Our results indicate that there is a geographical link between personal exposure to urban and traffic-related air pollution of 1-NP and PM_{2.5}, with Tijuana having increased concentrations vs. San Ysidro and neighboring California communities.

Secondary to this, a univariate regression model indicates that high personal exposure among “Border Commuters” to 1-NP is due in part to border concentrations and border wait times. Regression coefficients presented in Table 3.4 suggest that for every unit of change in “Border Exposure” there is a corresponding change in personal exposure with the autumn and winter season contributing more to the rate change. The effect of season could be a result of longer border wait time in Autumn and Winter vs. Spring and Summer for both pedestrians (79 ± 45 minutes vs 46 ± 25 minutes) and vehicles (93 ± 24 minutes vs 64 ± 12 minutes). Although “Border Exposure” loses significance in the multivariate regression, the covariate “Total Travel” accounts for time spent at the border, indicating that the border is still an important microenvironment to overall personal exposure to 1-NP. Interestingly, when comparing the beta coefficient for “Border Exposure”, which is expressed as a rate, indicates that the rate of exposure to 1-NP at the border is 2 times higher than the rate of exposure to PM_{2.5}. This suggests that the rate of accumulation while standing at the border is 2 times greater for 1-NP vs. PM_{2.5}. This could be explained by the structural design of SYPE as the pedestrian northbound pathway is located directly east of the bus lane and is within 3 meters (10 feet) of idling buses with no

barriers (such as trees or a wall) to prevent or reduce exposure. The remaining 23 lanes of vehicle traffic are located directly west of the bus lane and so exposure to $PM_{2.5}$ from vehicle emissions could potentially be attenuated by the buses, thereby, reducing personal exposure to non-diesel $PM_{2.5}$ to some extent. The contribution of border exposures to total personal exposure to 1-NP is also supported in that 1-NP concentrations are 4 fold higher (comparing geometric means) at the border compared to home indoor concentrations and 2 fold higher compared to home outdoor concentrations among “Border Commuters.” In contrast, $PM_{2.5}$ concentrations differ less dramatically between the different fixed site locations, with border and home indoor concentrations being equal and both being 1.3 fold higher than home outdoor concentrations.

Potential limitations to this study include modified study participants’ behavior as a result of participating in this study and having to carry personal monitors. However, they were instructed to go about their activities according to their regular routine to the extent possible. Model parameters that described location, time spent traveling, and time spent near diesel were collected on a time activity diary sheet that was filled out by study participants every two hours for 24-hours concurrently while wearing the personal monitoring equipment. Since the diaries were filled out promptly the likelihood of poor or inaccurate recall is reduced. This information could have further been refined to reduce estimate error by having participants wear a personal GPS unit; unfortunately, this pilot study had financial limitations.

3.6 Discussion

Although hybrid models that use time activity data, personal, and microenvironment monitoring are preferable, such models may not be feasible in large scale studies. However,

more refined exposure estimates can reduce exposure error and bias and ultimately minimize exposure misclassification. Thus the ability to use this powerful exposure assessment method in a pilot study has advantages for future larger studies within the same population, including, the ability to understand the impact of microenvironments on overall exposure and to identify and investigate important predictor variables. The main objective of this study was to assess if there were any covariates important in predicting personal exposure to DPM and $PM_{2.5}$ and to quantify exposure in various microenvironments to understand the extent of exposure to 1-NP and $PM_{2.5}$ among a susceptible population. The hybrid design of the exposure assessment in the current study helps ensure that inferences made from this study have minimized error and bias.

In summary, “Total Travel given season” is a significant predictor of personal exposure to 1-NP among “Border Commuters” following a multivariate regression analysis. In addition, “Season” is an important predictor of personal exposure to $PM_{2.5}$. Overall, “Border Commuters” had significantly higher overall exposures as compared to “Non-Border Commuters.” This confirms that geographic heterogeneity in ambient concentrations and subject demographics results in substantial heterogeneity in personal exposure to 1-NP and $PM_{2.5}$. Demographic heterogeneity includes differences in activity patterns such as having to wait hours in a pedestrian line within 3 meters (10 feet) of 24 lanes of idling vehicles and buses to cross into the U.S. to work and or go to school. Another important example of demographic heterogeneity includes socioeconomic disparities between a developed (i.e.. USA) and developing country (i.e.. Mexico) which inevitably impacts the quality of the infrastructure, including vehicle fleet, grade of fuel, industrial emissions, and housing characteristics. Solutions to reduce exposure to 1-NP and $PM_{2.5}$ must consider practicality. Considering that “Border Exposure” weighted by time is a

predictor in the univariate analysis for personal 1-NP, it seems a logical approach would be to reduce border wait times. However, this entails collaborative efforts by both the U.S. and Mexican governments.

Tables

Table 3.1 PM_{2.5} (µg/m³) Concentrations for Personal and Fixed Site Samples

	Border	“Border Commuters”			“Non-Border Commuters”		
	Area	Personal	Home Outdoor	Home Indoor	Personal	Home Outdoor	Home Indoor
N	34	56	14	22	15	3	3
Mean (SD)	24 (28)	39 (30)	19 (12)	25 (17)	21 (11)	4 (2)	7 (4)
GM	18	31	14	18	19	4	6
95% CI	[15, 23]	[26, 37]	[9, 22]	[13, 25]	[15, 24]	[1, 10]	[3, 14]
Median	15	26	16	17	18	4	5
IQR	13-22	18-49	6-27	13-37	13-22	2-6	4-9
Min	8	13	5	4	13	2	4
Max	167	146	45	77	48	7	12
RSD%	116	77	72	74	54	55	60
P*		<0.01	0.02	0.01			
ND (%)	18 (53)	0 (0)	2 (14)	2 (9)	0 (0)	0 (0)	0 (0)

*p-value for comparison between border crossers and non-border crossers using Wilcoxon -Mann-Whitney U-test, significant at the 0.05 level (2-tailed)

Table 3.2 1-NP (pg/m³) Concentrations for Personal and Fixed Site Samples

	Border	“Border Commuters”			“Non-Border Commuters”		
	Area	Personal	Home Outdoor	Home Indoor	Personal	Home Outdoor	Home Indoor
N	34	56	14	22	15	3	3
Mean (SD)	2.0 (2.3)	1.7 (2.3)	1.0 (0.93)	0.64 (0.81)	0.22 (0.21)	0.27 (0.24)	0.078 (0.075)
GM	1.1	0.73	0.63	0.27	0.15	0.20	0.059
95% CI	[0.78, 1.7]	[0.50-1.1]	[0.34, 1.2]	[0.15, 0.049]	[0.089, 0.24]	[0.056, 0.75]	[0.017, 0.20]
Median	1.3	1.0	0.73	0.20	0.15	0.18	0.040
IQR	0.49-2.6	0.33-1.9	0.19-1.6	0.079-0.83	0.054-0.30	0.11-0.43	0.040-0.11
Min	0.24	0.054	0.094	0.040	0.054	0.11	0.040
Max	9.5	13	2.8	3.0	0.86	0.61	0.19
RSD%	116	150	89	127	99	89	97
P*		<0.01	0.11	0.04			
ND (%)	6 (18)	6 (11)	0 (0)	5 (23)	4 (27)	0 (0)	1 (33)

*p-value for comparison between border crossers and non-border crossers using Wilcoxon -Mann-Whitney U-test, significant at the 0.05 level (2-tailed)

Table 3.3 Seasonal Difference for Personal Exposure to PM_{2.5} and 1-Nitropyrene

	N	Mean (SD)	GM	95% CI	Median	IQR	Min	Max	P*
1-NP									
Border Commuters									
Spring & Summer	13	1.2 (3.5)	0.22	[0.09, 0.55]	0.2	0.05-0.33	0.05	12.8	<0.00
Autumn & Winter	43	1.9 (2.3)	1.05	[0.72, 1.5]	1.2	0.49-2.5	0.05	11.4	
Non-Border Commuters									
Spring & Summer	8	0.15 (0.087)	0.12	[0.07, 0.22]	0.14	0.06-0.21	0.05	0.30	0.38
Autumn & Winter	7	0.30 (0.29)	0.18	[0.06, 0.52]	0.29	0.05-0.40	0.05	0.86	
PM_{2.5}									
Border Commuters									
Spring & Summer	13	17 (4)	16	[14, 19]	16	13-18	13	25	<0.00
Autumn & Winter	43	46 (31)	37	[31, 45]	38	23-55	13	146	
Non-Border Commuters									
Spring & Summer	8	22 (10)	20	[15, 28]	19	16-23	13	44	0.19
Autumn & Winter	7	19 (13)	17	[11, 27]	13	13-21	13	48	

*p-value for Wilcoxon -Mann-Whitney U-test, significant at the 0.05 level (2-tailed)

Table 3.4 Univariate Regression Predictors of Personal Measurements to 1-Nitropyrene among “Border Commuters”

Model Parameter	β (SE)	r^2	CI	P-value*
Season	1.55 (0.41)*	0.21	[0.73, 2.4]	<0.00
Total Travel	0.52 (0.93)	0.01	[-1.3, 2.4]	0.58
Total Travel Season	4.12 (0.81)*	0.28	[0.37, 7.9]	0.03
Total Travel U.S.	0.08 (0.43)	<0.00	[-0.78, 0.95]	0.84
Total Travel U.S. Season	1.4 (1.01)	0.25	[-0.58, 3.5]	0.16
Total Travel Mexico	0.46 (0.71)	0.01	[-0.97, 1.9]	0.52
Total Travel Mexico Season	1.2 (1.33)	0.22	[-1.5, 3.9]	0.38
Near Diesel	0.30 (0.40)	0.01	[-0.51, 1.1]	0.46
Near Diesel Season	0.06 (0.78)	0.22	[-1.5, 1.6]	0.94
$C_{\text{border}} \times T_{\text{border}}$	0.92 (0.34)*	0.12	[0.24, 1.6]	0.01
$C_{\text{border}} \times T_{\text{border}} \text{Season}$	-0.66 (0.33)*	0.26	[-3.6, 2.3]	0.65
$C_{\text{home}} \times T_{\text{home}}$	-0.41 (0.50)	0.01	[-1.4, 0.59]	0.42
$C_{\text{home}} \times T_{\text{home}} \text{Season}$	-1.21 (1.19)	0.23	[-3.6, 1.2]	0.32

*Significant at the 0.05 level (2-tailed), n=56

Table 3.5 Univariate Regression Predictors of Personal Measurements to PM_{2.5} among “Border Commuters”

Model Parameter	β (SE)	r²	CI	P-value*
Season	0.35 (0.08)*	0.28	[0.20, 0.51]	<0.01
Total Travel	0.23 (0.18)	0.03	[-0.14, 0.60]	0.21
Total Travel Season	0.34 (0.37)	0.30	[-0.40, 1.1]	0.36
Total Travel U.S.	0.04 (0.09)	<0.00	[-0.13, 0.21]	0.65
Total Travel U.S. Season	0.26 (0.19)	0.32	[-0.13, 0.65]	0.19
Total Travel Mexico	0.21 (0.14)	0.04	[-0.072, 0.49]	0.14
Total Travel Mexico Season	0.03 (0.26)	0.28	[-0.49, 0.54]	0.92
Near Diesel	-0.01 (0.07)	<0.00	[-0.17, 0.15]	0.93
Near Diesel Season	0.01 (0.15)	0.26	[-0.28, 0.31]	0.92
C _{border} xT _{border}	-0.02 (0.10)	<0.01	[-0.22, 0.18]	0.84
C _{border} xT _{border} Season	-0.33 (0.18)	0.33	[-0.68, 0.024]	0.07
C _{home} xT _{home}	-0.04 (0.059)	0.01	[-0.16, 0.074]	0.45
C _{home} xT _{home} Season	0.07 (0.12)	0.29	[-0.17, 0.31]	0.56

*Significant at the 0.05 level (2-tailed), n=56

Table 3.6. Multivariate Regression Predictors of Personal Measurements to 1-Nitropyrene among “Border Commuters”

Model Parameter	β (SE)	r^2	CI	p-value *
Season	-1.58 (1.41)		[-4.4, 1.3]	0.27
Total Travel	-2.66 (1.6)		[-5.9, 0.54]	0.10
Total Travel Season	3.9 (1.8)*	0.32	[0.23, 7.6]	0.04
$C_{border} \times T_{border}$	0.55 (0.32)		[-0.1, 1.2]	0.09

*Significant at the 0.05 level (2-tailed), adjusted $r^2=0.27$, n=56

Chapter 4

Urinary metabolites of 1-Nitropyrene in US-Mexico Border Residents Who Frequently Cross the San Ysidro Port of Entry

4.1 Abstract

The use of 1-nitropyrene (1-NP) and its major urinary metabolites as markers for occupational exposure to diesel exhaust (DE) exposure has previously been proposed. However, few studies have applied these markers to assess personal exposure to traffic-related concentrations of DE. We report herein results from a pilot community-based study that evaluated the suitability of using 1-NP and its major urinary metabolites as markers of exposure to traffic-related DE. Ninety-one participants were enrolled in the study; seventy-three were classified as the high exposure group and 18 as the low exposure group. Two metabolites of 1-NP were readily detected in urine samples, of which 8-hydroxy-1-nitropyrene (8-OHNP) was the most abundant metabolite followed by 8-hydroxy-N-acetyl-1-aminopyrene (8-OHNAAP). A subset of the participants had creatinine concentrations available and thus corrected for accordingly. The high exposure group had a 2-fold increased concentration of 8-OHNP vs the low exposure group for both creatinine adjusted (0.094 ± 0.12 pg/mg creatinine vs 0.047 ± 0.032 pg/mg creatinine, $p=0.11$ Mann-Whitney) and non-creatinine adjusted concentrations (0.071 ± 0.066 pg/mL vs 0.032 ± 0.021 pg/mL, $p<0.01$ Mann-Whitney). The same trend was seen for 8-OHNAAP with creatinine adjusted having a 2-fold increase concentration (0.11 ± 0.11 pg/mg creatinine vs 0.049 ± 0.032 pg/mg creatinine, $p=0.11$ Mann-Whitney) and non-creatinine adjusted having a 3-fold increase concentration (0.063 ± 0.11 pg/mL vs 0.021 ± 0.013 pg/mL,

p=0.11 Mann-Whitney) for the high exposure group vs the low exposure group. There was a positive correlation between personal 1-NP and both 8-OHNP and 8-OHNAAP (for both creatinine and non-creatinine adjusted) but significant only for 8-OHNAAP (for both creatinine and non-creatinine adjusted). There was a positive association between 8-OHNP and 1-NP concentrations at the border given the time spent at the border before and after creatinine adjustments. 1-NP concentrations inside the homes given the time spent at home was also positively associated with both metabolites with the exception of the unadjusted 8-OHNP metabolite in which no association was seen. Linear regression analysis revealed that “Total Travel” was a significant predictor of 8-OHNP for both adjusted and unadjusted concentrations. “Season” was a predictor of only unadjusted 8-OHNAAP concentrations.

4.2 Introduction

Epidemiological and toxicological studies have indicated that exposure to diesel exhaust (DE) is associated with a variety of adverse health effects. IARC has classified DE as a probable human carcinogen. The chemical substances in diesel exhaust (DE) that have been found to contribute to its mutagenicity include nitropolycyclic aromatic hydrocarbons (NPAHs). NPAHs are a group of organic compounds and are mainly originated from combustion sources such as diesel vehicle exhaust emissions or formed indirectly as a result of photo-oxidation processes of their parent compounds, PAHs, with atmospheric oxidants such as hydroxyl or nitrate radicals (49, 117-119). Their atmospheric concentrations usually range around a few picograms per cubic meter (25, 48). NPAHs are associated with fine particulate matter (PM_{2.5}) with stronger associations seen with ultrafine particles (UFP) (14). Particle size distribution of NPAHs towards

the fine particulate matter (PM_{2.5}) and even more so towards UFPs highlights their importance regarding health effects due to the fact that particle size controls the deposition behavior of particulate matter in the lung; While PM_{2.5} can reach the pulmonary alveoli, UFPs are deposited at much higher in efficiency due to their smaller aerodynamic size. In addition, direct acting mutagenicity per unit mass of UFP is significantly higher than PM_{2.5} (120, 121).

1-nitropyrene (1-NP) is a four ring NPAH and thus condenses onto particulate matter due to its low vapor pressure. It is one of the main contributors of the direct-acting mutagenicity of NPAH (122-124). 1-NP formation is derived directly from diesel combustion with no evidence of photochemical formation (106, 125). 1-NP is greatly enriched in DE compared to other sources of particulate air pollution, and has been used in a number of studies as a unique marker for DE levels with concentrations ranging from ~1-1200 picograms per cubic meter (36, 48, 50, 102, 125-127). In addition, metabolites of 1-NP have been postulated as suitable urinary biomarkers for exposure to DE. Metabolism of 1-NP has been studied extensively in vitro (in human and animal cell lines) and in vivo in animals. Metabolism proceeds via P450 mediated C-oxidation, acetylation, and nitroreduction (55-59, 128). The major urinary metabolites that have been observed in vivo are hydroxy-1-nitropyrenes (OHNPs), hydroxy-N-acetyl-1-aminopyrenes (OHNAAPs), N-acetyl-1-aminopyrene (NAAP) and 1-aminopyrene (1-AP) (51, 55, 64, 129-132). The most abundant isomers in human urine are 6- and 8-OHNAAP and 6-, and 8-OHNP (41, 42). Human and animal studies have demonstrated that the metabolites of 1-NP in urine are elevated following acute exposure to DE (51-53). With human research demonstrating an association between urinary 1-NP metabolites and elevated occupational or environmental exposure to DE (38, 40, 41), these markers may be useful in exposure assessment studies.

Exposure to DE occurs in conjunction with exposures to other traffic-related or urban air pollutants including gasoline exhaust and industrial sources, which are also complex mixtures; thus, complicating estimates of DE exposure among people. Previous studies have used common surrogate markers to indicate levels of exposure to DE, including black carbon, elemental carbon, and PAH ratios (28, 30). However, these surrogate markers are subject to interference from non-diesel sources, which can lead to misclassification of personal exposure. Thus, the specificity of 1-NP and its urinary metabolites allows for reliable quantification of personal exposure to DE and an assessment of internal dose. The overarching goal of this study is to understand the relationship between 1-NP and its urinary metabolites and to evaluate predictors of urinary metabolites in a susceptible population.

4.3 Materials and Methods

4.3.1 Study Population

Two different cohorts were recruited for participation in this study. One cohort served as the comparison group (“Non-Border Commuters”) to a cohort that had potentially high exposure to traffic-related pollutants (“Border Commuters”). “Border Commuters” were those that lived in Tijuana, Baja California, Mexico and crossed the San Ysidro Port of Entry (SYPOE) to work or go to school in or near San Ysidro, California, U.S.A. “Non-Border Commuters” were participants that lived and worked or went to school in or near San Ysidro and did not cross into Mexico. Recruitment details and descriptive statistics are described in detail elsewhere (102,

127). To quickly summarize sampling efforts, a total of 91 participants (73 “Border Commuters” and 18 “Non-Border Commuters) were enrolled in the study; all self-classified as Hispanic.

4.3.2 Sample Collection

All participants provided spot urinary samples for analysis of 1-NP urinary metabolites, using the method described in detail by Miller-Schulze (41) which is able to detect up to seven urinary metabolites (3-, 6-, and 8-OHNP, 3-, 6-, and 8-OHNAAP, and NAAP). Measurements of two of the seven metabolites were considered sufficiently reliable and robust for inclusion in this study and included 8-OHNP and 8-OHNAAP. Urine samples were collected in polyethylene (HDPE) bottles and immediately placed on ice and transferred to the San Diego State University School of Public Health Laboratory and stored in a -20°C freezer. Prior to analysis they were shipped to the University of Washington Department of Environmental and Occupational Health Sciences. Urine volumes collected ranged from 20-482 with an average of 165 ml. Extractions required 100 ml of urine. Of the 91 urine samples, 25 had volumes <100 ml and thus were diluted with DI water to bring volume to 100 ml. Metabolite concentrations in mass per volume of sample extract were calculated from the HPLC/MS/MS chromatograms, by taking the ratio of the metabolite peak area to the internal standard, then dividing by the slope of the calibration curve. The calibration slope was determined by linear regression with a 1/x weighting and forcing to an intercept of zero. Concentrations were then adjusted for total urine volume collected and final extraction volume. Limit of detection (LOD) was 0.015 pg/mL for 8-OHNP and 0.020 pg/mL for 8-OHNAAP, and calculated by taking the average of the water blanks that were spiked with internal standard. Concentrations below the limit of detection were substituted

with $LOD/\sqrt{2}$. Final metabolite concentrations are reported both unadjusted, and after adjusting for creatinine to control for diuresis.

Seventy-one of the 91 participants (56 “Border Commuters” and 15 “Non-Border Commuters”) had personal 24-hour measurements of 1-NP. Personal 1-NP sampling has previously been described in detail (102). To quickly summarize 1-NP sampling, personal 1-NP was collected with a PM_{2.5} impactor (BGI HPEM, Waltham, MA and SKC PEM, Eighty Four, PA) connected to a SKC personal air sampling pump (SKC AirChek Pump XR5000, Eighty Four, PA) operated at 4 liters per minute. Analysis of personal 1-NP data are reported elsewhere (102, 127).

4.3.3 Statistical Analysis

STATA1C ver 11 (STATA Corp, LP, Texas) was the statistical software used for all data analysis except for the generation of the 3-D plots in which SigmaPlot ver 10 (Systat Software Inc, San Jose, CA) was used. Comparisons between “Border Commuters” and “Non-Border Commuters” for urinary metabolites were done using a Wilcoxon-Mann-Whitney U-test. A linear regression model for each metabolite was developed to identify predictor variables of importance. Log transformations were used for all continuous linear regression variables due to data not being normally distributed. Independence of errors, linearity, homoscedasticity of residuals, multicollinearity, and influential outliers were tested prior to modeling. Comparisons and linear regressions were calculated with 95% confidence. Predictor variables of interest included “Season”, “Total Travel”, “Total Travel Mexico”, “Total Travel U.S.”, “Near Diesel”, “Personal 1-NP”, “ $C_{border} \times T_{border}$ ” (henceforward referred to as “Border Exposure”), and

“ $C_{\text{home}} \times T_{\text{home}}$ ” (henceforward referred to as “Home Exposure”). “Season” was dichotomized into Spring and Summer (3/20-9/21) vs. Autumn and Winter (9/22-3/19); “Total Travel” was the amount of time (in minutes) spent on or near a roadway; “Total Travel Mexico” was the amount of time (in minutes) spent on or near a roadway in Mexico; “Total Travel U.S.” was the amount of time (in minutes) spent on or near a roadway in the U.S.; “Near Diesel” was the amount of time (in minutes) spent near any diesel powered vehicles or equipment; “Personal 1-NP” was the 24-hour personal concentration of 1-NP in pg/m^3 ; “Border Exposure” was the concentration of 1-NP (in pg/m^3) at the border, multiplied by the amount of time (in minutes) spent at the border; “Home Exposure” was the concentration of 1-NP (in pg/m^3) at the home, multiplied by the amount of time (in minutes) spent at the home.

The exposure model is defined as follows:

$$\begin{aligned} \text{Log}(Y_i) = & \beta_0 + \beta_1(\text{season}) + \beta_2\text{Log}(\text{total travel}) + \beta_3\text{Log}(\text{total travel Mexico}) + \beta_4\text{Log}(\text{total} \\ & \text{travel U.S.}) + \beta_5\text{Log}(\text{near diesel}) + \beta_6\text{Log}(\text{personal 1-NP}) + \beta_7\text{Log}(C_{\text{border}} \times T_{\text{border}}) + \\ & \beta_8\text{Log}(C_{\text{home}} \times T_{\text{home}}) + \varepsilon_i \end{aligned}$$

where Y_i is the concentration of 8-OHNP or 8-OHNAAP for personal exposure among “Border Commuters”, β_1 - β_8 are the slope estimates for the corresponding covariates, and ε_i is the error term.

4.4 Results

Of the seven urine metabolites quantified, only 8-OHNP and 8-OHNAAP were considered reliable and included in the analysis. Table 1 and 2 summarizes the descriptive

statistics for the two metabolites between “Border Commuters” and “Non-Border Commuters” for both unadjusted and adjusted concentrations. Fifty-six of the urine samples for 8-OHNAAP were below the LOD (43 “Border Commuters” and 13 “Non-Border Commuters”) while 9 of the urine samples were below the LOD for 8-OHNP (6 “Border Commuters” and 3 “Non-Border Commuters”). “Border Commuters” had a 2-fold higher mean concentration for both unadjusted and adjusted 8-OHNP (with unadjusted having a statistically significant difference), a 3-fold higher mean concentration for unadjusted 8-OHNAAP, and 2-fold higher average concentrations for adjusted 8-OHNAAP concentrations.

The relationship between each metabolite (for both adjusted and unadjusted) vs personal 1-NP, “Border Exposure”, and “Home Exposure” was assessed. Figure 4.1 shows there is a positive correlation between personal 1-NP and unadjusted concentrations of 8-OHNP and 8-OHNAAP (significant for 8-OHNAAP). The same trend was seen for adjusted concentrations of 8-OHNP ($r=0.1$, $p=0.39$, $n=48$) and 8-OHNAAP ($r=0.4$, $p=0.03$, $n=27$). To further explore the relationship between personal 1-NP and metabolites without bias in either direction as a result of non-detects (NDs) a scatterplot using only detectable values were plotted (Figure 4.2); similar trends were seen.

Unadjusted 8-OHNP and 8-OHNAAP concentrations were positively associated with “Border Exposure” (Figure 4.3) with the same trend seen for adjusted 8-OHNP concentrations ($r=0.2$, $p=0.19$, $n=34$); however, no association was seen for 8-OHNAAP ($r=-0.2$, $p=0.38$, $n=22$). Figure 4.4, which excludes NDs, shows a similar trend between the adjusted metabolites and “Border Exposure.”

There was no association between “Home Exposure” and unadjusted 8-OHNP even after excluding the NDs (Figure 4.5 & 4.6). These figures do show a positive association between “Home Exposure” and unadjusted 8-OHNAAP. Adjusted concentrations for both metabolites reported positive associations (8-OHNP: $r=0.2$, $p=0.36$, $n=37$; 8-OHNAAP: $r=0.1$, $p=0.77$, $n=24$).

Univariate analysis for “Border Commuters” resulted in one significant predictor variable for each metabolite. Results are only shown for non-adjusted 8-OHNP concentrations (Table 4.2). “Total Travel” was the only significant predictor variable for 8-OHNP for both adjusted and non-adjusted concentrations. Predictor variable for 8-OHNAAP included “Season” but only for unadjusted concentrations ($\beta=0.28$, $SE=0.11$, $r^2=0.09$, $p=0.01$). Season was an interaction term for some of the predictor variables, with concentrations increasing during the autumn and winter season vs the spring and summer season, and thus accounted for accordingly in each univariate analysis.

Exploratory analysis was conducted to try and understand the relationship between external exposure (personal 1-NP) and internal uptake (urinary metabolites) among all study participants. The association between 8-OHNP and 8-OHNAAP was slightly positive with no difference in trend with and without the NDs (Figure 4.7) but after normalizing each metabolite for personal 1-NP concentrations a significantly strong relationship was seen (Figure 4.8). To further understand the variability of 1-NP on each metabolite, a 3-dimensional plot was created with and without the NDs. The plot revealed that each metabolite is more strongly associated with personal 1-NP compared to each other (Figure 4.9 and 4.10). This led to the idea that 1-NP

metabolites could potentially be predictive of personal 1-NP. To explore this, a linear regression model was tested. The exposure model is defined as follows:

$$\text{Log}(Y_i) = \beta_0 + \beta_1 \text{Log}(8\text{-OHNP}) + \beta_2 \text{Log}(8\text{-OHNAAP}) + \varepsilon_i$$

where Y_i is the concentration of personal 1-NP, β_1, β_2 are the slope estimates for the corresponding covariates, and ε_i is the error term.

The results indicate that only 8-OHNAAP was a significant predictor of personal 1-NP (Table 4) with or without the NDs, explaining between 12-15% of the variance. However, exclusion of NDs did increase the β coefficient from 1.1 to 1.5 but there was no significant difference between β coefficients.

4.5 Discussion

This is the first quantitative study done using both a biomarker of external exposure (1-NP) and biomarkers of internal exposure (1-NP metabolites) to understand the extent of exposure in this disproportionately impacted population. As described previously, the use of 1-NP and its metabolites show great promise as markers for DE due to their specificity and sensitivity. Results demonstrated that “Border Commuters” have higher concentrations of both metabolites compared to “Non-Border Commuters.” Previous analysis showed that “Border Commuters” had higher personal 1-NP concentrations than “Non-Border Commuters” (102), concluding that “Border Commuters” have higher external and internal exposure. However, the relationship between personal 1-NP and urinary metabolites was only slightly positive with a stronger and

significant relationship seen for 8-OHNAAP. Miller-Schulze also reported a stronger and significant relationship between 1-NP and 8-OHNAAP compared to 8-OHNP (41). The stronger relationship between 1-NP and 8-OHNAAP suggests a difference in clearance pattern between 8-OHNP and 8-OHNAAP. This raises the concern that an unmeasured factor (ie. physiological difference such as genetics) is an important determinant of urinary metabolite concentrations of 1-NP that could present a significant source of misclassification. Previous research has hypothesized that N-acetyltransferase (NAT) is active in the metabolism of 1-NP (133). NAT1 and NAT2 are isozymes of NAT that catalyze acetylation of which common polymorphisms exist for both isozymes. However, polymorphisms of NAT2 tend to have a greater influence on the overall acetylation (134). Given the expected role of NAT2 in formation of acetylated compounds (and 8-OHNAAP being an acetylated compound) and the common frequency of the corresponding slow and fast acetylator NAT genotypes, it is possible that the rate of clearance of 1-NP metabolites could be influenced by polymorphisms of NAT enzymes. Given the ability to measure acetylation rate phenotype for NAT2 by measuring metabolism of caffeine (135)², it is recommended that future work involving 1-NP metabolites explore the association between 8-OHNAAP and NAT2 genotype.

To understand if the variability between personal 1-NP and urinary metabolites was due to between person 1-NP measurements, urinary metabolites were normalized to personal 1-NP. This resulted in a very strong relationship between urinary metabolites, suggesting that variability between personal 1-NP and urinary metabolite concentrations is due to some other

² Walker et al. comment that the caffeine assay is not specific to NAT2 and may include the effects of other metabolizing enzymes such as NAT1. Yet phenotypes determined using caffeine as a probe are consistent with other means of measuring NAT2 activity, so the caffeine test is considered a reliable indicator for NAT2 phenotypes (134).

factor(s) after controlling for personal exposure to 1-NP. Other factors may include the need to account for time as a result of the metabolic half-life of 1-NP, genetics (as mentioned previously), or environment. Overall, this study confirms the utility of being able to use 1-NP metabolites as biomarkers for DE for further research in this study population as urinary metabolite concentrations for the two metabolites were both detectable and demonstrated the ability to detect differences across two different exposure groups. However, further research needs to be done to further understand the between-person variability for 1-NP metabolite concentrations.

In addition this is the first quantitative study to characterize important predictor variable of 1-NP urinary metabolites. Results reported that “Total Travel” was a significant predictor of 8-OHNP. Previous results also reported “Total Travel” to be a significant predictor of personal 1-NP in the same study cohort (127). “Total Travel” included the amount of time spent at the border and given that there was positive association between 8-OHNP and “Border Exposure” suggests the importance of border having an impact on personal exposure to DE given both the concentration of 1-NP and the amount of time spent at the border.

Tables and Figure

Figure 4.1 Relationship Between Unadjusted Urinary Metabolites and Personal 1-NP Among all Study Participants

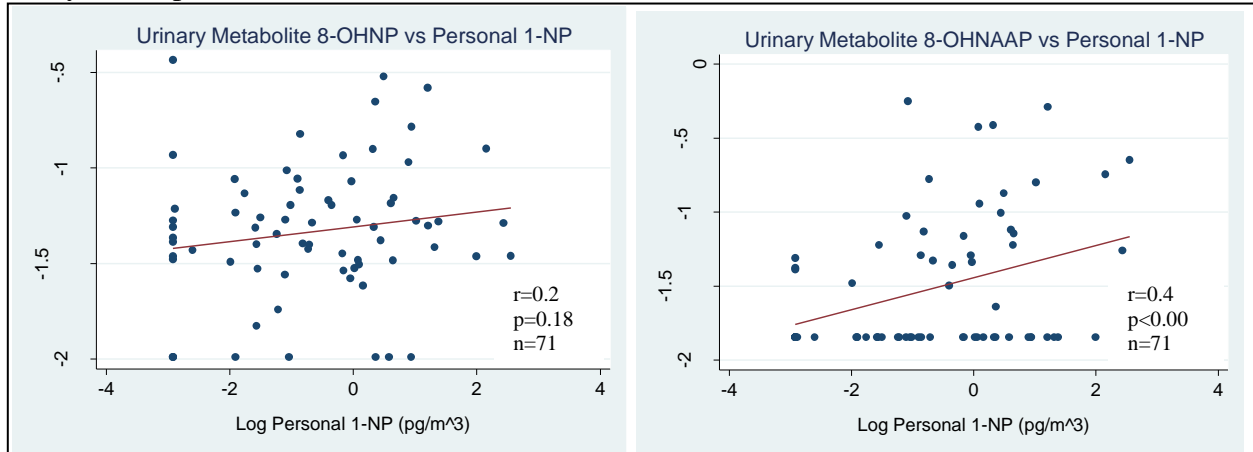


Figure 4.2 Relationship Between Unadjusted Urinary Metabolites and Personal 1-NP Among all Study Participants Excluding Values Below Limit of Detection

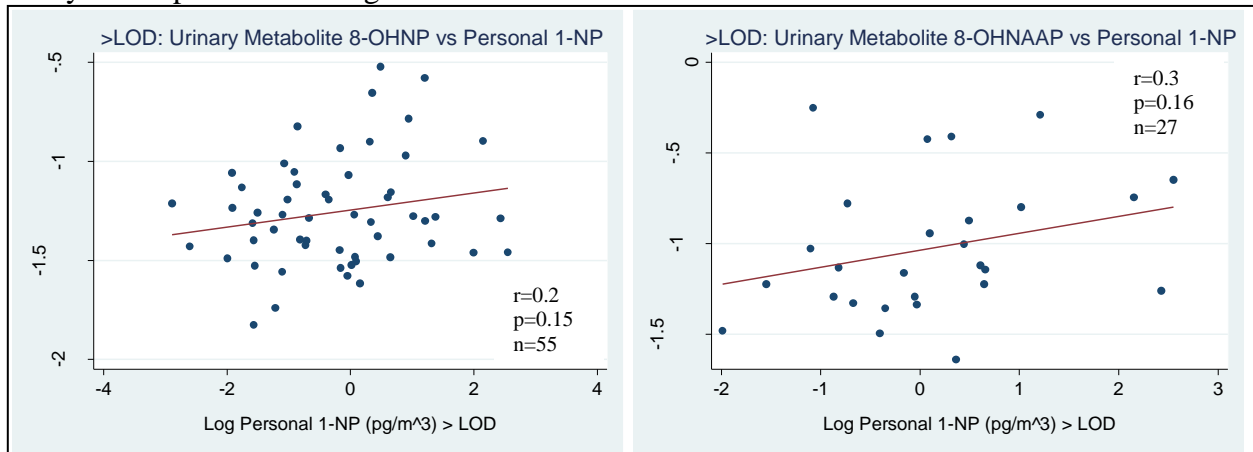


Figure 4.3 Relationship Between Unadjusted Urinary Metabolites and Border Exposure Among “Border Commuters”

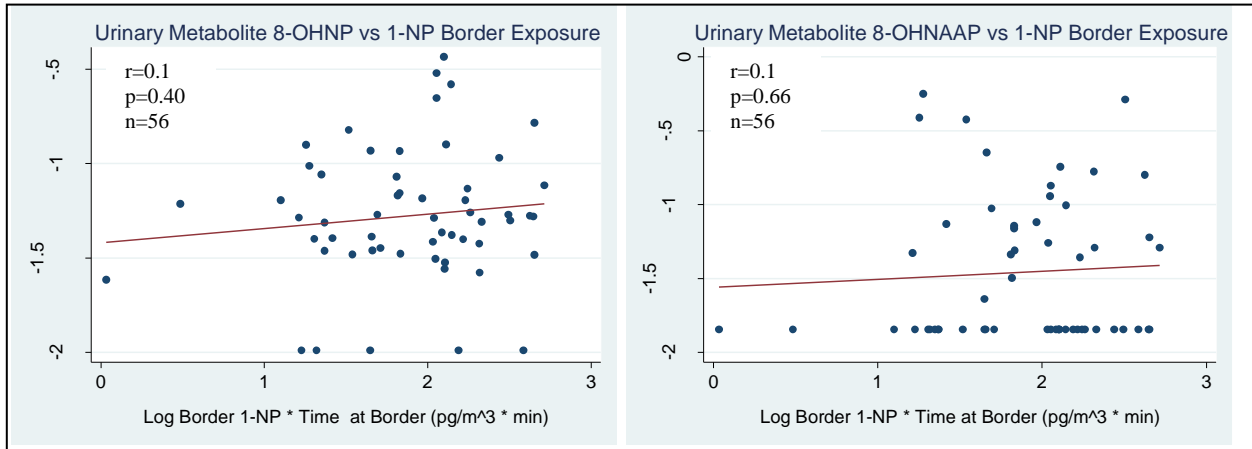


Figure 4.4 Relationship Between Unadjusted Urinary Metabolites and Border Exposure Among “Border Commuters” Excluding Values Below Limit of Detection

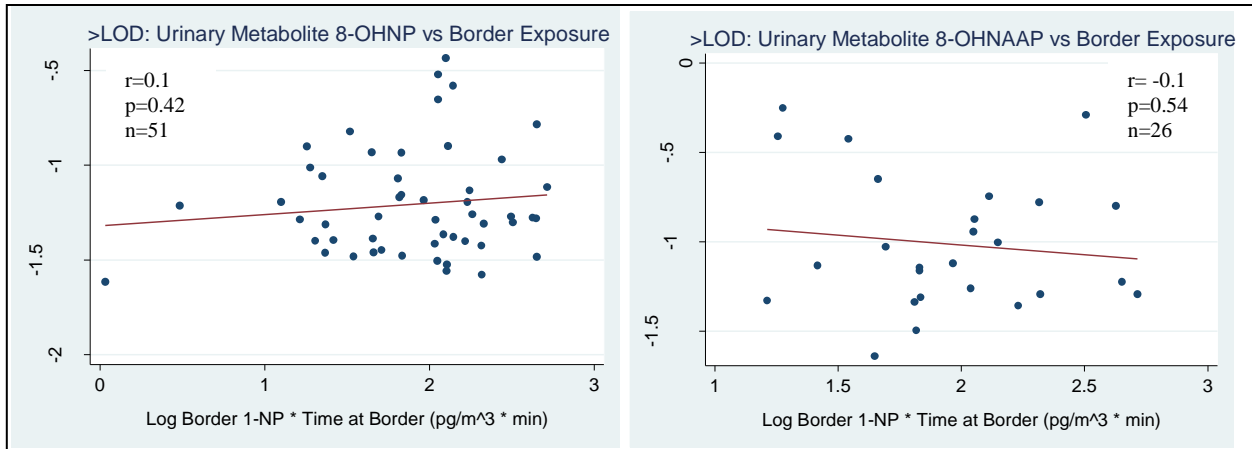


Figure 4.5 Relationship Between Unadjusted Urinary Metabolites and Home Exposure Among all Study Participants

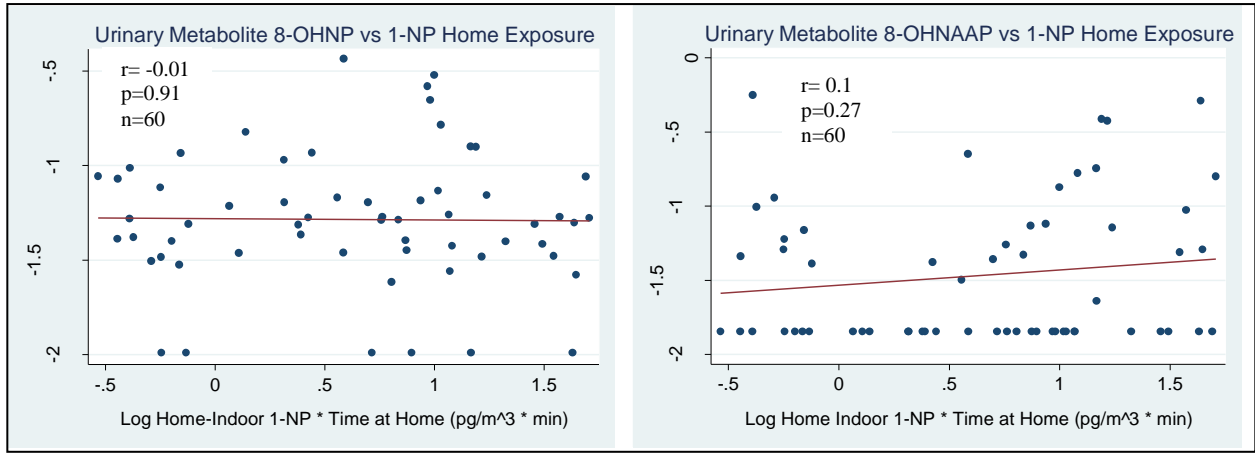


Figure 4.6 Relationship Between Unadjusted Urinary Metabolites and Home Exposure Among all Study Participants Excluding Values Below Limit of Detection

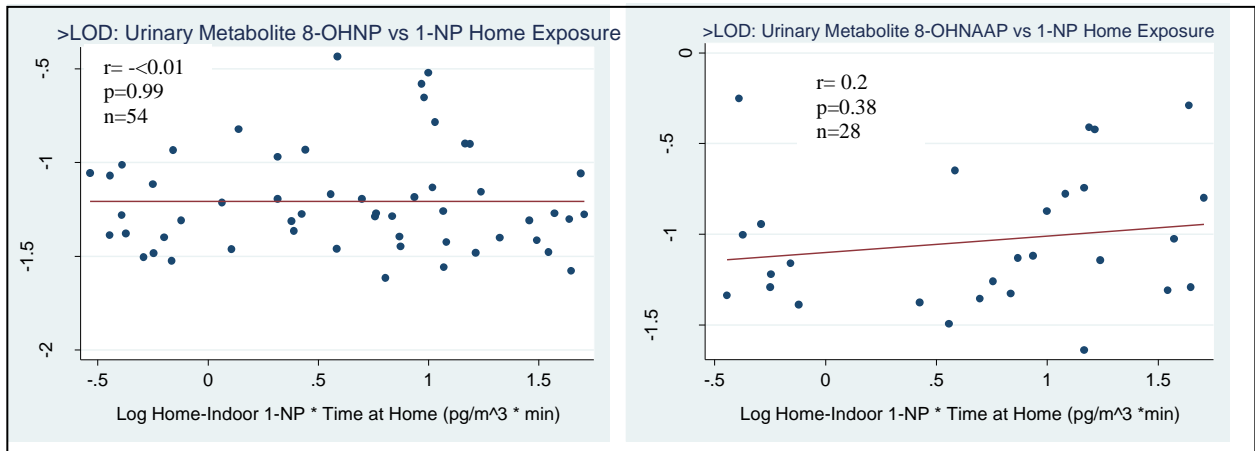


Figure 4.7 Relationship Between Unadjusted Urinary Metabolites for all Study Participants

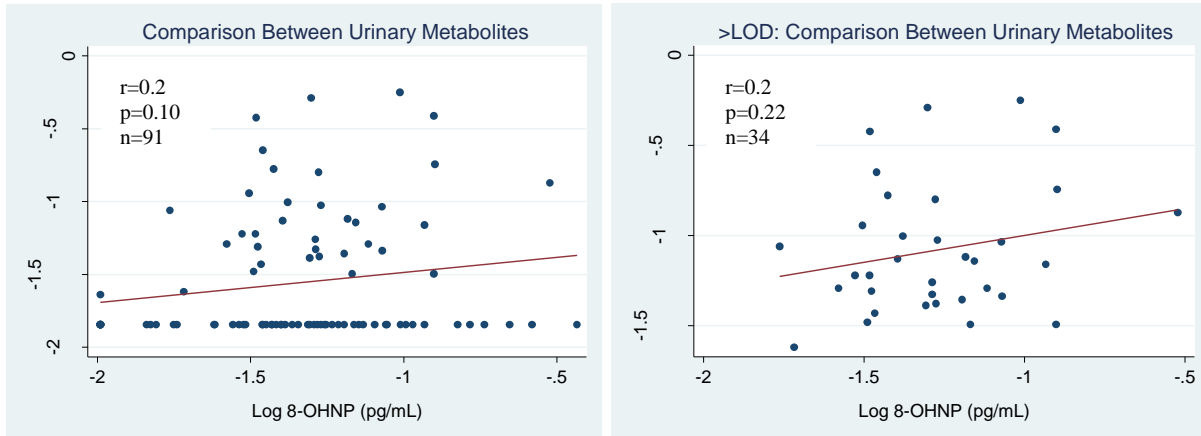


Figure 4.8 Relationship Between Unadjusted Urinary Metabolites Normalized to Personal 1-NP Among all Study Participants Excluding Values Below Limit of Detection

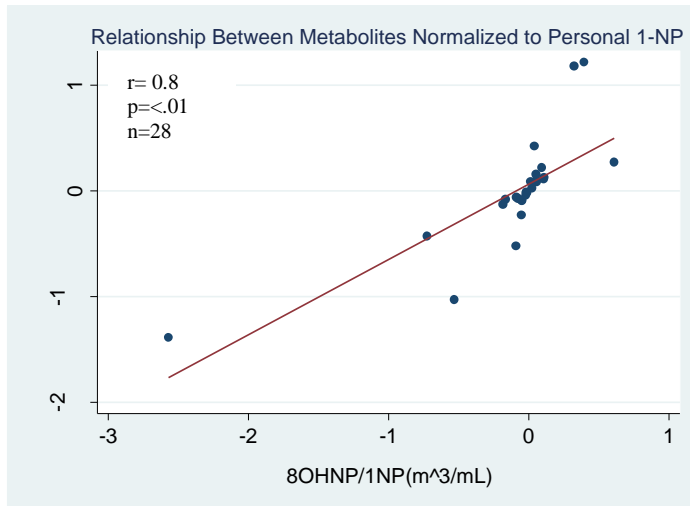


Figure 4.9 3-D Relationship Between Unadjusted Urinary Metabolites and Personal 1-NP Among all Study Participants

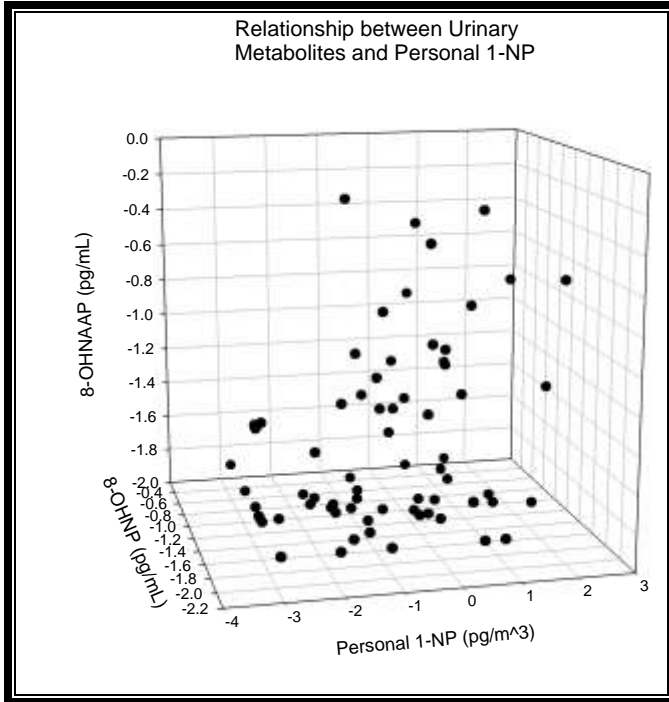
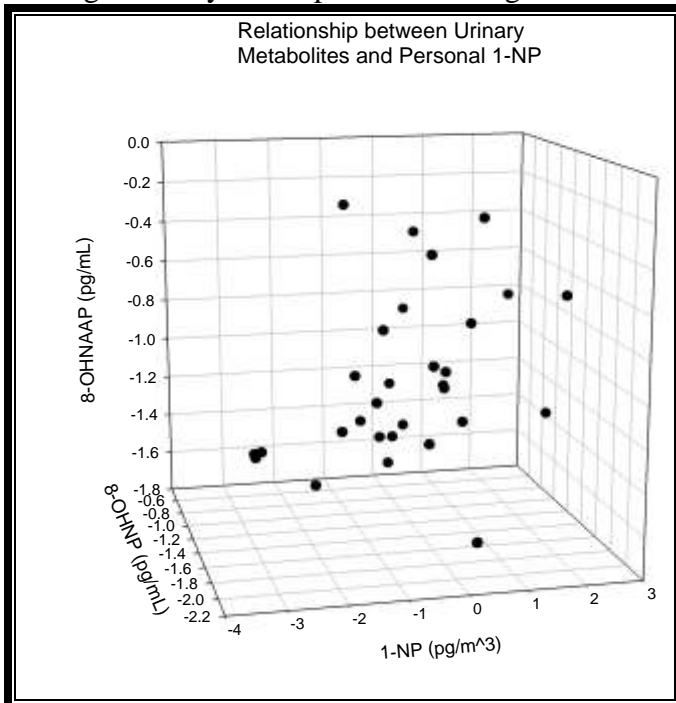


Figure 4.10 3-D Relationship Between Unadjusted Urinary Metabolites and Personal 1-NP Among all Study Participants Excluding Values Below Limit of Detection



Tables and Figures

Table 4.1 Comparison Between 8-OHNP Before and After Adjusting for Creatinine

	UnAdjusted		Adjusted	
	8-OHNP (pg/mL)		8-OHNP (pg/mg creatinine)	
	Border Commuters	Non-Border Commuters	Border Commuters	Non-Border Commuters
N	73	18	49	17
Mean (SD)	0.071 (0.066)	0.032 (0.021)	0.094 (0.12)	0.045 (0.032)
GM	0.052	0.027	0.059	0.038
95% CI	[0.043, 0.062]	[0.019, 0.037]	[0.045, 0.077]	[0.027, 0.054]
Median	0.052	0.029	0.048	0.037
IQR	0.034 - 0.085	0.016 – 0.045	0.034 – 0.098	0.022 – 0.062
Range	0.010 – 0.37	0.010 – 0.088	0.010 – 0.71	0.011 – 0.13
p-value*	<0.01		0.11	

* p-value for Mann-Whitney U-test comparing “Border Commuters” and “Non-Border Commuters” with a significance of $p < 0.05$

Table 4.2 Comparison Between 8-OHNAAP Before and After Adjusting for Creatinine

	UnAdjusted		Adjusted	
	8-OHNAAP (pg/mL)		8-OHNAAP (pg/mg creatinine)	
	Border Commuters	Non-Border Commuters	Border Commuters	Non-Border Commuters
N	73	18	26	6
Mean (SD)	0.063 (0.11)	0.021 (0.013)	0.11 (0.11)	0.049 (0.032)
GM	0.031	0.019	0.072	0.040
95% CI	[0.024, 0.039]	[0.015, 0.024]	[0.050, 0.10]	[0.018, 0.089]
Median	0.014	0.014	0.066	0.051
IQR	0.014 – 0.06	0.014 – 0.024	0.039 – 0.14	0.017 – 0.062
Range	0.014 – 0.56	0.014 – 0.06	0.012 – 0.57	0.014 – 0.10
p-value*		0.11		0.11

* p-value for Mann-Whitney U-test comparing “Border Commuters” and “Non-Border Commuters” with a significance of $p < 0.0$

Table 4.3 Univariate Regression Predictors of Unadjusted 8-OHNP (pg/mL) Among “Border Commuters”

Model Parameter	B (SE)	r ²	CI	P-value*
Season	0.11 (0.084)	0.023	[-0.060, 0.28]	0.20
Total Travel	0.43 (0.20)	0.06	[0.030, 0.83]	0.036
Total Travel U.S.	0.18 (0.092)	0.055	[<0.00, 0.37]	0.060
Total Travel U.S. Season	-0.028 (0.22)	0.074	[-0.15, 0.60]	0.90
Total Travel Mexico	0.084 (0.16)	<0.00	[-0.24, 0.41]	0.61
Total Travel Mexico Season	0.20 (0.33)	0.017	[-0.50, 0.44]	0.56
Near Diesel	<-0.00 (0.10)	<0.00	[-0.20, 0.19]	0.96
Near Diesel Season	<-0.14 (0.20)	0.038	[-0.24, 0.43]	0.48
Personal 1-NP	0.010 (0.033)	<0.00	[-0.061, 0.072]	0.86
Personal 1-NP Season	-0.059 (0.082)	0.035	[-0.22, 0.11]	0.48
C _{border} xT _{border}	0.076 (0.088)	0.014	[-0.10, 0.25]	0.39
C _{border} xT _{border} Season	-0.25 (0.41)	0.035	[-0.52, 1.1]	0.55
C _{Home} xT _{Home}	-0.021 (0.073)	<0.00	[-0.17, 0.12]	0.77
C _{Home} xT _{Home} Season	0.15 (0.17)	0.038	[-0.41, 0.16]	0.39

*Significant at the 0.05 level (2-tailed)

Table 4.4 Univariate Regression Predictors of Personal 1-NP (pg/m³) Among all Study Participants with and without Non-Detects (NDs)

Model Parameter	N	B (SE)	r ²	CI	P-value*
8-OHNP	71	0.68 (0.50)	0.026	[-0.31, 1.7]	0.18
8-OHNP excluding NDs	63	0.52 (0.66)	0.010	[-0.80, 1.8]	0.43
8-OHNAAP	71	1.1 (0.37)	0.12	[0.41, 1.9]	<0.01
8-OHNAAP excluding NDs	30	1.4 (0.66)	0.15	[0.092, 2.8]	0.04

*Significant at the 0.05 level (2-tailed)

Chapter 5

Conclusions

Traffic-related air pollution is an insidious problem, causing health effects at various levels of exposure; especially diesel exhaust (DE) which is now considered a carcinogen. Lower socioeconomic status populations suffer higher exposure to urban and traffic-related air pollution, consequently increasing their risk for associated health effects. People who live in Tijuana, Mexico are particularly vulnerable to this threat compared to San Diego, CA residents as a result of socioeconomic differences between the developed and developing countries. Risk factors for developing countries, such as Mexico, include increased home indoor and outdoor air pollution due to consequential effects of their urban environment including but not limited to industry, poor vehicle fleet, lax of air pollution standards, domestic heating from fossil fuels, and absences of advances in clean technology. Additionally, Tijuana residents who commute on foot across the border for work or school in San Diego experience augmented exposure due to long border wait times and close proximity to highly concentrated traffic, specifically diesel buses. However, it is the pursuit of higher income from better paying jobs or a U.S. education that can provide greater opportunities for socioeconomic success that drives the sacrifice of pedestrians to endure long border wait times coupled with toxic exposure to exhaust fumes from vehicles within feet of their border wait. The primary goal of this study was to characterize traffic-related exposure in this disproportionately impacted population of pedestrians where quantitative data was lacking in the efforts of understanding the extent of exposure. In addition, investigation of exposure as a result of border delays and other possible exposure risk factors is essential in that it

can provide necessary information used to aide community input on the San Ysidro border crossing now in redevelopment planning, and can provide quantitative data on the benefits of reducing border delays.

This study estimated exposure on seventy-three border commuters who live in Tijuana and a comparison group of 18 non-border commuters who live in San Diego. Questionnaires, time activity diaries, and urine samples were collected from all participants. Home indoor and outdoor measurements of PM_{2.5} and 1-NP and personal exposures to PM_{2.5}, 1-NP, and CO were measured for single 24-hour periods on a subset of participants. Border measurements of PM_{2.5}, 1-NP, CO, UFPs, and BC were taken at the border during participants' commutes. Personal and at-home concentrations were all significantly higher among border commuters. Border concentrations were similar to other near-road studies. Area border measurements of 1-NP given the amount of time border commuters spent at the border was a predictor of personal 1-NP. In addition, the rate of accumulation for 1-NP vs. PM_{2.5} was 2 times greater. To estimate body burden of DE two urinary metabolites of 1-NP were quantified. Border crossers were found to have 2-fold and 3-fold increased concentrations of 8-OHNP and 8-OHNAAP, respectively. Positive associations between external exposure (1-NP) and metabolite concentrations were seen.

These results reinforce the idea that socioeconomics modify exposure to ambient air pollutants with increased external exposure and uptake of body burden for those disproportionately impacted. Residents of Tijuana not only have increased background exposure but their frequent northbound commute across SYPOE augments their exposure to toxic air pollutants as a result of standing within feet of idling stop-and-go vehicle traffic. Measures to decrease exposure are necessary to minimize risk of adverse health effects. Reduction of

background exposure would be challenging and lengthy as this would require drastic infrastructure changes in Tijuana. Such changes would demand advances in clean technology and much more stringent air pollution standards for industry, vehicle fleet, and trash burning as a means of waste disposal. On the other hand, measures to reduce pedestrian exposure to traffic-related pollutants at the border would be more feasible, immediate, and could potentially have a large impact on their overall exposure to toxic air pollutants as levels at the border are similar to studies that show associations between exposure and health risk. Practical and effective measures include decreasing border wait times and placing a barrier between the vehicles and the pedestrians. An inexpensive barrier could include the use of vegetation that requires minimal maintenance. Such preventative measures are the most effective tool for maximizing health and socioeconomic well being.

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Appendix A: Pictures of Recruitment Ad in Spanish Only

IconAdMedia
Icon AdMedia Location Form

Site Name: San Ysidro Trolley---Blue Line

Date: 05-26-10

Location & Number: West Side Center.--#1-(Single)



Picture #: 101

Description: Left Side Panel

Picture #: 102

Description: Front Header

Picture #: 103

Description: Right Side Panel



Picture #:

Description: Approach Angle

Graphics Changed to: University of Washington Graphics

Gane hasta \$50.00

Estudio de Salud Ambiental
de la frontera de los Estados Unidos-México

**Gane hasta
\$50.00**



Estudio de Salud Ambiental
de la frontera de los Estados Unidos-México

Comuníquese al
619 947-4319

Correo electrónico
healthyborders@gmail.com



SCHOOL OF PUBLIC HEALTH
UNIVERSITY of WASHINGTON



SAN DIEGO STATE
UNIVERSITY



www.vendsight.com

CLIENT: Univ. of Washington
 DATE: 05.18.10 VERSION: 3.10
 PROJECT: Refreshment Center



Appendix B: Recruitment Flyer in English



You can join a US-Mexico Border “Environmental Health Study”!



Casa Familiar, in collaboration with San Diego State University, is performing an environmental health study near the San Diego/Tijuana border. This study needs participants who answer YES to the following questions:



- Are you between 18 and 60 years of age and have no chronic lung, liver, or heart conditions?
- Are you free of diesel exposure both non-occupationally and occupationally?

If you participate, you will be asked to:

- Carry small air monitoring equipment for 24-hours
- Answer five questions every two hours for 24-hours
- Answer a short questionnaire about your commute.
- Provide a sample of urine

You can receive up to \$50 for participation in the study

Contact Dr. Penelope Quintana from San Diego State University at healthyborders@gmail.com or (619) 813-4265

Appendix C: Recruitment Flyer in Spanish



¡Participe en el Estudio de Salud Ambiental de la frontera de los Estados Unidos-México!



Casa Familiar, en colaboración con la Universidad Estatal de San Diego está llevando a cabo un estudio de salud ambiental cerca de la frontera San Diego/Tijuana.



- ¿Tiene usted entre 18 y 60 años de edad y está usted libre de enfermedades crónicas de pulmón, hígado o del corazón??
- ¿Es usted una persona que no está expuesta a diesel tanto de manera ocupacional o no ocupacional?

Si usted desea participar, se le pedirá lo siguiente:

- Llevar un pequeño equipo de monitoreo durante 24 horas.
- Contestar cinco preguntas cada dos horas durante 24 horas.
- Contestar un pequeño cuestionario acerca de sus traslados de un lugar a otro.
- Proporcionar una muestra de orina.

Usted puede recibir hasta \$50 por participar en el estudio

Contacto Dr. Penelope Quintana de la Universidad Estatal de San Diego at healthyborders@gmail.com or (619) 813-4265

Appendix D: Eligibility Script for “Border Commuters” in English

Exposure Monitoring Eligibility Script: Subjects
San Diego State University School of Public Health

Study of Environmental Health among Border Pedestrians

Casa Familiar, in collaboration with San Diego State University, is performing an environmental health study near the San Diego/Tijuana border. Before you give your permission to participate, it is important that we identify if you are eligible to participate by asking you a few questions.

Part I. Exclusion Criteria for Subjects

- Yes / No 1. Are you over 18 years of age?
- Yes / No 2. Are you and the people in your household non-smokers?
- Yes / No 3. Are you free of any chronic lung, liver, or heart conditions?
- Yes / No 4. Are you free of diesel exposure both non-occupationally and occupationally. Examples include: aircraft ground crew, fisherman, bus driver, diesel gas-station employee, 18-wheeler trucker, and diesel mechanic (including aircraft, locomotive, and 18-wheeler mechanic).
- Yes / No 5. Do you live in Tijuana, B.C. and cross the border **more than** 4 days per month?
- Yes / No 6. Are you willing to consent to participate for one day and cross at the San Ysidro border crossing for this study, per study parameters?
- Yes / No 7. Are you willing to answer questions about home/work/school exposures and border crossing experiences?

If you answered **NO** to any of the questions we want to thank you for participating in our questionnaire however by answering *no* to any of the questions has disqualified you from being able to participate in our study. Your time was greatly appreciated. Thank you again for participating.

If you answered **YES** to all the questions you may continue on to Part II.

Part II. Personal Information

Name: _____

Contact Information: _____

Preferred language: _____

Dates Available to Participate: _____

ID Number

Appendix E: Eligibility Script for “Border Commuters” in Spanish

Guía de Elegibilidad del Seguimiento de Exposición: Candidatos

Escuela de Salud Pública de la Universidad Estatal de San Diego

Salud ambiental entre los peatones de la frontera

Casa Familiar, en colaboración con la Universidad Estatal de San Diego está llevando a cabo un estudio de salud ambiental cerca de la frontera San Diego/Tijuana. Antes de que nos de su permiso para participar es importante que le hagamos unas cuantas preguntas para identificar si usted es elegible.

Parte I. Criterios de exclusión para los candidatos

- Si / No 1. ¿Tiene usted más de 18 años de edad?
- Si / No 2. ¿Usted o algunas personas que viven en su hogar no son fumadores?
- Si / No 3. ¿Está usted libre de enfermedades crónicas de pulmón, hígado o del corazón?
- Si / No 4. ¿Es usted una persona que no está expuesta a diesel tanto de manera ocupacional o no ocupacional? Por ejemplo: personal de tierra de aviones, pescadores, chofer de camiones, empleado de estación de gasolina, camionero de 18 ejes, y mecánico de diesel (incluyendo mecánico de aviones, locomotoras y camiones de 18 ejes).
- Si / No 5. ¿Viven en Tijuana, B.C. y cruza la frontera **más de** 4 días por mes?
- Si / No 6. ¿Está usted dispuesto a participar en el estudio durante un día de acuerdo con los parámetros del estudio?
- Si / No 7. ¿Está usted dispuesto a contestar las preguntas acerca de su exposición en el hogar/trabajo/escuela y experiencias del cruce fronterizo?

Si contestó que **NO** a cualquiera de las preguntas queremos agradecerle por participar en nuestro cuestionario puesto que el haber contestado que *no* a cualquiera de las preguntas lo ha descalificado de participar en nuestro estudio. Su tiempo es muy importante. Gracias de nuevo por participar.

Si usted contestó que **SI** a todas las preguntas usted puede continuar con la Parte II.

Part II. Información Personal

Nombre: _____

Información de contacto: _____

Idioma de preferencia: _____

Fechas disponibles para participar: _____

Número de identificación:

Appendix F: Eligibility Script for “Non-Border Commuters” in English

Exposure Monitoring Eligibility Script: Controls San Diego State University School of Public Health

Study of Environmental Health among Border Pedestrians

Casa Familiar, in collaboration with San Diego State University, is performing an environmental health study near the San Diego/Tijuana border. Before you give your permission to participate, it is important that we identify if you are eligible to participate by asking you a few questions.

Part I. Exclusion Criteria for Controls

- Yes / No 1. Are you between 18 and 60 years of age?
- Yes / No 2. Are you and the people in your household non-smokers?
- Yes / No 3. Are you free of any chronic lung, liver, or heart conditions?
- Yes / No 4. Are you free of diesel exposure both non-occupationally and occupationally. Examples include: aircraft ground crew, fisherman, bus driver, diesel gas-station employee, 18-wheeler trucker, and diesel mechanic (including aircraft, locomotive, and 18-wheeler mechanic).
- Yes / No 5. Have **not** crossed the border in the past 4 months?
- Yes / No 6. Live and work/attend school in San Ysidro (92173), Otay Mesa West (92154), National City (91950), or Chula Vista (91910 or 91911) and live within two miles of a major freeway?
- Yes / No 7. Are you willing to consent to participate for one day, per study parameters?
- Yes / No 8. Are you willing to answer questions about home/work/school exposures?

If you answered NO to any of the questions we want to thank you for participating in our questionnaire however by answering *no* to any of the questions has disqualified you from being able to participate in our study. Your time was greatly appreciated. Thank you again for participating.

If you answered YES to all the questions you may continue on to Part II.

Part II. Personal Information

Name: _____

Contact Information: _____

Preferred language _____

Days Available to Participate: _____

ID Number

Appendix G: Eligibility Script for “Non-Border Commuters” in Spanish

Guía de Elegibilidad del Seguimiento de Exposición: Controles

Escuela de Salud Pública de la Universidad Estatal de San Diego Salud ambiental entre los peatones de la frontera

Casa Familiar, en colaboración con la Universidad Estatal de San Diego está llevando a cabo un estudio de salud ambiental cerca de la frontera San Diego/Tijuana. Antes de que nos de su permiso para participar es importante que le hagamos unas cuantas preguntas para identificar si usted es elegible.

Parte I. Criterios de exclusión para los candidatos

- Si / No 1. ¿Tiene usted entre 18 y 60 años de edad?
- Si / No 2. ¿Usted o algunas personas que viven en su hogar no son fumadores?
- Si / No 3. ¿Está usted libre de enfermedades crónicas de pulmón, hígado o del corazón?
- Si / No 4. ¿Es usted una persona que no está expuesta a diesel tanto de manera ocupacional o no ocupacional? Por ejemplo: personal de tierra de aviones, pescadores, chofer de camiones, empleado de estación de gasolina, camionero de 18 ejes, y mecánico de diesel (incluyendo mecánico de aviones, locomotoras y camiones de 18 ejes).
- Si / No 5. ¿No ha cruzado la frontera en los últimos 4 meses?
- Si / No 6. Vive, trabajo o asiste a la escuela en San Ysidro (92173), Otay Mesa West (92154), National City (91950), o Chula Vista (91910 or 91911) y vive a dos millas de una vía rápida (freeway)?
- Si / No 7. ¿Está usted dispuesto a participar en el estudio durante un día de acuerdo con los parámetros del estudio?
- Si / No 8. ¿Esta usted dispuesto a contestar preguntas acerca de su hogar/trabajo/escuela?

Si contestó que NO a cualquiera de las preguntas queremos agradecerle por participar en nuestro cuestionario puesto que el haber contestado que *no* a cualquiera de las preguntas lo ha descalificado de participar en nuestro estudio. Su tiempo es muy importante. Gracias de nuevo por participar.

Si usted contestó que SI a todas las preguntas usted puede continuar con la Parte II.

Part II. Información Personal

Nombre: _____

Información de contacto: _____

Idioma de preferencia: _____

Fechas disponibles para participar: _____

Número de identificación:

Appendix H: Questionnaire in English

Healthy Borders Research Study Exposure Questionnaire

Interviewer: _____

Subject ID# _____

Date: ____/____/____
Month day year

Time: ____:____ (am/pm)
hour min circle one

Gender: _____ (1=female/2=male)

Personal Data

1. What is your address:

Street Address

City

Zip code

2. What is your date of birth? _____ (month) _____ (day) _____ (year)

3. What is your height _____ ft _____ in -OR- _____ cm

4. What is your weight _____ lbs -OR- _____ kg

5. What is your race? (Interviewer check all that are reported)

___ White₁

___ African American₂

___ Asian/Pacific₃

___ Hispanic (Mexican-American, Chicano, Latino, Puerto Rican, Central or South American)₄

___ Other₅— Please Specify _____

6. What is the highest grade you completed in school? (Interviewer check all that are reported)

___ elementary (6 or fewer years)₁

___ junior high (7-8 years)₂

___ some high school₃

___ high school graduate (12 years)₄

___ technical/vocational training₅

___ some college₆

___ college graduate₇

___ some graduate school₈

___ graduate/professional/doctorate degree₉

7. Which of the following best describes you? (Check one)

___ Employed₁ **If check then go to 7a**

___ Not Employed₂ **If check then go to 8**

___ Student₃ **If check then go to 8**

___ Homemaker₄ **If check then go to 8**

___ Retired₅ **If check then go to 8**

___ Disabled₆ **If check then go to 30**

Healthy Borders Research Study Exposure Questionnaire

Interviewer: _____

Subject ID# _____

Border Area Air Filter #: _____

Personal Air Filter #: _____

Border Area Air Filter Blank #: _____

Personal Air Filter Blank #: _____

Urine Sample ID # _____

7a. What type of work do you do? (check one)

___ Management, Business, or Financial₁ (*manager, accountant, health & safety, financial specialist, tax preparer*)

___ Science, Engineering, or Computer Professional₂ (*scientists, engineer, architect, computer programmer*)

___ Healthcare Practitioner₃ (*dentist, physician, chiropractor, physician asst, RN, therapist, pharmacists, nutritionists*)

___ Other Professional Worker₄ (*social worker, teacher, human resources, counselor, lawyer, writer, coach*)

___ Technician₅ (*EMT, dental hygienist, biological/chemical technician, medical record technician*)

___ Sales Worker₆ (*cashier, telemarketer, sales representative, travel agent*)

___ Administrative Support₇ (*receptionist, secretary, paralegal, teaching assistant, customer service, teller*)

___ Construction₈ (*carpenter, electrician, painter, plumber, plaster, roofer, dry wall, iron and steel*)

___ Installation, Maintenance, or Repair₉ (*mechanic, maintenance, tailor, upholstery, cabinet maker*)

___ Production Operative Worker₁₀ (*laundry/dry cleaning, packaging, plating and coating, metal washing or cleaning*)

___ Transportation and Material Moving₁₁ (*parking lot attendant, truck/bus/taxi driver*)

___ Laborer or helper₁₂ (*agricultural worker, fisher, hunter, service station attendant, logging and forestry*)

___ Protective Service Worker₁₃ (*security guard, law enforcement, firefighter, lifeguard*)

___ Service Worker₁₄ (*housekeeping, medical/dental asst, janitor, dishwasher, cook, waiter, child care, barber, cosmetologist*)

Healthy Borders Research Study Exposure Questionnaire

Interviewer: _____

Subject ID# _____

Border Area Air Filter #: _____ Personal Air Filter #: _____

Border Area Air Filter Blank #: _____ Personal Air Filter Blank #: _____ Urine Sample ID # _____

General Exposure – Does NOT include exposures during commute across the U.S.-Mexico border at San Ysidro, CA

8. Answer as best as possible for the past 14 days, including today

Day	Day of the week	Date m/d/yr	How much time did you spend in or near busy roadways	About how much time did you spend in or near diesel powered equipment or vehicles (example: buses, trains, tractors, diesel trucks)	Where there any smokers nearby? If yes, how long were you around smokers?
1			minutes	minutes	___ yes ___ no _____ min
2			minutes	minutes	___ yes ___ no _____ min
3			minutes	minutes	___ yes ___ no _____ min
4			minutes	minutes	___ yes ___ no _____ min
5			minutes	minutes	___ yes ___ no _____ min
6			minutes	minutes	___ yes ___ no _____ min
7			minutes	minutes	___ yes ___ no _____ min
8			minutes	minutes	___ yes ___ no _____ min
9			minutes	minutes	___ yes ___ no _____ min
10			minutes	minutes	___ yes ___ no _____ min
11			minutes	minutes	___ yes ___ no _____ min
12			minutes	minutes	___ yes ___ no _____ min
13			minutes	minutes	___ yes ___ no _____ min
14			minutes	minutes	___ yes ___ no _____ min

Healthy Borders Research Study Exposure Questionnaire

Interviewer: _____

Subject ID# _____

Border Area Air Filter #: _____ Personal Air Filter #: _____

Border Area Air Filter Blank #: _____ Personal Air Filter Blank #: _____ Urine Sample ID# _____

Border Crossing Experience: Subjects Only

9. Answer as best as possible for the past 14 days including today

Day	Day of the week	Date m/d/yr	Time of day crossed	Did you cross the border as a pedestrian or in a vehicle? How long did it take to cross the border?	Mode of transport <u>TO</u> <u>THE BORDER</u> and commute time <u>TO GET</u> <u>TO THE BORDER</u>	Where there any smokers nearby during your border commute? If yes, how long were you around smokers?
1			am/pm	min	min	yes no min
2			am/pm	min	min	yes no min
3			am/pm	min	min	yes no min
4			am/pm	min	min	yes no min
5			am/pm	min	min	yes no min
6			am/pm	min	min	yes no min
7			am/pm	min	min	yes no min
8			am/pm	min	min	yes no min
9			am/pm	min	min	yes no min
10			am/pm	min	min	yes no min
11			am/pm	min	min	yes no min
12			am/pm	min	min	yes no min
13			am/pm	min	min	yes no min
14			am/pm	min	min	yes no min

Appendix I: Questionnaire in Spanish Healthy Borders Research Study Exposure Questionnaire

Interviewer: _____ Subject ID# _____

Date: ____/____/____ Time: ____:____ (am / pm) Gender: _____ (female/male)
Month day year hour min circle one

Personal Data

1. ¿Cuál es su dirección?

Calle	Ciudad	Código postal
-------	--------	---------------

2. ¿Cuál es su fecha de nacimiento? _____ (mes) _____ (día) _____ (año)

3. ¿Cuál es su altura? _____ pies _____ pulgadas -O- _____ cm

4. ¿Cuál es su peso? _____ libras -O- _____ kg

5. ¿Cuál es su origen étnico? (Entrevistador: Marque todas las que correspondan)

- Blanco₁
- Afroamericano₂
- Asiático/Islands del Pacífico₃
- Hispano₄ (México-Americano, Chicano, Latino, Puertorriqueño, América Central o Sudamérica)
- Otros₅ – Por favor especifique _____

6. ¿Cuál es su grado escolar más alto? (Entrevistador: Marque todas las que correspondan)

- Secundaria o menos₁
- Algo de Preparatoria₂
- Terminó la Preparatoria₃
- Entrenamiento técnico /entrenamiento vocacional₄
- Algo de estudios universitarios₅
- Título universitario₆
- Algún estudio de postgrado₇
- Terminó los estudios de postgrado₈
- Doctorado₉

7. Cual de las siguientes opciones describe mayor su ocupación? (marque uno)

- Empleado₁ *Si marco esta opción continúe con la pregunta 7a*
- No empleado₂ *Continúe con la pregunta 8*
- Estudiante₃ *Continúe con la pregunta 8*
- Dueña de casa?₄ *Continúe con la pregunta 8*
- Jubilado₅ *Continúe con la pregunta 8*
- Invalido₆ *Continúe con la pregunta 8*

Healthy Borders Research Study Exposure Questionnaire

Interviewer: _____

Subject ID# _____

Border Area Air Filter # : _____

Personal Air Filter # : _____

Border Area Air Filter Blank # : _____

Personal Air Filter Blank # : _____

Urine Sample ID # _____

7a. Que tipo de trabajo hace usted? (marque uno)

- Administración, Negocios, Financiero₁ (*administrador, especialista en finanzas, preparador de declaraciones de impuestos, salud y seguridad*)
- Ciencia, Ingeniería, Profesional en computación₂ (*científico, ingeniero, arquitecto, programador de computadores*)
- Salud₃ (*dentista, médico, ayudante de médico, enfermera/o, quiropractor, farmacéutico, nutricionista*)
- Otro trabajo profesional₄ (*asistente social, profesor, recursos humanos, consejero, abogado, escritor, entrenador*)
- Técnico₅ (*EMT, higienista dental, técnico biológico/químico, técnico de registro médico*)
- Vendedor₆ (*cajero, vendedor por teléfono, representante de ventas, agente de viajes*)
- Apoyo administrativo₇ (*repcionista, secretaria/o, paralegal, ayudante de profesor, servicio a clientes, cajero*)
- Construcción₈ (*carpintero, electricista, pintor, plomero, techador, fierro y acero*)
- Instalación, Mantenimiento, o Reparación₉ (*mecánica, mantención, tapicería, mueblería*)
- Operador de producción₁₀ (*lavandería, empaquetar, enchapado, limpieza de metal*)
- Transporte y movimiento de materiales₁₁ (*operador de estacionamiento, chofer de camión, bus o taxi*)
- Obrero o ayudante₁₂ (*trabajador agrícola, pescador, cazador, operador de estación de servicio, explotación forestal*)
- Trabajador de Servicio de Protección₁₃ (*guardia de seguridad, policía, bombero, salvavidas*)
- Trabajador de Servicio, excepto de Protección₁₄ (*empleado doméstico, asistente médico/dental, portero, lavaplatos, cocinero, mesero, cuidado de niños, peluquero, cosmetólogo*)

Healthy Borders Research Study Exposure Questionnaire

Interviewer: _____

Subject ID# _____

Border Area Air Filter # : _____

Personal Air Filter # : _____

Border Area Air Filter Blank # : _____

Personal Air Filter Blank # : _____

Urine Sample ID # _____

General Exposure – Does NOT include exposures during commute across the U.S.-Mexico border at San Ysidro, CA

8. Por favor provea información de los últimos 14 días, incluyendo el día de hoy

Día	Día de la semana	Fecha mes/día/año	¿Cuánto tiempo pasó en o cerca de algunas carreteras con mucho tráfico?	¿Cuánto tiempo pasó usted en o cerca de equipo o vehículos que funcionan con diesel (por ejemplo: camiones, trenes, tractores, camiones de diesel)?	¿Había fumadores cerca de usted? Si la respuesta es si, cuánto tiempo estuvo usted cerca de esos fumadores?
1			minutos	minutos	__ si __ no ____ min
2			minutos	minutos	__ si __ no ____ min
3			minutos	minutos	__ si __ no ____ min
4			minutos	minutos	__ si __ no ____ min
5			minutos	minutos	__ si __ no ____ min
6			minutos	minutos	__ si __ no ____ min
7			minutos	minutos	__ si __ no ____ min
8			minutos	minutos	__ si __ no ____ min
9			minutos	minutos	__ si __ no ____ min
10			minutos	minutos	__ si __ no ____ min
11			minutos	minutos	__ si __ no ____ min
12			minutos	minutos	__ si __ no ____ min
13			minutos	minutos	__ si __ no ____ min
14			minutos	minutos	__ si __ no ____ min

Healthy Borders Research Study Exposure Questionnaire

Interviewer: _____

Subject ID# _____

Border Area Air Filter #: _____

Personal Air Filter #: _____

Border Area Air Filter Blank #: _____

Personal Air Filter Blank #: _____

Urine Sample ID #: _____

Border Crossing Experience: Subjects Only

9. Por favor provea la información de los últimos 14 días, incluyendo el día de hoy

Día	Día de la semana	Fecha mes/día/año	Hora en la que cruzó	Tiempo de espera en la frontera y medio de transporte que utilizó para cruzar la línea		Medio de transporte que utilizó para IR A LA LÍNEA y tiempo de viaje para IR A LA LÍNEA	¿Había fumadores cerca de usted durante su viaje? Si la respuesta es si, ¿cuánto tiempo estuvo usted cerca de los fumadores?
1			am/pm		min	min	___ si ___ no ___ min
2			am/pm		min	min	___ si ___ no ___ min
3			am/pm		min	min	___ si ___ no ___ min
4			am/pm		min	min	___ si ___ no ___ min
5			am/pm		min	min	___ si ___ no ___ min
6			am/pm		min	min	___ si ___ no ___ min
7			am/pm		min	min	___ si ___ no ___ min
8			am/pm		min	min	___ si ___ no ___ min
9			am/pm		min	min	___ si ___ no ___ min
10			am/pm		min	min	___ si ___ no ___ min
11			am/pm		min	min	___ si ___ no ___ min
12			am/pm		min	min	___ si ___ no ___ min
13			am/pm		min	min	___ si ___ no ___ min
14			am/pm		min	min	___ si ___ no ___ min

Appendix J: Time Activity Diary in English

Subject ID: _____

Tech 1: _____

Instruction Page

The purpose of this “diary” is to get a better idea of your activities and where you were while doing those activities. We are especially interested in understanding how close you are to exhaust, smoke and/or fumes. We ask that you fill out the diary every hour.

1. Fill out the diary accurately as possible to help us better understand how close you are to exhaust, smoke and/or fumes.
2. If you happen to be doing the same thing over a period of hours (example: sleeping from 10pm to 6am), you can just write in “Same as Above” under the section, “Describe your activities during this time period”

Subject ID: _____

Tech 1: _____

Time 8:00am to 10:00am	Date	(mth)	(day)	(year)
<p>1. Where you standing near any of the following below during this time frame? _____ yes₁ _____ no₀ (If yes, fill out 1a-1d)</p> <p>a. Standing near trash fires _____ minutes _____ distance from you (meters/feet) Circle One</p> <p>b. Standing near fireplace _____ minutes _____ distance from you (meters/feet) Circle One</p> <p>c. Standing near bonfire _____ minutes _____ distance from you (meters/feet) Circle One</p> <p>d. Standing near brushfire _____ minutes _____ distance from you (meters/feet) Circle One</p>				
<p>2. Where you working with or around any of the following below during this time frame? _____ yes₁ _____ no₀ (If yes, fill out 2a)</p> <p>a. Working with or around diesel powered equipment or vehicles _____ minutes</p>				
<p>3. Where you cooking during this time frame? _____ yes₁ _____ no₀ (If yes, fill out 3a-3c)</p> <p>a. Cooking with gas _____ minutes</p> <p>b. Cooking with wood _____ minutes</p> <p>c. Cooking with charcoal _____ minutes</p>				
<p>4. Where you in or near a vehicle during this time frame? _____ yes₁ _____ no₀ (If yes, fill out 4a-4h)</p> <p>a. In Taxi _____ minutes</p> <p>b. Near Taxi _____ minutes _____ distance from you (meters/feet) Circle One</p> <p>c. In Car _____ minutes</p> <p>d. Near Car _____ minutes _____ distance from you (meters/feet) Circle One</p> <p>e. In Bus _____ minutes</p> <p>f. Near Bus _____ minutes _____ distance from you (meters/feet) Circle One</p> <p>g. In Diesel Vehicle _____ minutes</p> <p>h. Near Diesel Vehicle _____ minutes _____ distance from you (meters/feet) Circle One</p>				
<p>5. Where there any smokers nearby during this time frame? _____ yes₁ _____ no₀ (If yes, fill out 5a-5b)</p> <p>a. Cigarettes _____ minutes _____ distance from you (meters/feet) Circle One</p> <p>b. Cigars _____ minutes _____ distance from you (meters/feet) Circle One</p>				
<p>What did you eat during this time period: _____</p> <p>Describe your activities during this time period: _____</p> <p>Specify Location: _____ Home (Mexico/U.S.A) _____ Work (Mexico/U.S.A) _____ School (Mexico/U.S.A) _____ Other (Mexico/U.S.A)</p> <p>Time at Location: _____ Min _____ Min _____ Min _____ Min</p>				

Appendix K: Time Activity Diary in Spanish

Subject ID: _____

Tech 1: _____

Instrucciones

El propósito de este "diario" es tener un mayor conocimiento de sus actividades y dónde se encontraba usted mientras las estaba realizando. Estamos muy interesados en saber qué tan cerca estuvo usted de tubos de escape, humo y/o gases. Le pedimos que llene el diario cada hora.

1. Por favor llene el diario con la mayor precisión que pueda para poder entender qué tan cerca se encontraba de tubos de escape, humo y/o gases.
2. Si usted realiza la misma actividad durante varias horas (por ejemplo, dormir de 10pm a 6am), usted puede escribir "La misma actividad que la anterior" en la sección "Describa sus actividades durante ese periodo de tiempo".

Subject ID: _____

Tech 1: _____

Hora: 4:00am a 6:00am	Fecha	(mes)	(día)	(año)
1. ¿Estaba usted parado cerca de los siguientes lugares durante ese periodo de tiempo? _____ si _____ no (Si la respuesta es afirmativa conteste la pregunta 1a-1d)				
a. Parado cerca de un lugar donde queman basura	_____ minutos	_____ distancia de usted (metros/pies)	Indique uno	
b. Parado cerca de una chimenea	_____ minutos	_____ distancia de usted (metros/pies)	Indique uno	
c. Parado cerca de una hoguera	_____ minutos	_____ distancia de usted (metros/pies)	Indique uno	
d. Parado cerca de un lugar donde se quema la vegetación	_____ minutos	_____ distancia de usted (metros/pies)	Indique uno	
2. ¿Estaba usted trabajando con o alrededor de los siguientes vehículos durante ese periodo de tiempo? _____ si _____ no (Si afirmativa conteste la pregunta 2a)				
a. Trabajando con o cerca de vehículos o equipo que funcionan con diesel	_____ minutos			
3. ¿Estaba usted cocinando durante ese periodo de tiempo? _____ si _____ no (Si la respuesta es afirmativa conteste la pregunta 3a-3c)				
a. Cocinando con gas	_____ minutos			
b. Cocinando con madera	_____ minutos			
c. Cocinando con carbón	_____ minutos			
4. ¿Estaba usted dentro o cerca de un vehículo durante ese periodo de tiempo? _____ si _____ no (Si la respuesta es afirmativa conteste la pregunta 4a-4b)				
a. En un taxi	_____ minutos			
b. Cerca de un taxi	_____ minutos	_____ distancia de usted (metros/pies)	Indique uno	
c. En un automóvil	_____ minutos			
d. Cerca de un automóvil	_____ minutos	_____ distancia de usted (metros/pies)	Indique uno	
e. En un autobús	_____ minutos			
f. Cerca de un autobús	_____ minutos	_____ distancia de usted (metros/pies)	Indique uno	
g. En un vehículo que funciona con diesel	_____ minutos			
h. Cerca de un vehículo que funciona con diesel	_____ minutos	_____ distancia de usted (metros/pies)	Indique uno	
5. ¿Había algunos fumadores cerca de usted durante ese periodo de tiempo? _____ si _____ no (Si la respuesta es afirmativa conteste la pregunta 5a-5b)				
a. Cigarrillos	_____ minutos	_____ distancia de usted (metros/pies)	Indique uno	
b. Puros	_____ minutos	_____ distancia de usted (metros/pies)	Indique uno	
Describa sus actividades durante ese periodo de tiempo: _____				
Lugar:	_____ Casa (Mexico/E.U.A.)	_____ Trabajo (Mexico/E.U.A.)	_____ Escuela (Mexico/E.U.A.)	_____ Otro (Mexico/E.U.A.)
Tiempo en Lugar:	_____ Minutos	_____ Minutos	_____ Minutos	_____ Minutos

Appendix L: Equipment Information Sheet

Healthy Borders Study 2010 Equipment Information Sheet

Technician: _____ Subject ID# _____
Date: ____/____/____ Time: ____:____ (am/pm) Gender: _____ (1=female/2=male)
Month day year hour min circle one

For Study Participant to Fill Out

1. For the past three days, on average, how many hours did you spend at home? _____ hours
2. For the past two weeks, on average, how many hours did you spend at home? _____ hours
3. Do you work? ____ Yes₁ ____ No₀
 - a. For the past three days, on average, how many hours did you spend at work? ____ hours
 - b. For the past two weeks, on average, how many hours did you spend at work? ____ hours
 - c. If yes, what is your work address:

Street Address City Zip code

4. Do you go to school? ____ yes₁ ____ no₀ ____ vacation₂ ____ how long
 - a. For the past three days, on average, how many hours did you spend at school? ____ hours
 - b. For the past two weeks, on average, how many hours did you spend at school? ____ hours
 - c. If yes, what is the name and address? _____ name

Street Address City Zip code

For Study Personnel to Fill Out

Personal Equipment Start time: _____ (am/pm) Date: _____ (month) _____ (day) _____ (year)
Personal Equipment Stop time: _____ (am/pm) Date: _____ (month) _____ (day) _____ (year)

Check if used

- _____ 1. HOBO CO, serial # _____
- _____ 2. EL-USB-CO, # _____
- _____ 3. HOBO RH/Temp, serial # _____
- _____ 4. AirChek XR5000, model#210-5000 serial # _____ UW/SDSU (circle one)
Pump flow rate before (LPM): _____
Pump flow rate after (LPM): _____
Total minutes: _____
Filter ID number: _____ HPEM / PEM (circle one)
Field blank number: _____ HPEM / PEM (circle one)

Appendix M: “Border Commuters” Crossing Information Sheet

Healthy Borders Study 2010 Border Crossing Information Sheet

1. Exact Time Arrived in Live to Cross the SY Border by Foot: _____ am/pm (circle one)

a. Smokers nearby: _____ yes/no

- i. Smoker 1: _____ distance (m/ft) (circle one) _____ minutes
- ii. Smoker 2: _____ distance (m/ft) (circle one) _____ minutes
- iii. Smoker 3: _____ distance (m/ft) (circle one) _____ minutes
- iv. Smoker 3: _____ distance (m/ft) (circle one) _____ minutes
- v. Smoker 3: _____ distance (m/ft) (circle one) _____ minutes
- vi. Smoker 3: _____ distance (m/ft) (circle one) _____ minutes
- vii. Smoker 3: _____ distance (m/ft) (circle one) _____ minutes

b. Buses nearby: _____ yes/no

- i. Bus 1: _____ distance (m/ft) (circle one) _____ minutes
- ii. Bus 2: _____ distance (m/ft) (circle one) _____ minutes
- iii. Bus 3: _____ distance (m/ft) (circle one) _____ minutes
- iv. Bus 4: _____ distance (m/ft) (circle one) _____ minutes
- v. Bus 5: _____ distance (m/ft) (circle one) _____ minutes
- vi. Bus 6: _____ distance (m/ft) (circle one) _____ minutes
- vii. Bus 6: _____ distance (m/ft) (circle one) _____ minutes
- viii. Bus 6: _____ distance (m/ft) (circle one) _____ minutes
- ix. Bus 6: _____ distance (m/ft) (circle one) _____ minutes
- x. Bus 6: _____ distance (m/ft) (circle one) _____ minutes

c. Diesel Trucks Nearby: _____ yes/no

- i. Diesel Truck 1: _____ distance (m/ft) (circle one) _____ minutes
- ii. Diesel Truck 2: _____ distance (m/ft) (circle one) _____ minutes
- iii. Diesel Truck 3: _____ distance (m/ft) (circle one) _____ minutes
- iv. Diesel Truck 4: _____ distance (m/ft) (circle one) _____ minutes
- v. Diesel Truck 5: _____ distance (m/ft) (circle one) _____ minutes
- vi. Diesel Truck 6: _____ distance (m/ft) (circle one) _____ minutes

2. Exact Time Exited the San Ysidro Border by Foot: _____ am/pm (circle one)

Appendix N: At Home Area Monitoring Information Sheet

Date Sampled: _____

Subject(s): _____

Healthy Borders Study 2010 At Home Area Monitoring Information Sheet

FOR STUDY PARTICIPANT TO FILL OUT

1. Location of home: Tijuana / South San Diego (*circle one*)
2. Address: _____
3. Near Major Roadway or Freeway: Yes / No (*circle one*) Distance: _____ meters / ft (*circle one*)
4. Residential Area: Yes / No (*circle one*)
5. Near Bus Stop: Yes / No (*circle one*) Distance: _____ meters / ft (*circle one*)
6. GPS: N _____ W _____

Pump Inside Home:	Start time: _____ AM/PM	End time: _____ AM/PM
	Start time: _____ AM/PM	End time: _____ AM/PM
	Start time: _____ AM/PM	End time: _____ AM/PM
	Start time: _____ AM/PM	End time: _____ AM/PM

Pump Outside Home:	Start time: _____ AM/PM	End time: _____ AM/PM
	Start time: _____ AM/PM	End time: _____ AM/PM
	Start time: _____ AM/PM	End time: _____ AM/PM
	Start time: _____ AM/PM	End time: _____ AM/PM

FOR STUDY PERSONNEL TO FILL OUT

1. HOBO RH/Temp Inside home (serial # _____) Outside home (serial # _____)
2. AirChek XR5000, model #210-5000, serial # _____ UW / SDSU (*circle one*)
Pump flow rate before (LPM): _____
Pump flow rate after (LPM): _____
Total minutes: _____
Filter ID number: _____ HPEM / PEM (*circle one*)
Field blank number: _____ HPEM / PEM (*circle one*)
Location: Outside home / Inside home living room (*circle one*)
3. AirChek XR5000, model #210-5000, serial # _____ UW / SDSU (*circle one*)
Pump flow rate before (LPM): _____
Pump flow rate after (LPM): _____
Total minutes: _____
Filter ID number: _____ HPEM / PEM (*circle one*)
Location: Outside home / Inside home living room (*circle one*)

*Wind direction, wind speed, and weather from weather report

Appendix O: “Border Commuters” Information Sheet About Crossing the Border in English



**U.S. Customs and Border Protection
San Ysidro, CA
Frequently Asked Questions**



San Diego State University “Healthy Borders Study”

The Port Directors office is aware of this study

CBP Contact

SCBPO Stephen C. Ford
Cell (619) 843-9824
Office (619) 662-2277

SDSU Contact

Vanessa E. Galaviz
Cell (619) 813-4265
Office (619) 594-2091

1. What will the officer want to know when I cross with the equipment?

The officer will most likely ask you, “Of what country are you a Citizen?” and “What are you bringing from Mexico?” At that time you should present your identification and/or Immigration documents and declare the items you are bringing, including the study equipment.

2. When should I tell the officer about the equipment?

During the initial questioning by the primary officer, if you are questioned further, offer to present the documentation you have been given regarding the study. Documentation will include: this “Question and Answer” flyer, a flyer approved by SCPBO Stephen C. Ford that demonstrates the study equipment to be carried across the border as a result of this study and a flyer that provides instructions for breakdown and inspection of equipment if secondary inspection is necessary.

3. Will having this equipment mean that I will be sent to Secondary inspection?

The decision for further inspection is done on a case by case basis and is done at the discretion of the primary officer. You will most likely not be sent in to secondary based solely on the equipment you possess, however, you are still subject to inspection as are all travelers.

4. What should I do if the officer takes the equipment or if I lose the equipment?

If the equipment should be taken from you tell the officer to contact SCBPO Stephen C. Ford to collect the equipment, as he is the local CBP contact for this study. If equipment should be lost, immediately contact Vanessa E. Galaviz.

5. My friend wants to see how this study works and what I will be doing. As a participant, may I bring him as an observer?

Yes - Anyone may cross the border, if they have the proper identification. As travelers, keep in mind that everyone is subject to inspection.

Appendix P: “Border Commuters” Information Sheet About Crossing the Border in Spanish



U.S. Customs and Border Protection (CBP)
San Ysidro, CA
Preguntas frecuentes



Universidad Estatal de San Diego (SDSU) “Estudio de Fronteras Saludables”

La oficina de los directores del Puerto tiene conocimiento de este estudio

Persona de contacto en CBP:

Stephen C. Ford, oficial de CBP
Celular (619) 843-9824
Oficina (619) 662-2277

Persona de contacto en SDSU:

Vanessa E. Galaviz
Celular (619) 813-4265
Oficina (619) 594-2091

1. ¿Qué es lo que el oficial desea saber cuando cruce la frontera con el equipo?

El oficial le preguntará muy probablemente lo siguiente: ¿De qué país es usted ciudadano? y ¿qué está usted trayendo de México? En ese momento usted deberá presentar su identificación y/o documentos de migración así como declarar los artículos que usted trae incluyendo el equipo del estudio.

2. ¿Cuándo debo informar al oficial que traigo el equipo?

Durante el interrogatorio inicial con el oficial primario. Si le hacen más preguntas puede presentar la documentación que le entregamos sobre el estudio. La documentación incluye: el volante de “Preguntas y Respuestas,” un volante aprobado por el oficial Stephen C. Ford de CBP el cual describe el equipo del estudio que usted está cruzando como parte del estudio y un volante con las instrucciones en caso de que se descomponga el equipo o para revisarlo en caso de que lo manden a inspección secundaria.

3. ¿El hecho de traer el equipo significa que me enviarán a inspección secundaria?

La decisión de enviarlo a inspección secundaria dependerá de cada caso y será a discreción del oficial primario. Muy probablemente usted no será enviado a inspección secundaria únicamente porque tiene el equipo en posesión, sin embargo, usted estará sujeto a inspección como todos los viajeros.

4. ¿Qué debo hacer si el oficial toma el equipo o lo pierdo?

Si le quitan el equipo usted necesita pedirle al oficial que se comunique con Stephen C. Ford quien es la persona de contacto en CBP que puede recuperar el equipo. Si el equipo se pierde por favor comuníquese de inmediato con Vanessa E. Galaviz.

5. Un amigo quiere ver cómo se realiza el estudio y lo que yo voy a hacer. Como participante, ¿puedo traer a mi amigo como observador?

Sí, cualquiera puede cruzar la frontera si es que cuenta con los documentos necesarios. Como viajeros tome en cuenta que toda persona está sujeta a inspección.

Appendix Q: U.S.-Mexico Border at San Ysidro Area Monitoring Information Sheet

Healthy Borders Study 2010 U.S. - Mexico Border at San Ysidro Area Monitoring Information Sheet

Date Sampled: _____ Initials: _____
 Start time: _____ AM/PM End time: _____ AM/PM
 Subjects: _____

Equipment Location

Location: GPS: N 32°32.566' W117°01.697'
 Ft from gate: 17 North Ft from vehicles: 35 East

Check if used

- _____ 1. TSI 3007, serial #6228
- _____ 2. HOBO CO, serial # _____, serial # _____, serial # _____
- _____ 3. EL-USB-CO, # _____, # _____, # _____
- _____ 4. HOBO RH/Temp, serial # _____
- _____ 5. pDR 1500, serial #1006440800
- _____ 6. AirChek XR5000, model#210-5000 serial # _____ UW/SDSU, attached to HPEM/PEM
 Pump flow rate before (LPM):
 Pump flow rate after (LPM):
 Total minutes:
 Filter ID number:
 Field blank number:
- _____ 7. AirChek XR5000, model#210-5000 serial # _____ UW/SDSU, attached to HPEM/PEM
 Pump flow rate before (LPM):
 Pump flow rate after (LPM):
 Total minutes:
 Filter ID number:
- _____ 8. AirChek XR5000, model#210-5000 serial # _____ UW/SDSU, attached to HPEM/PEM
 Pump flow rate before (LPM):
 Pump flow rate after (LPM):
 Total minutes:
 Filter ID number:

*Wind direction, wind speed, and weather from weather report
 *Wait time of Cars (provided by 619.690.8999) by hour is being extrapolated every hour
 *Number of Buses by hour

Time: number:
 Time: number:
 Time: number:
 Time: number:
 Time: number:

Appendix R: Check Off List

Healthy Borders Study 2010

Check Off List

The Day Before

1. PERSONAL MONITORING

- _____ 1. Activate HOBOS (CO and Temp/RH) in HT 10
- _____ 2. Calibrate SKC Air Pump and Prepare PEM filters in HT 10
- _____ 3. Print out study materials
 - _____ a. Eligibility Script
 - _____ b. Consent Form
 - _____ c. Equipment Information Sheet
 - _____ d. TAD
 - _____ e. Exposure Questionnaire
 - _____ f. Intercept Questionnaire
 - _____ g. Reimbursement Form
 - _____ h. Area Monitoring Information Sheet
 - _____ i. Customs Equipment Breakdown
 - _____ j. Customs Q&A
 - _____ k. Customs Pictures of Equipment

2. AREA MONITORING

- _____ 1. Activate TSI 3007 in HT 10
- _____ 2. Activate pDR in HT 10
- _____ 3. Activate HOBOS (CO and Temp/RH) in HT 10
- _____ 4. Prepare PEM filters in HT 10
- _____ 5. Calibrate two SKC air pumps: one for pDR and another for PEM
- _____ 5. Print out Area Monitoring Information Sheet (one for border and one for area)

The First Day

1. PERSONAL MONITORING

- _____ 1. Bring Equipment
 - _____ a. HOBOS (CO & Temp/RH)
 - _____ b. Backpack(s)
 - _____ c. SKC Air pump with PEM
- _____ 2. Bring TAD and Consent
- _____ 3. Bring Equipment Information Sheet
- _____ 4. Bring Customs Equipment Breakdown, Customs Q&A, Customs Pictures of Equipment
- _____ 4. Bring ID sticker labels

2. AREA MONITORING

- _____ 1. Bring Equipment
 - _____ a. TSI 3007
 - _____ b. SKC Air pump and pDR
 - _____ c. SKC Air pump with PEM
 - _____ c. HOBOS (CO and Temp/RH)
- _____ 2. Fill out Area Monitoring Information Sheet (one for border and one for area)

Healthy Borders Study 2010
Check Off List

The Second Day

1. PERSONAL MONITORING

- _____ 1. Bring Calender for Exposure Questionnaire
- _____ 2. Bring Exposure Questionnaire
- _____ 3. Bring Intercept Questionnaire for border crossers
- _____ 4. Bring Reimbursement Form
- _____ 5. Bring Urine cup(s)
- _____ 6. Bring Gloves
- _____ 7. Bring Ice Cooler with secondary container and ice packs
- _____ 8. Bring ID sticker labels
- _____ 9. Bring Money
- _____ 10. Collect TAD
- _____ 11. Collect Equipment
 - _____ a. HOBOs (CO & Temp/RH)
 - _____ b. Backpack(s)
 - _____ c. SKC Air pump with PEM

2. AREA MONITORING

- _____ 1. Collect Equipment
 - _____ a. TSI 3007
 - _____ b. SKC Air pump and pDR
 - _____ c. SKC Air pump with PEM
 - _____ c. HOBOs (CO and Temp/RH)
- _____ 2. Fill out Area Monitoring Information Sheet (one for border and one for area)
- _____ 3. Print out weather report for the past 24 hours

Appendix S: Instructions for Equipment if “Border Commuters” are Inspected by U.S. Customs and Border Protection Officer



SAN DIEGO STATE UNIVERSITY “Healthy Borders Study”



San Diego State University is performing an environmental health study on pedestrians crossing the US-Mexico border at the San Ysidro Port of Entry. Pedestrians will cross carrying air monitoring equipment. To minimize interference with SDSU study results and to maximize U.S. Customs and Border Protection security efforts instructions for breakdown and inspection of equipment has been provided below. All equipment may go through x-ray.

The Port Directors office is aware of this study

CBP contact

SCBPO Stephen C. Ford
Cell (619) 843-9824 Office (619) 662-2277

SDSU Contact

Vanessa E. Galaviz
Cell (619) 813-4265 Office (619) 594-2091

Instructions for Breakdown and Inspection of Equipment

SKC Air Pump XR5000 connected to a filter



SKC PUMP INSTRUCTIONS

- Please do not turn off or press any buttons.
- Please do not unscrew, internal parts are sensitive
- Please do not remove tubing from the pump

FILTER INSTRUCTIONS

- Please do not disconnect any parts or open the cap
- Please do not remove tubing from the filter or block airflow

HOBO RH and Temperature Data Logger



HOBO Carbon Monoxide Data Logger



HOBO INSTRUCTIONS

- You may unscrew the back but do not take out the batter

Appendix T: U.S. Customs and Border Protection Letter of Support

720 East San Ysidro Boulevard
San Ysidro, CA 92173



**U.S. Customs and
Border Protection**

January 4, 2010

To: Graduate and Research Affairs

Division of Research Affairs
San Diego State University
5500 Campanile Drive
San Diego, CA 92182-8220

Re: SDSURF research project **Healthy Borders San Ysidro**
(vIRB #381041, PIs Dr. PJE Quintana/ Dr. J Elder)

Dear IRB representative:

US Customs and Border Protection at San Ysidro/Otay Mesa has reviewed the referenced protocol and has advised Dr. Quintana of procedures to minimize disruption to study participants and to CBP personnel. We will work closely with Dr. PJE Quintana and her staff to expedite data collection for this study.

Sincerely,

A handwritten signature in black ink, appearing to read "Stephen C. Ford".

SCBPO Stephen C. Ford
Special Projects/Health and Safety
San Ysidro/Otay Mesa POE's
V 619.662.2277 C 619.843.9824 Fax 619.690.8929
Email Stephen.C.Ford@dhs.gov

Appendix U: Procedure for Collection of Gravimetric PM_{2.5}

VG 7/26/11

v.1.0

Procedure for Collection of Gravimetric PM_{2.5}

1. Gravimetric mass for PM_{2.5} levels determined from 37 mm Teflon filters (PN 224-1709, SKC Inc.)
2. Pre-weighed 200 filters in Environmental Health Laboratory in HSB F-466, following Simpson Lab SOP v2.0
 - a. All filters equilibrated to room temperature and humidity for a minimum of 2 hours
 - b. Anti-static device used, inside of glass draft shield cleaned with static guard spray using a swab, lab coat placed near vent on ceiling to prevent high wind draft onto ultra-microbalance
 - c. 200mg standard weighed 3 times, first 5 filters weighed 3 times, 10% filters weighed twice to verify consistency of weights, periodically (every ½ hour or so) weigh standard. For any repeated measurement, if 1st and 2nd measurement to not closely agree weigh again (maximum acceptable variance +/-5ug, communication Tim Gould)
 - d. 50 filters weighed on 1/18/10
 - e. 81 filters weighed on 6/27/10
 - f. 69 filters weighed on 6/28/10
 - g. Filters placed in a newly purchased PetriSlide and given an ID number (PD1504700, Millipore). ID numbers started with HB (healthy borders) followed by a number (0-200)
 - i. HB-XXX
3. PEM and filter assembly and disassembly performed in a glove bag (Aldrich AtmosBag PN Z530220, Sigma Aldrich Co.) to minimize contamination from ambient dust.
 - a. Two types of PEMs were used
 - i. 4 from UW (HPEMs)
 - ii. 4 from SDSU (PEM 761-203B, SKC Inc.)
 - b. Cleaning of PEMs before each use, including screens and screws
 - i. Silicone grease was removed
 - ii. PEMs placed in beaker with DDI water and detergent and soaked for ~15 minutes
 - iii. PEMs were rinsed with DDI water until soap film was rinsed away
 - iv. PEMs were dried either by using air line or left to dry overnight in glove bag on a new paper towel.
4. Following assembly of PEM with filter, covering of PEM was taped, PEM was placed in foil, and then placed in ziplock baggy to be taken to field.
5. Personal, border, and indoor/outdoor sampling
 - a. Each filter was given a field name followed by date (XX-XX-XX)
 - i. PF = personal filter
 - ii. PFB = personal filter blank
 - iii. PFBdr = personal filter at border crossing ONLY
 - iv. ABF = area border filter
 - v. ABF2 = duplicate
 - vi. ABFB = area border filter blank

- vii. AHFI = at home filter inside
 - viii. AHFO = at home filter outside
 - b. 1-NP sampled with a PM_{2.5} impactor connected to a SKC personal air sampling pump (AirChek Pump XR5000, SKC Inc.) operated at 4 liters per minute in order to achieve size selective sampling, as indicated by the manufacturer.
 - c. Personal and border blanks collected. No indoor/outdoor blanks collected. Blank filters assembled and taken to field in same manner as filter samples. Blanks were taken out of zip lock baggy, foil taken off, take over hole taken off and left to sit for ~30 seconds. Tape placed back on, new foil used to cover, and placed back in zip lock baggy to be taken back to lab.
6. Pumps calibrated before and after use (Defender 520, Bios International Corp.). Greater than 10% flow rate variability resulted in sample exclusion. None were greater than 10% flow rate.
- a. 3 post-flow rates were calibrated with a rotameter
 - b. 10 filters, no pre-flow rate noted in field notes
 - c. 2 filters, no post-flow rate noted in field notes
 - d. 1 filter, no pre- or post-flow rate noted in notes
7. Following disassembly of PEM with filter
- a. Filters placed into same PetriSlide and stored in -20C until later shipped to UW for NP analysis
8. Filters shipped to UW overnight on dry ice
9. Filters post-weighed in Environmental Health Laboratory in HSB F-466, following Simpson Lab SOP v2.0
- a. All filters equilibrated to room temperature and humidity for a minimum of 2 hours
 - b. Anti-static device used, inside of glass draft shield cleaned with static guard spray using a swab, lab coat placed near vent on ceiling to prevent high wind draft onto ultra-microbalance
 - c. 200mg standard weighed 3 times, first 5 filters weighed 3 times, 10% filters weighed twice to verify consistency of weights, periodically (every ½ hour or so) weigh standard. For any repeated measurement, if 1st and 2nd measurement to not closely agree weigh again (maximum acceptable variance +/-5ug, communication Tim Gould)
 - d. 188 filters weighed on 6/29/11
10. Blanks
- a. Maximum acceptable variance +/- 10ug mass difference. Outliers were omitted from inclusion of filter concentration. Resulted in 4 personal blank filters and 3 border area blank filters from being omitted; leaving 7 personal blank filters and 5 border area blank filters.
 - b. Final filter concentrations were calculated using blanks that were pre-weighed on the same day. Blanks for that day were averaged
 - c. Blanks were stratified by day of pre-weight. There were 3 pre-weight days. Day 2 had high variance so a pooled variance was done to determine
 - d.
11. Concentration (ug/m³) calculated using:

$$\frac{(M_f - M_i) - (B_f - B_i)}{[\text{Flow rate (LPM)} * \text{sample time (min)}] * 1000}$$

Appendix V: Standard Operation Procedure for Gravimetric Analysis

File:SOP Filter Gravimetric Analysis 2.0

STANDARD OPERATING PROCEDURE (SOP) FOR GRAVIMETRIC ANALYSIS OF PARTICULATE MATTER ON FILTERS

Prepared by:	Mike Paulsen	Date:	2/11/09
Revised by:	Vanessa Galaviz	Date:	1/18/10
Revised by:	Vanessa Galaviz	Date:	6/27/11
Revised by:		Date:	
Revised by:		Date:	
Reviewed by:		Date:	
Approved by:		Date:	

Section No.	<u>1</u>
Revision No.	<u>2.0</u>
Date	<u>06/27/09</u>
Page	<u>1 of 3</u>

Standard operating procedure for the gravimetric analysis of particulate matter on filters

This document describes the standard operating procedure for weighing filters using the Metler Toledo ultramicrobalance in the Environmental Health Laboratory in HSB F-466.

Supplies:

1. Teflon filters
2. Stainless steel forceps (flat tip tweezers) to handle filters
3. Plastic or Teflon-coated forceps¹ to handle weight standard
4. 100% methanol in squirt bottle
5. Box of Kimwipes
6. Nitrile gloves
7. Foil
8. PetriSlides (ie. PetriSlides for Contamination Analysis, Millipore Cat: PD1504700, <http://www.millipore.com/catalogue/module/C10719#2>)
9. Ultramicrobalance: Metler Toledo model UMX SN:1121113960
10. PC with Excel and script for transferring data

Notes:

- Wear anti-static clothing. Avoid wearing long-sleeve sweaters or other high-lint clothing without a lab coat. Remove any jewelry or watches. Always wear lab gloves when handling filters.
- Environmental conditions: 1) Avoid direct sunlight and 2) Avoid strong drafts (ie. from fans or air conditioning). There is a vent located west of the ultramicrobalance in F-466 that may create a strong draft. To prevent, hang a lab coat from the ceiling to block the draft (personal communication with Russell Dills).
- Squirt Kimwipe with 100% methanol. Use damp Kimwipe to wipe stainless steel forceps prior to handling filters. DO NOT touch filters until solvent has evaporated completely from forceps.
- Pre-weighing: clean forceps once prior to use. Post-weighing: clean forceps prior to touching each filter (unless care is taken, preventing forceps from contacting mass) to prevent cross-contamination.

¹ Protects metal weight standard from being scratched

Section No.	1
Revision No.	2.0
Date	06/27/09
Page	2 of 3

Procedure:

1. Allow filters to equilibrate to room temperature and humidity for a minimum of 2 hours. Place a clean sheet of foil paper on a 25-7/8" x 17-3/4" x 1" metal tray (trays are located west of ultramicrobalance). Using clean metal forceps, grab the Teflon filter (preferably by the outer support ring if existent) and place on the metal tray containing the clean sheet of foil paper. Cover filters with foil paper to prevent dust from settling on the filters (foil paper should not touch filters). Air should be able to enter to allow for equilibration.
2. Prior to filter weighing, clean the inside of the glass draft shield with the static guard spray using a swab.
3. Prior to opening Excel, turn on the Ultramicrobalance, which allows the Ultramicrobalance and Excel to sync upon opening.
4. Open Excel
5. Open the script "Metler Toledo Balance" from an icon in the Start menu
6. There are two optical sensors on the balance. The left side zero's the balance and the right side opens the door.
7. To transfer data to Excel, hit F2. Note that the data will be entered wherever the cursor is in the spreadsheet
8. Turn on the antistatic device. Use the antistatic device to reduce static charges on the filters. Allow the filter to rest directly on the horizontal bar of the device for a few seconds.
9. Monitor the performance of the balance using a weight standard. The weight is not actually used to calibrate the balance. Calibration is automatic. There are no specified accuracy criteria for the weight standards. The 200 mg standard is reasonably close in mass to a Teflon filter and is a good choice for this control. Weigh three times to verify consistency.
10. For the first five filters, weigh three times
11. Periodically (every ½ hour or so), weigh the standard again.
12. Periodically (10% or so), weigh filters twice to verify consistency of weights
13. For any repeated measurement, if the first and second measurement do not closely agree (maximum acceptable variance is +/- 5 ug), weigh again
14. After filter weighing, place the Teflon filter in a labeled petri dish.
15. When finished weighing samples, turn the antistatic device off, save data to a flash drive (and turn off the balance and PC).

Section No.	<u>1</u>
Revision No.	<u>2.0</u>
Date	<u>06/27/09</u>
Page	<u>3 of 3</u>

Appendix W: Standard Operating Procedure for 1-NP Filter Extraction and Analysis

STANDARD OPERATING PROCEDURE (SOP) FOR NPAH ANALYSIS IN PARTICULATE MATTER SAMPLES

Prepared By:	Justin P. Miller-Schulze Graduate Student	Date:	2/19/08
Revised By:	Mike Paulsen Vanessa E. Galaviz (Graduate Student) Vanessa E. Galaviz	Date:	8/18/09 6/17/11 7/19/11
Reviewed By:		Date:	
Approved By:		Date:	

I. Introduction

1. This document describes the standard operating procedure for the analysis of nitro polycyclic aromatic hydrocarbons (NPAHs) in particulate matter (PM) samples. The extraction method is an ultrasonic liquid extraction, and the analysis method is a two-dimensional high performance liquid chromatography with tandem mass spectrometry (2D-HPLC-MS/MS) method. Initially, this method was intended to quantify only 1-nitropyrene (1-NP), the most abundant NPAH in diesel exhaust particulate matter (DPM). However, this method has been expanded to include two additional NPAH species, 2-nitropyrene (2-NP) and 2-nitrofluoranthene (2-NF1). Both 2-NP and 2-NF1 are formed primarily through atmospheric processes. The method has the potential to include additional NPAHs, most likely 4-nitropyrene (4-NP) and 3-nitrofluoranthene (3-NF1). The 2D-HPLC-MS/MS method employs a deuterated internal standard (d_9 -1dNP) for quantification of all NPAHs at this time.
2. Other SOPs to be consulted are:
 - a. SOP _____
3. Other documents to be consulted are:
 - a. Miller-Schulze, J.P., et al. *J. Chromatogr. A*. **1167** (2007) 154-160.
4. Forms to use with the assay:
 - a. Form: 'NPAH stock solns.xls'
 - I. Location: simpsonlab on 'Storage-a\projects' (S:) → vgalaviz folder → NPAH Stock Solutions folder
 - II. Useful to refer to when making stock solution in III. 9. Vii. 1.
 - b. Form: 'FilterBenchsheet_1NP_Batches_template.xls'
 - I. Location: simpsonlab on 'Storage-a\projects' (S:) → vgalaviz folder → BenchSheets folder
 - II. Useful to make detailed notes when doing extractions and running HPLC
 - c.
5. Known limitations and interferences:
 - a. The efficacy of the 1-dNP internal standard for each NPAH analyte is not uniform. For example, the 1-dNP was chosen as an internal standard for 1-NP, and does an adequate job of correcting for extraction and analysis losses for this analyte.

However, the efficacy of the 1-dNP as an internal standard for 2-NP is noticeably less than for 1-NP, with 2-NFl somewhere in between 1-NP and 2-NFl.

II. Sample Collection

1. For the most part, as of the writing of this SOP, this extraction and analysis method has been applied to PM samples that were either purchased from NIST (i.e., standard reference materials, SRMs), or collected by a 3rd party (NIEHS filter samples, KC metro samples, etc). As such, this SOP will not address procedures and/or issues associated with sample collection.

III. Materials

1. Chromasolve ethanol for HPLC (denatured) 95% + 5% IPA by volume, Sigma Aldrich cat#270741-2L. Used for rinsing/cleaning glassware, making reconstitute extraction solution, making mobile phase for the HPLC-MS/MS, and NPAH stock solution used for calibrants and spiking solution.
2. Acetic acid. Glacial, reagent ACS, spectrum cat#A1010. 500mL. Density = 1.053g/mL. Used for making buffer¹
3. Sodium acetate anhydrous. Sigma. Cat: S8750-1KG. M = 82.03g/mol. Used for making buffer
4. Methanol. Fisher Optima cat#A454-4. Used for rinsing/cleaning glassware, and making mobile phase for the HPLC-MS/MS.
5. Formic acid. GR ACS EM Science cat#FX0440-1. Used for making mobile phase for the HPLC-MS/MS.
6. L-L-Ascorbic acid. 99+% Sigma Aldrich cat#25564. 100g. M = 176.12g/mol. Used for making mobile phase for the HPLC-MS/MS.
7. Aminopyrene??? From supplier or synthesized standard. Used as a positive control for HPLC-MS/MS.
8. Dicloromethane (Methylene Chloride). Baker analyzed A.C.S. Reagent Lot H50B42. Cat# 4L 9324-03 used for extracting 1-NP from filters, and rinsing/cleaning conical-bottom and round-bottom screw top glass tubes and teflon-lined screw caps.
9. Extraction:
 - a. Each individual PM Sample/Filter requires:
 - i. One (1) five ml conical-bottom screw-top glass tube with one (1) Teflon-lined screw cap used in step V.8 (Kimble Chase No.73785-5) and one (1) 15mL round-bottom screw-top glass tube with one (1) Teflon-lined screw cap used in step V.2 (Catalog Number???). The glass tubes should be rinsed with methylene chloride then silanized then rinsed again with methylene chloride prior to use. Teflon-lined screw caps should be rinsed with methylene chloride prior to use. (Note: the conical-bottom screw-top glass tubes are nominally identified by the supplier and throughout this procedure as “five ml”, but the actual capacity is 8 ml.) (Note: Always purchase new glass tubes for extraction to prevent contamination. Personnel communication with Chris Simpson.)

¹ Buffer is referring to 20mM Sodium Acetate/Acetic Acid, pH 5.5 solution. Refer to step V.1.iii and V.2.iii on how to prepare. Buffer is stable for 7 days. Buffer will be used throughout the SOP.

- ii. One (1) amber autosampler vial with aluminum (PTFE-lined rubber septa) crimp cap. (Autosampler vial does not need to be silanized prior to use because sample will be in a silanized insert.)
 - iii. One (1) silanized glass vial insert, with plastic “foot”.
 - iv. Two (2) glass 9” pasteur pipette, silanized.
 - v. One (1) plastic 1 ml syringe (Fisher cat# 14-823-2E),
 - vi. One (1) 13mm Syringe filter with 0.2 μm PTFE membrane (Pall Life Sciences PN 4542)
 - vii. One (1) disposable flow control valve liner (Supelco cat 57059).
- b. The extraction procedure as a whole requires:
- i. One (1) ultrasonic water bath, with rack submerged in water.
 - ii. One (1) low-volume Turbovap evaporative concentrator at $T=45^{\circ}\text{C}$, and equipped with N_2 gas cylinder (N_2 tank lasts 2-6 weeks unused).
 - iii. One (1) sample vortexer.
 - iv. One (1) 25 μl positive displacement pipettor. For sensitive trace analysis, this pipettor should be segregated from general use so as to prevent high levels of NPAHs from being in contact with pipettor (particularly 1-NP and 1-AP) (Mike Paulsen changed tip on 5/10/11). To be used ONLY for spiking 1-dNP on filters and calibrants.
 - v. One (1) filter cutter for the separation of filters from PMP support rings.
 - vi. One (1) diaper pad to cover lab bench to prevent contamination of samples, glassware, etc., by lab bench.
 - vii. One (1) Gilson pipettor (negative displacement type with plastic tips) for aqueous solvents, for dispensing volumes of 150 μl .
 - viii. Autosampler vial crimper and decrimper.

10. Analysis:

- a. The 2D-HPLC-MS/MS analysis of PM extracts requires:
- i. Agilent 1100 HPLC
 - ii. Agilent 6410 Triple Quad Mass Spectrometer
 - iii. Two (2) external LC pumps
 - iv. One (1) Helium gas cylinder with line split into 2, with inline filters attached, for sparging of reservoirs hooked up to external LC pumps
 - v. LC columns:
 1. AC-1: Waters Xterra RP18 2.1 x 150 mm, $d_p=3.5\ \mu\text{m}$, with Waters guard column. P/N 186000410. Store in 100% ACN (4+ days).
 2. AC-2: Agilent Zorbax SB-Phenyl, 2.1 x 150 mm, $d_p=3.5\ \mu\text{m}$. Special order.
 3. TC: Waters Atlantis RP18 2.1 x 30 mm, $d_p=3\ \mu\text{m}$. 186001287.
 4. RC: Specially made reduction column, Pt/Rh, 4 x 10 mm. DO NOT store in ACN.
 - vi. Required PEEK tubing for all connections
 - vii. A set of calibrants, with ascending levels of NPAH, i.e., from 0.5 fg/ μl to 5000 fg/ μl 1-NP, 2-NP, 2-NFl, etc., and constant level of 1-dNP internal standard, i.e., 400 fg/ μl . Exact [1-dNP] will depend on anticipated NPAH levels in PM. Calibrants should be made day of or day before run LCMS. Run 100 fg/ μl calibrant, zero calibrant, and blank every 10 – 20 samples. Silanize 2mL glass amber vials. Reuse same 2mL glass amber vials

throughout your procedure. Rinse with methylene chloride before each re-use.

1. Stock solution is 6667 fg/ μ l each: 1-NP, 2-NP, 2-NFl in 100% chromosolve ethanol [Stored at -20°C in 20 ml amber vial.] Stock solution is used to make both calibrants and 1000 fg/uL spiking solution. Refer to form listed in 4.a. for details on preparing stock and spiking solutions (preferable to make enough stock and spiking solution to be used for all your samples). Refer to IV.1.iii.1. on making buffer and IV.1.iii. on making EtOH/buffer solution. Make a fresh stock of 20mM Acetate buffer. Use plastic pipette tips for transferring. Reuse same vials for calibrants. Prior to each use 1) empty left over calibrants from low to high concentration using plastic pipette tips into waste container (to be later disposed into bulk HPLC waste jug) then 2) with plastic pipette tip transfer 100% chromosolve EtOH into vial, shake, then remove with pipette and transfer into EtOH waste container.
 2. 1.125 ml stock + 0.375 ml 20mM Acetate buffer = 5000 fg/ μ l
 3. 300 μ l of 5000 + 1.2 ml EtOH/buffer (75/25) = 1000 fg/ μ l
 4. 150 μ l of 5000 + 1.35 ml EtOH/buffer (75/25) = 500 fg/ μ l
 5. 150 μ l of 1000 + 1.35 ml EtOH/buffer (75/25) = 100 fg/ μ l
 6. 150 μ l of 500 + 1.35 ml EtOH/buffer (75/25) = 50 fg/ μ l
 7. 150 μ l of 100 + 1.35 ml EtOH/buffer (75/25) = 10 fg/ μ l
 8. 150 μ l of 50 + 1.35 ml EtOH/buffer (75/25) = 5 fg/ μ l
 9. 375 μ l of 10 + 0.75 ml EtOH/buffer (75/25) = 2.5 fg/ μ l
 10. 150 μ l of 10 + 1.35 ml EtOH/buffer (75/25) = 1 fg/ μ l
 11. 150 μ l of 5 + 1.35 ml EtOH/buffer (75/25) = 0.5 fg/ μ l
 12. Zero Calibrant = 0.0 fg/uL
 13. Transfer 1 ml of each calibrant to an empty, silanized HPLC vial. For zero calibrant, transfer 1mL of EtOH/buffer (75/25, pH=5.5) into silanized HPLC vial.
 14. Remove 15.6 μ l from each vial (DON'T USE SEGREGATED 25 uL POSITIVE DISPLACEMENT PIPETTOR)
 15. Add 15.6 μ l internal standard solution (10^{-7} M 1-dNP) (USE SEGREGATED 25 uL POSITIVE DISPLACEMENT PIPETTOR)
 16. Blank Calibrant. Consisting of only EtOH/buffer (75/25) (make 3 of them). No need for exact volume. Can use 9" pasteur pipet to transfer into silanized HPLC vial.
- viii. AP check standard for evaluation of reduction column efficiency. Make fresh prior to each HPLC-MS/MS run. AP in EtOH/buffer (75/25.). Refer to form listed in 4.a. for details on preparing.
1. Presently (5/11/11) we are making as follows:
 - a. Transfer 2mL 100% EtOH with 2mL pasteur pipet into 5mL amber vial, silanized.
 - b. Pipet 9.9ul of 10.1ng/mL AP made by GO on 2/4/10 into 2mL 100% EtOH.

IV. Reagents

C:\Users\Vanessa Galaviz\Documents\1. UW Dissertation\1. UW\4. Simpsons Lab\NP_Air Filters\SOP\LCMS NPAH SOP Ver 3c woTrackChanges.doc

4

1. Extraction

- a. The extraction procedure requires the following reagents/solvents:
 - i. Seven (7) ml CH_2Cl_2 per sample for use as an extraction solvent. The CH_2Cl_2 will also be used to wash filter cutters, conical bottom vials, pipettes, etc.
 - ii. 1-dNP filter spiking solution. This solution should be prepared such that the concentration of 1-d-NP in the final extract (150 μl volume) is the same as the concentration in the calibrants (assuming 100% recovery from the spiked filter).
 1. Presently (8/18/09) we are spiking as follows:
 - a. calibrants with 15.6 μl of 10^{-7} M (1000 μL final volume)
 - b. filters with 23.4 μl of 10^{-8} M 1-dNP (150 μl final extract volume)
 - iii. 150 μl 75% ethanol/25% 20 mM Sodium Acetate/Acetic Acid, pH=5.5 sample matrix per sample extract. (Note: the sample matrix is different from the mobile phase, which is a mixture of 85:15 of the same solvents.) Solution is stable for 7 days. Store 4C. Total volume 100mL. To dispose pour in "bulk HPLC waste" jug.
 1. First make buffer. Total volume 0.5L. Solution is stable for 7 days. Store 4C.
 - a. Base: 20mM of sodium acetate ($0.02\text{M} = x \text{ mol}/82.03\text{g/L} \rightarrow x = 1.64\text{g per liter}$). Total volume 0.5L. Solution is stable for 7 days. Store 4C.
 - i. Using graduated cylinder measure 500mL of DDI water and pour into a 1L glass bottle.
 - ii. Weigh out 0.82grams of sodium acetate and pour into DDI water. Shake until dissolved.
 - b. Acid: 20mM acetic acid. [$0.02\text{moles/L} = (1.053\text{g}/x \text{ ml}/1\text{L}) * (\text{mol}/60.05\text{g}) \rightarrow x = 1.14\text{mL per liter}$]. Total vol 0.5L. Solution is stable for 7 days. Store 4C.
 - i. Using graduated cylinder measure out 0.57mL of acetic acid then fill to 500mL with DDI water then pour into 1L glass bottle. Shake until dissolved.
 - c. Calibrate pH meter
 - d. Pour 300mL sodium acetate into 1L bottle
 - e. Pour 25mL acetic acid
 - f. Using 9" glass pasteur pipette transfer needed solution until pH is 5.5
 2. Transfer 75mL of ethanol into a 100mL glass bottle
 3. Transfer 25mL of buffer into 75mL of ethanol
 4. Shake.
 - iv. Bottles of methanol and/or ethanol for rinsing/washing of glassware, pipettors, etc.

2. Analysis

- a. The 2D-HPLC-MS/MS analysis method requires the following reagents/solvents (All glass bottles are reused and prior to each use should be rinsed with DDI water):

- i. One (1) liter methanol w/ 0.01% formic acid (Volume is dependent on length of sequence, which is dependent on number of samples). Stable for 7 days. Store RT. Dispose of down drain.
 1. Measure 1L of methanol with graduated cylinder and pour into 1L glass bottle.
 2. Pipet 100uL formic acid into 1L methanol with plastic pipet tip
 3. Shake
- ii. One (1) liter DDI H₂O w/ 0.01% formic acid (See above regarding volume). Stable for 7 days. Store RT. Dispose of down drain
 1. Measure 1L of DDI water with graduated cylinder and pour into 1L glass bottle
 2. Pipet 100uL formic acid into 1L DDI water with plastic pipet tip.
 3. Shake
- iii. ½ Liter (0.5 L) 85% ethanol/15% 0.020 M Sodium Acetate/Acetic Acid, pH=5.5. Stable for 7 days. Store 4C. Dispose in “bulk HPLC waste” jug.
 1. Refer to V.3.1. on making buffer.
 2. Transfer 425mL of ethanol into a 1L glass bottle
 3. Transfer 75mL of buffer into 425mL of ethanol
 4. Shake.
- iv. One (1) liter 0.01 M L-Ascorbic Acid in H₂O. Reactive so make day of running HPLC-MS/MS. Dispose of down drain
 1. 0.01moles/L = (176.12g/ x mole) * 1L → 1.76g
 2. Using graduated cylinder measure out 1L of DDI water and pour into 1L glass bottle
 3. Weigh out 1.76 grams of L-Ascorbic acid then pour into 1L glass bottle. Shake until dissolved.

V. Extraction Procedure (takes about 4 hrs)

- Notes: 1. ALWAYS use new gloves when touching silanized glassware
 2. Use benchsheet to record ID numbers (filter, QC, and standards) and notes relating to sample processing and dates prepared.
 3. Extraction procedure should be done under the hood
 4. When using a positive displacement pipettor, rinse tip with 100% chromosolve EtOH from squirt bottle. Put capillary tip on and pipette with 100% chromosolve EtOH (pour some from stock into silanized tube), dispensing into waste beaker (DON'T touch waste beaker with tip). Do this a couple of times. 5. Before transferring filters into silanized glass vials ALWAYS rinse twissors and scissors with methylene chloride from squirt bottle and wipe down with kimwipe to prevent touching filter with wet scissors and/or twissors.
1. Quality Assurance (QA) samples are to be prepared with each set of PM samples and/or filter samples. Specifically, these QA samples should include, in duplicate:
 - a. 1-dNP Controls/Deuterated Filters. This QA sample is either a blank filter spiked with the same 1-dNP spike volume and solution as the rest of the samples (if the sample set being analyzed is comprised of filters) or simply spiking the 5 ml tube with this spike volume/solution. The 1-dNP control is designed to give a measure of the recovery of the extraction procedure.
 1. Presently (5/11/10) we are spiking as follows:
 - i. 23.4 µl of 10⁻⁸ M 1-dNP (150 µl final extract volume)

- b. Positive Controls/Fortified Filters. This QA sample is either a blank filter spiked with both the equivalent 1-dNP spike (as described for the 1-dNP Controls) as well as a moderately high concentration spike of the NPAH analytes being targeted in the assay (in the 0.5-5000 fg/ μ L calibrant set described above, the positive control spike was 25 μ L of a 1000 fg/ μ L 1NP, 2NP, and 2NFI solution). The positive control is designed to give a measure of the recovery for each NPAH and possible interaction of the NPAH analytes with each other.

1. Presently (5/11/10) we are spiking as follows

- i. 23.4 μ L of 10^{-8} M 1-dNP (150 μ L final extract volume)
- ii. 25 μ L of 1000 fg/ μ L NPAH (150 μ L final extract volume)

- c. Extract Blanks/Field Blanks. This QA sample is simply an unspiked 5 ml vial that is put through the entire extraction procedure. If the sample set being analyzed consists of filters, and the Simpson Lab has the same filters as used in the sampling, then an additional set of "Filter Blanks" can be done, which are identical to the Extract Blanks except a blank filter is inserted into the 5 ml vial, and then the full extraction procedure is performed on this sample. The extract blank is designed to give a measure of the contamination of the glassware and/or solvents used in the extraction procedure.

Controls (CN): This QA sample consists of spiking 1-dNP and NPAH analytes (as described for the positive controls/fortified filters) into a silanized 5ml conical-bottom screw-top glass tube. This QA sample is designed to give an idea of the recovery for just the 2D-LC-MS/MS analysis, i.e., excluding the extraction. **BEGIN PROCEDURE AT STEP V.10**

1. Presently (5/11/10) we are spiking as follows

- i. 23.4 μ L of 10^{-8} M 1-dNP (150 μ L final extract volume)
- ii. 25 μ L of 1000 fg/ μ L NPAH (150 μ L final extract volume)

2. For filter samples, if filters have PMP support rings, cut filter out of ring using extraction solvent-rinsed (methylene chloride) filter cutter, and with solvent-rinsed (methylene chloride) forceps deposit detached filter into silanized, labeled, extraction-solvent rinsed 5 ml conical screw-top round bottom tube. For PM mass samples, weigh out 10-15 mg PM mass into 5mL conical screw-top round bottom tubes. Some PM samples may require the de-staticizer to obtain consistent, accurate weighings. (Note: PMP support rings may or may not need to be cut off depending on analytes to be measured and detection system to be used. PMP support ring interferes with PAH (by GC-MS) analysis. It does not interfere with the NPAH and the LG. Personal communication with Chris Simpson)
3. Assign sample numbers to filters in randomized order for extraction and print labels for each step of analysis
4. Spike filters/ PM masses with 23.4 μ L of 1-dNP spiking solution (using the aforementioned calculated spike concentration and volume) using a segregated 25 μ L positive displacement pipettor.
 - a. As quality control filters, include:
 - i. 2 deuterated filters with 23.4 μ L of 10^{-8} M 1-dNP (noted above in V.1.a.)
 - ii. 2 fortified filters with 23.4 μ L of 10^{-8} M 1-dNP and 25 μ L of 1000fg/ μ L NPAH spiking solution (noted above in V.1.b.)

- iii. 2 blank filters without any spikes (can be field or lab blanks) (noted above in V.1.c.)
5. Let spiked samples age for 30 minutes. (EtOH is evaporating and NPAHs are binding to filter and air particles on the filter.
6. Add 7 ml of CH₂Cl₂ extraction solvent to each sample tube with 10 ml graduated pipette or with a pipette dispenser. These 7 ml volumes need not be quantitative.
7. Sonicate all capped tubes for 60 minutes at full power. Use the lid on the sonicator to exclude light. Use care to avoid spilling samples and wipe tubes with kim wipe after take out of bath
8. Decant supernatant CH₂Cl₂ into a second silanized, rinsed, and labeled 5 ml conical shaped bottom tube using a 9" silanized glass Pasteur pipet to transfer.
9. Place tubes with supernatant CH₂Cl₂ into low volume Turbovap (equilibrated ~30 minutes prior such that water bath is at T=45 °C when tube being inserted into Turbovap). Position tubes such that the nozzles extend slightly into the vials (the tube should be several mm off the bottom of the rack). Start with low nitrogen flow to avoid splattering samples. Increase flow gradually while observing liquid to confirm the sample is not splattering. Nozzles should be wiped with methanol or ethanol-soaked kimwipe prior to use. After blowing stream of N₂ into tubes for a count of 10 seconds, tubes should be tightly capped and stored in freezer.
10. Evaporate CH₂Cl₂ extraction solvent to dryness.
 - a. Include two control (CN) samples at this step. To prepare refer to V.1.d.
11. If extraction procedure is to be suspended at this point, all tubes should be evacuated with N₂ using sample concentrator in hood (Techne DB-3A). If N₂ evacuated tubes have been stored in freezer overnight, allow them to temperature equilibrate for ~30 minutes.
12. Reconstitute dried extracts with 150 µl 75% EtOH/OAc sample matrix using 200 µl Gilson pipettor (negative displacement type with plastic tips). Pipettor tip need not be changed after dispensing a single aliquot unless tip touches glass during process. Ask Mike
13. Sonicate all extract Tubes for 10 minutes at full power in ultrasonic bath.
14. Vortex all extract tubes in rack for 10 minutes at power level = 4 using sample vortexer.
15. Using sample matrix-rinsed glass 9" or 5.5" pasteur pipette, transfer entire reconstituted extract volume from 5 ml tube into plastic 1 ml syringe equipped with 0.2 µm syringe filter and filter attachment tip. A fresh syringe/filter/tip set should be used for each sample.
16. Depress plunger into syringe to expel reconstituted volume into silanized glass autosampler vial insert situated in "foot" inside labeled amber autosampler vial. Repeat process 2-3 times to insure that entire extract volume is expelled into vial insert.
17. After expelling entire volume into vial insert, cap vial with aluminum crimp cap with PTFE-lined rubber septa.
18. Analyze extract or store in -20C freezer (EtOH content high enough to not freeze sample. Communication with Mike Paulsen)

VI. Instrumental Analysis

1. Analyze all extracts and QA samples using 2D-HPLC-MS/MS method with internal standard calibration. Run time per sample is 38 minutes
2. To prepare 2D-HPLC system, use the following procedure:
 - a. Use the “degas” function of the ultrasonic bath in F442 to “degas” all mobile phases listed in IV. 2. a. , i.e., MeOH w/0.01% FA, H₂O w/ 0.01% FA, 0.01 M L-Ascorbic Acid, and 85% EtOH/15% OAc. To do this:
 - i. Insert mobile phases, one at a time, into ultrasonic bath, and verify that water level is such that at least ¼ of reservoir bottle is submerged.
 - ii. Remove cap from reservoir and place lead donut over reservoir bottle.
 - iii. Attach vacuum line with #4 black rubber stopper to reservoir bottle.
 - iv. Turn on ultrasonic bath and start the “degas” function. Red indicator should then illuminate, showing a reversed “6”.
 - v. Turn vacuum on.
 - vi. Degassing should take about 9 minutes.
 - vii. Repeat for all mobile phases.
 - b. Once all mobile phases have been degassed, all pumps should be purged with the appropriate solvent. The Agilent pump should be purged with MeOH w/0.01% FA (Channel B1) and H₂O w/0.01% FA (Channel A1). The Biorad external pump should be purged with the 85/15 EtOH/OAc mobile phase, and the Shimadzu external pump should be purged with the 0.01 M L-Ascorbic Acid mobile phase. The Agilent pump can be purged simply by opening the purge valve on the pump and the setting the flow rate to 5 ml/min and running at this flow rate for ~5 minutes. The external pumps have to have their PEEK tubing outlets run into a beaker and run at ~9 ml/min. for 3-5 minutes.
 - c. To prep the reduction column (RC) for the analysis, it should be “back flushed” (have mobile phase run through the column in the reverse direction) with the 85/15 EtOH/OAc mobile phase for 10 minutes at 0.15 ml/min. This can be done with the autosampler, guard column, and AC-1 (all situated in the “forward” direction) upstream of the RC. Back flushing times much longer than 10 minutes should be avoided.
 - d. After back flushing the reduction column and purging all pumps with the appropriate mobile phases, the system should be configured as shown in the attached figure 1.
 - e. There are a variety of operational parameters for the 2D-HPLC method that are detailed in Appendix A, HPLC operational parameters. When the 2D-HPLC system is fully configured, and the appropriate temperatures have been input into the Agilent Masshunter Data Acquisition software as per Appendix A, the system should be equilibrated (i.e., run without analyzing samples) for ~ 30 minutes prior to injecting the 1st sample. [NOTE: I CANNOT LOCATE THE APPENDIX CONTAINING HPLC OPERATING INSTRUCTIONS- IS IT SUPPLEMENTAL MATERIALS FOR THE MANUSCRIPT? - MIKE 8/18/09]
3. Additional notes regarding HPLC operation
 - a. Present method is “NP dNP 2NP 2F1 MRM 3 wide 1”
 - b. Biorad pump → autosampler (port 1) → AC1
 - i. Flow is 0.1 ml/min
 - ii. This pump has pressure limits that have to be re-set each time the power is cycled. Set high limit to 325 and low limit to 7.
 - c. Pressure during trapping
 - i. 230-240 bar

- ii. 120 bar
 - d. Pressure during non-trapping period
 - i. 115-130 bar
 - ii. 25 bar
 - e. Shimadzu pump (10 mM L-Ascorbic acid)
 - i. Diluent
 - ii. 0.5 ml/min
 - f. Trapping window is 7-12 minutes
 - g. Be careful with the backpressure needle valve because it is sensitive to small adjustments!
4. Instrument checkout sequence
 - a. Standard 50 (50 fg/μl X 40 μl injection = 2000 fg on column)
 - b. AP (50 fg/μl X 40 μl injection = 2000 fg on column)
 - c. Blanks
 - d. Calibration curves
 5. Worklist order using method "NPAH 062311"
 - a. Calibration curve from low to high standard
 - b. 3 blanks
 - c. 12 samples
 - d. 2 blanks
 - e. Check standard
 - f. AP standard. Use method "NP dNP 2NP 2NFL MRM_WithoutReduction."

<u>System Component</u>	<u>Upstream Connection</u>	<u>Downstream Connection</u>
BioRad pump	85/15 EtOH/OAc	Agilent autosampler port 1
Guard col/AC-1/RC	Agilent autosampler port 2	Mixing T (side port)
Shimadzu pump	10 mM L-Ascorbic acid	Mixing T (side port)
Switching valve port 1		AC-2
Switching valve port 2	Agilent pump	
Switching valve port 3	Trapping column outlet	
Switching valve port 4		Waste
Switching valve port 5	Mixing T	
Switching valve port 6	Trapping column inlet	
AC-2	Switching valve port 1	Detector
Agilent pump	MeOH/water/ 0.01% formic acid	Switching valve port 2

Backpressure Log

Pump	BioRad	BioRad	Shimadzu	Shimadzu	Agilent	Agilent
Switch Valve Position	Eluting: Column 1	Trapping: Column 2	Eluting	Trapping	Eluting: 0.2mL/min	Trapping
Pressure Date: 8/26/09	143-155	238-263	60	135	185	142
9/4/2009					182	140
New AC-1 9/17/09	148-166	195-230	60	130	182	142
2/4/2010	150	160:w/pump#2 0.3mL/min & 260w/pump#2 0.3mL/min	0	90	117	90

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A 5/13/11	124-142		185			
5/17/11	125-136	288-263	2	145	154-158	115-117
6/24/11	120-134	269-296	20	180	198	136

Note: Shimadzu pressure depends partly on the positioning of the backpressure valve

1. Biorad back pressure too high (>325 during trapping). Back flushing did not resolve problem. TC and AC-2 stored in 100% ACN.

A. Full system not configured. Biorad connected to GC-AC-1. Shimadzu connected to AC-2

VII. Data Analysis

1. File/New Batch (choose file location and name)
2. File/Add Samples (select all samples for batch and edit sample type to sample/cal, etc and assign cal levels)
3. Select the highest calibrant (highlight)

Appendix Y: Standard Operating Procedure for Urinary Metabolite Extraction and Analysis

STANDARD OPERATING PROCEDURE (SOP) FOR EXTRACTION AND ANALYSIS OF NITROPYRENE METABOLITES IN URINE USING LC/MS/MS

Prepared by:	Mike Paulsen	Date:	11/23/07
Revised by:	Mike Paulsen	Date:	4/9/08
Revised by:	Mike Paulsen	Date:	5/5/08
Revised by:	Mike Paulsen	Date:	5/12/08
Revised by:	Vanessa Galaviz	Date:	
Reviewed by:		Date:	
Approved by:	Chris Simpson Assistant Professor	Date:	

Section No.	<u>1</u>
Revision No.	<u>3.3</u>
Date	<u>6/2/10</u>
Page	<u>1 of 16</u>

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Table of Contents

List of Tables	2
Supplies:.....	3
Equipment:.....	3
Preparation of Solutions:.....	3
Urine Sample Collection:.....	4
Urine Extraction.....	4
Blue Rayon Extraction.....	4
Alumina Sep-Pak Cleanup:.....	5
Quality Control Samples:.....	6
Instrumental Analysis:	7
Reporting, Data Analysis and QC Review:	8
Preparation of Calibrants and Spike Solutions:	9

List of Tables

Table 1 Preparation of High Calibration Standard (200 pg/ μ l)	9
Table 2 Dilution of Calibrants 200 to 0.025 pg/ μ l.....	9
Table 3 Concentration of Calibrants	10
Table 4 Preparation of Deuterated Spike Solution	10
Table 5 Preparation of Protonated Spike Solution.....	11
Table 6 LC/MS/MS Method From Mass Hunter Software	11
Table 7 Data QC Flags.....	16

Section No.	<u>1</u>
Revision No.	<u>3.3</u>
Date	<u>6/2/10</u>
Page	<u>2 of 16</u>

Supplies:

1. Silanized glassware: amber HPLC vials, HPLC vial micro inserts, Pasteur pipettes, TurboVap tubes
2. 125 mL glass bottle with Teflon-lined cap
3. HCl, Fisher, A144s-500
4. Sodium Acetate anhydrous, Fluka, Chemika, 71185
5. β -Glucuronidase/Arylsulfatase (100,000 Fishman units/mL / 800,000 Roy units/mL, Roche, 127 698)
6. Blue Rayon
 - a. MP Biomedicals, 808687 or
 - b. Funakoshi Co (Japan), BR-001
7. SPE tube (20 cc size), Supelco, 57177
8. Polyethylene frit for blue rayon filtration, Supelco 57181
9. Ammonium hydroxide, Sigma, ACS reagent, 28-30% NH₃, 221228-500ML-A
10. TurboVap tubes, 50ml size (silanized and methanol-rinsed)
11. Sep-Pak Alumina A cartridge, Waters, WAT020500
12. Methanol, Fisher, optima, A454-4
13. Ethylacetate (JT Baker, Baker Analyzed ACS Reagent, 9280-05)
14. Syringe for extract filtration, disposable, 1 cc, BD, with slip tip, 309602
15. Syringe filters
 - a. Acrodisc 3 mm HPLC 0.45 μ m, 3 CR PTFE or
 - b. National Scientific 4 mm Teflon 0.2 μ m, F2504-4
16. Extension tube for syringe filters, Supelco 57059
17. HPLC vials with caps
18. Nylon membrane filters 0.45 μ m 100 discs, cat #7404-004, Whatman Int
19. Disposable flow control valve liners for the visiprep-DL, cat#57059 pack 150, Supelco

Equipment:

1. Shaking Water-Bath
2. TurboVap
3. Sample Concentrator (heated with nitrogen source) (Techne, Dri Block DB-3A)
4. Wrist-Action Shaker
5. Sonic Bath
6. SPE Vacuum Manifold
7. LC/MS/MS, Agilent 1100/6410

Preparation of Solutions:

1. 1 M HCl (75 μ L per sample) (0.825 ml concentrated HCl + 9.175 ml H₂O) (Stable for couple of months)

Section No.	<u>1</u>
Revision No.	<u>3.3</u>
Date	<u>6/2/10</u>
Page	<u>3 of 16</u>

2. 4 M acetate buffer (pH 5) (5 ml per sample) (Stable for couple of months). Discard: neutralize with baking soda and pour down drain
 - a. 4 M sodium acetate
 - b. 4 M acetic acid (23 ml glacial/ 100 ml final volume)
 - c. Start out with 100 ml (a) in a 250 mL Wheaton bottle and then add (b) until pH 5
Requires approximately 2:1 (a:b), so start by adding 40 ml (b), then slowly adding more (b) until pH 5 is reached.
3. Methanol: Ammonium (50:1) (20 mL per sample) (prepared by diluting ammonia water to 7% of concentrate) (Make day of) (discard polar waste)
4. Methanol: ethyl acetate (1:1) (40 mL per sample) (measure equal volumes of each solvent and combine in a Wheaton bottle) (Stable for couple months) (discard polar waste)
5. Mobile phase B: Methanol with 0.01% ammonium hydroxide (350 μ l of 28-30% stock per liter). Prepare fresh and keep refrigerated or on ice. (Make day of)
6. Mobile phase A: Water with 0.01% ammonium hydroxide (350 μ l of 28-30% stock per liter). Prepare fresh and keep refrigerated or on ice. (Make day of)

Urine Sample Collection

1. Collect urine sample in 500 ml Nalgene polypropylene bottles (methanol-rinsed)
2. Store frozen at -20°C until analysis

Urine Extraction

1. Thaw urine samples and transfer 100 ml portions into 125 ml silanized glass Wheaton bottles (methanol-rinsed)
2. Label bottles near the top using a sharpie and label caps using tape
3. Add 75 μ l of 1 M HCl (can use plastic pipette tip)
4. Add 5 ml of 4 M acetate buffer (pH 5) (use 5mL glass pipette)
5. Add 25 μ l D spike (recover standards OHNPs-d₈, OHNAAPs-d₈ and 1-NAAP-d₉) (use positive displacement pipette)
6. Add β -Glucuronidase/aryl sulfatase (75 μ L) (can use plastic pipette tip)
7. Prepare water spike samples and blanks (Unspiked water)
 - a. D Spike (N=2): 100 ml water amended with HCl, buffer, protonated and deuterated standards, and glucuronidase
 - b. D+H Spike (N=2): 100 ml water amended with HCl, buffer, protonated and deuterated standards, and glucuronidase
 - c. Blank (N=2): 100 ml water amended with HCl, buffer, and glucuronidase
8. Incubate at 37°C for 4 hours in a shaking water-bath (note: should start incubation by around 10:30 am)

Section No.	<u>1</u>
Revision No.	<u>3.3</u>
Date	<u>6/2/10</u>
Page	<u>4 of 16</u>

Blue Rayon Extraction

1. Weigh 100 mg portions of blue rayon into new weigh boats. Store individual portions in ziplock bags until samples finish deconjugation. Longer-term storage of 100 mg portions may be in clean glass headspace vials vials sealed with Teflon-lined caps.
2. Add 100 mg blue rayon to each sample
3. Incubate samples at room temperature while shaking on wrist-action shaker for 1 hour (covered with black plastic bag to protect from light)
4. Extraction of blue rayon
 - a. Pour the urine through a funnel (to capture blue rayon if it is accidentally poured out of the bottle) directly to waste leaving the blue rayon in the bottle. (It is not necessary to use the vacuum manifold for this step)
 - b. Rinse the bottle and blue rayon with water (3 x 10 ml) and pour through the funnel
 - c. After the third rinse, use clean tweezers (MeOH rinse) to transfer the blue rayon from the bottle to a clean, empty 20 cc SPE tube. Place the tube on a vacuum manifold and vacuum excess water out of blue rayon
 - d. After transferring blue rayon to the SPE tube, rinse the bottle with approximately 25 ml water followed by two rinses of several ml methanol to remove residual water (Important to remove as much residual water as water will delay the evaporation step)
 - e. Return the blue rayon to the bottle
 - f. Add 20 ml extraction solvent (50:1 methanol: ammonium hydroxide solution)
 - g. Sonicate for 30 minutes (covered to protect from light)
 - h. Prepare 6 cc empty SPE tubes by inserting polyethylene frits and rinsing twice with 6 ml methanol
 - i. Place a TurboVap tube inside the vacuum manifold, using a small beaker to hold the tub upright. Use a disposable flow control valve (cut leaving ~1/2 inch) to guide liquid into turbovac tubes
 - j. Pour extract through the 6 cc tubes into TurboVap tubes, leaving Blue Rayon in the bottle
 - k. Rinse the bottle and Blue Rayon with methanol (3 x 2 ml). Pour solvent through syringe to combine with extract in TurboVap tube
 - l. Add 50 µl DMSO to each sample as a keeper solvent
 - m. Evaporate extract to near-dryness in TurboVap at 45°C (add 10 ml acetonitrile when volume is reduced to approximately 5 ml to assist evaporation of water → TK did not do this step).
 - n. CAN STOP AT THIS STAGE TO CONTINUE THE FOLLOWING DAY.
PLACE TURBOVAC TUBES IN -20c
 - o. Re-dissolve residue in 5 ml methanol: ethyl acetate (1:1)
 - p. Vortex ~10 secs individually then sonicate 15 minutes with plastic cap and Parafilm sealing the top of TurboVap tube

Section No.	1
Revision No.	3.3
Date	6/2/10
Page	5 of 16

Alumina Sep-Pak Cleanup

1. Place new Alumina A Sep-Paks on a clean vacuum manifold
2. Precondition Sep-Paks with 5 x 5 ml methanol:ethyl acetate (1:1)
3. Place clean 15 ml test tubes inside manifold
4. Clean silanized Pasteur pipets by drawing up and expelling methanol three times
5. Rinse sides of TurboVap tube and transfer extract to Sep-Paks. Use a disposable flow control valve (cut leaving ~1/2 inch) to guide liquid into test tubes
6. Add a second 5 ml portion of eluant to TurboVap tubes and transfer to Sep-Paks
7. Add a third 5 ml portion of methanol: ethyl acetate (1:1) directly to Sep-Paks
8. Pour extracts from test tubes back to original TurboVap tubes. Rinse test tubes with 5 ml methanol and pour into TurboVap tubes
9. Evaporate methanol:ethyl acetate to near-dryness in TurboVap at 45°C
10. Re-dissolve residue by adding 300 µl methanol to TurboVap tubes
11. Cover TurboVap tubes with red plastic caps (methanol-rinsed)
12. Vortex briefly
13. Rinse apron of TurboVap tube 10 times using a silanized, methanol-rinsed Pasteur pipet
14. Filter into silanized HPLC vial inserts
15. Make control solutions
 - a. Add 50µL D spike and 50µL H spike
16. Cap vials and store samples in freezer until day of analysis
17. On day of analysis, remove samples from freezer and evaporate methanol to approximately 50 µl at room temperature under nitrogen
18. Add 20 µl water. Cap vial, flick vials several times and gently invert vials to mix

Quality Control Samples

1. Water Blanks
 - a. N=2 per batch
 - b. 100 ml water process like urine, but no spike solutions are added
2. Deuterated spiked water
 - a. N=2 per batch
 - b. 100 ml water process like urine
 - c. Spike with 25 µl deuterated internal standards only
3. D and H spiked water
 - a. N=2 per batch
 - b. 100 ml water process like urine
 - c. Spike with 25 µl deuterated internal standards and with 25 µl protonated spike mix

Section No.	<u>1</u>
Revision No.	<u>3.3</u>
Date	<u>6/2/10</u>
Page	<u>6 of 16</u>

4. Deuterated and protonated spike solution controls. Spike solutions added directly to solvent for injection
 - a. N=2 per batch
 - b. 50 µl deuterated spike and 50 µl protonated spike plus 40 µl water
5. Benchmark urine
 - a. N=2 per batch
 - b. Prepared from composited Chinese and UW lab staff urine (fortified with 1 pg/ml protonated NP metabolites)

Instrumental Analysis

1. Analyze by LC//MS/MS (Agilent 1100 HPLC with a 6410 tandem mass spectrometer)
2. Method: NP Metab MRM Hi pH 042308.m (See attached method printout)
3. Prepare mobile phases by adding 350 µl ammonium hydroxide per liter of either water or methanol
 - a. Note: It is important to keep the mobile phases cold to slow evaporation of ammonia. As the ammonia concentration decreases, the retention times increase for these analytes. Pre-chill the water and methanol and keep solutions on ice after adding the ammonium hydroxide. Mobile phases can be kept cold during analysis by placing the bottles in Styrofoam coolers packed with ice. Use a lid with a hole cut in the top to fit tightly against the cap of the mobile phase bottle. Ice will last approximately 24 hours.
4. Install column (Agilent Zorbax Extend-C18, 2.1 X 100 mm, 3.5 µm particles (761753-902, with guard column)
5. Purge pumps with fresh mobile phase for 5-10 minutes at 5 ml/ min, 50% each channel A and channel B
6. Sample naming (examples, where MMDDYY is the preparation date of the sample or standard and AA is the run number):
 - a. Std 10 MMDDYY AA.d
 - b. D H2O Spk #1 MMDDYY AA.d
 - c. D+H H2O Spk #1 MMDDYY AA.d
 - d. D+H 100% Ctl #1 MMDDYY AA.d
 - e. H2O Blk #1 MMDDYY AA.d
 - f. BM #1 MMDDYY AA.d
 - g. Sample ID MMDDYY AA.d
7. Sample log: Analyze the samples and standards in the following order
 - a. Instrument checkout standard
 - b. Blank
 - c. Std 0.01
 - d. Std 0.025
 - e. Std 0.1

Section No.	<u>1</u>
Revision No.	<u>3.3</u>
Date	<u>6/2/10</u>
Page	<u>7 of 16</u>

- f. Std 0.25
- g. Std 1
- h. Std 2.5
- i. Std 10
- j. Std 25
- k. Std 100
- l. Blank
- m. Blank
- n. QC and Benchmark sample extracts
- o. Std 1
- p. Blank
- q. 10 Samples
- r. Std 1
- s. Blank
- t. 10 Samples
- u. Std 1
- v. Blank
- w. Repeat pattern
- x. Reanalysis of 1 QC (D+H H2O Spk)
- y. Reanalysis of 2 urine extracts (1 per 15 samples analyzed)
- z. Std 1
- aa. Blank (shutdown method NP Metab Shutdown 050908.m)

Reporting, Data Analysis and QC Review

1. Set up calibration curves using 1/x weighting
2. Print the worklist
3. Generate quantitative analysis report
4. In Excel, print each report
5. Review reports
 - a. Were peaks correctly chosen?
 - b. Were peaks free of apparent interfering peaks?
 - c. Were peaks integrated properly?
 - d. Do values in summary report match the values in the printed reports being edited?
 - e. Generate a summary table for export to Excel
6. Open file in Excel for assigning QC flags

Section No.	<u>1</u>
Revision No.	<u>3.3</u>
Date	<u>6/2/10</u>
Page	<u>8 of 16</u>

Preparation of Calibrants and Spike Solutions

1. Calibrants:
 - a. See Table 1. Individual stock solutions are prepared in methanol
 - b. Prepare calibrants by diluting Standard 200 with methanol according to Table 2
 - c. Store at -20°C in freezer 5 in F-455

Table 1 Preparation of High Calibration Standard (200 pg/μl)

Standard No.	Chemical	Stock Conc. (ug/ml)	Conc. of 1:100 dil (pg/ul)	Calculated Vol into STD 200 (ul)	Actual Vol into STD 200 (ul)
9	3-OHNP	235	2350	170.2	170.2
6	6-OHNP	505	5050	79.2	79.2
8	8-OHNP	51	510	784.3	784.3
13	3-OHNAAP	77	771	519.0	200.0
	6-OHNAAP	650	6504	61.5	200.0
	8-OHNAAP	478	4782	83.6	200.0
	NAAP	444	4440	90.1	90.1
		Vol MeOH (μl)			276
		Total vol (μl)			2000

Table 2 Dilution of Calibrants 200 to 0.025 pg/μl

STD	STD Conc After adding ISTD (pg/μl)	Vol Standard (μl)	STD Used	Vol Diluent (μl)	Final Vol Before Adding ISTD and Water (μl)	Volume ISTD (μl)	Vol Water (μl)
1	100	250	200	0	250	250	200
2	25	62.5	200	187.5	250	250	200
3	10	31	200	279	250	250	200
4	2.5	7.75	200	302	250	250	200
5	1	60	10 (20) ^a	240	250	250	200
6	0.25	60	2.5 (5) ^a	240	250	250	200
7	0.1	50	1 (2) ^a	200	250	250	200
8	0.025	50	0.25 (0.5) ^a	200	250	250	200
9	0			250	250	250	200

Sum of STD 100 used 357.45

^a Number in () is nominal concentration before adding ISTD and water

Dilution of standards to 29% water

Add 0.2 ml water to 0.5 ml standard

Each set of standards will require 357 ul of STD 200 stock

Section No.	<u>1</u>
Revision No.	<u>3.3</u>
Date	<u>6/2/10</u>
Page	<u>9 of 16</u>

Table 3 Concentration of Calibrants

Compound	100	25	10	2.5	1	0.25	0.1	0.025	0
3-OHNP	71.43	17.86	7.14	1.79	0.714	0.179	0.0714	0.0179	0
6-OHNP	71.43	17.86	7.14	1.79	0.714	0.179	0.0714	0.0179	0
8-OHNP	71.43	17.86	7.14	1.79	0.714	0.179	0.0714	0.0179	0
3-OHNAAP	27.52	6.88	2.75	0.69	0.275	0.069	0.0275	0.0069	0
6-OHNAAP	232.30	58.08	23.23	5.81	2.323	0.581	0.2323	0.0581	0
8-OHNAAP	170.80	42.70	17.08	4.27	1.708	0.427	0.1708	0.0427	0
NAAP	71.43	17.86	7.14	1.79	0.714	0.179	0.0714	0.0179	0

Spike Solutions**Table 4 Preparation of Deuterated Spike Solution**

Standard No.	Chemical	Stock Conc. (ug/ml)	Conc. of 1:10 dil (pg/ul)	Calculated Vol into ISTD Spk Solution (ul)	Actual Vol into ISTD Spk Solution (ul)	Actual Conc ISTD Spk Solution (pg/ul)	Actual Conc in Sample (pg/ul)
17	d8-3-OHNP	190	18967	36.91	10	7.6	2.71
17	d8-6-OHNP	604	60352	11.60	10	24.1	8.62
17	d8-8-OHNP	509	50928	13.74	10	20.4	7.28
25	d8-3-OHNAAP	19	1910	367	440	33.6	12.0
23	d8-6-OHNAAP	282	28193	24.83	75	84.6	30.2
23	d8-8-OHNAAP	201	20067	34.88	75	60.2	21.5
	d9-NAAP	117	11700	59.83	150.0	70.2	25.1
			Vol MeOH (µl)			24230	
			Total vol (µl)			25000	

Section No.	<u>1</u>
Revision No.	<u>3.3</u>
Date	<u>6/2/10</u>
Page	<u>10 of 16</u>

Table 5 Preparation of Protonated Spike Solution

Compound	Actual Used Vol of 1:100 dil stock for 100 samples (ul)	Actual Used Vol of 1:1000 dil stock for 100 samples (ul)	Corrected Stock Conc. (ng/ul)	Mass in 2.5 ml Spike solution (pg)	Actual High Spike Mass per sample (pg)	Actual High Spike Extract Conc (pg/ul)
3-OHNP	21.3		235	50000	500	7.14
8-OHNP	98.0		51	50000	500	7.14
6-OHNP		99.0	505	50000	500	7.14
3-OHNAAP	20.0		77		154	2.20
8-OHNAAP			478		956	13.66
6-OHNAAP			650		1301	18.58
NAAP		112.6	444	50000	500	7.14
Spike volume						
Volume						
MeOH						
Final volume = 2.5 ml						

Table 6 LC/MS/MS Method From Mass Hunter Software

Acquisition Method Info

Method Name NP Metabolite MRM Hi pH_0.2mL 10uL longer dwell_A1 B2-NH3.m
Method Path D:\MassHunter\methods\NP Metabolite MRM Hi pH_0.2mL 10uL longer dwell_A1 B2-NH3.m
Method Description ESI Positive MS2 Background Scan Method

Device List

ALS
 Bin Pump
 Column
 MS QQQ

QQQ Mass Spectrometer

Ion Source ESI
Tune File atunes.tune.xml
Stop Mode No Limit/As Pump
Stop Time 20
Time Filter On
Time Filter Width 0.07

Time Segments

Section No.	<u>1</u>
Revision No.	<u>3.3</u>
Date	<u>6/2/10</u>
Page	<u>11 of 16</u>

Time Seg #	Time	Scan Type	Ion Mode	Polarity	Div Valve	Delta EMV	Store
1	0	MRM	ESI	Negative	To Waste	0	<input type="checkbox"/>
2	2	MRM	ESI	Negative	To MS	600	<input checked="" type="checkbox"/>
3	10.5	MRM	ESI	Negative	To MS	600	<input checked="" type="checkbox"/>
4	18.5	MRM	ESI	Negative	To MS	600	<input checked="" type="checkbox"/>
5	23	MRM	ESI	Negative	To Waste	0	<input type="checkbox"/>

Time Segment 1

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)
Compound1	<input type="checkbox"/>	350	Unit	200	Unit	500	140	0

Source Parameters

Parameter	Value
Gas Temp (°C)	350
Gas Flow (l/min)	11
Nebulizer (psi)	50
Capillary (V)	1500

Time Segment 2

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)
d8-OHNAAP	<input type="checkbox"/>	282	Wide	239	Wide	800	170	25
OHNAAP	<input type="checkbox"/>	274	Wide	231	Wide	800	170	25

Source Parameters

Parameter	Value
Gas Temp (°C)	350
Gas Flow (l/min)	10
Nebulizer (psi)	40
Capillary (V)	2000

Time Segment 3

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)
d8-OHNP	<input type="checkbox"/>	270	Wide	240	Wide	800	160	25
OHNP	<input type="checkbox"/>	262	Wide	232	Wide	800	160	25

Source Parameters

Parameter	Value
Gas Temp (°C)	350
Gas Flow (l/min)	10
Nebulizer (psi)	40
Capillary (V)	1500

Section No.	<u>1</u>
Revision No.	<u>3.3</u>
Date	<u>6/2/10</u>
Page	<u>12 of 16</u>

Time Segment 4

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CI (V)
d9-NAAP	<input type="checkbox"/>	267	Wide	225	Wide	200	150	25
NAAP	<input type="checkbox"/>	258	Wide	216	Wide	200	150	25

Source Parameters

Parameter	Value
Gas Temp (°C)	350
Gas Flow (l/min)	12
Nebulizer (psi)	45
Capillary (V)	2500

Time Segment 5

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CI (V)
Compound1	<input type="checkbox"/>	350	Unit	200	Unit	200	140	0

Source Parameters

Parameter	Value
Gas Temp (°C)	350
Gas Flow (l/min)	11
Nebulizer (psi)	50
Capillary (V)	1500

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	0	10000000

Instrument Curves

Actual
#N/A

Autosampler

Name	ALS	Model	G1313A
Ordinal #	1	Options	
Stop Time (min)	As Pump	Post Time (min)	Off
Injection Type	Standard Injection	Injection Volume	10
Overlap Time	Disable Overlapped Injection	Draw Position	3
Draw Speed	50	Eject Speed	50
Wash Vessel	N/A		
Ready Temp. Range		Temp.	

Section No.	1
Revision No.	3.3
Date	6/2/10
Page	13 of 16

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Binary Pump

Name	Bin Pump	Model	G1312A
Ordinal #	1	Options	SSV
Stop Time (min)	27	Post Time (min)	10
Flow (µl/min)	0.2	Pressure Min (bar)	0
Pressure Max (bar)	250	Max Flow Gradient (ml/min)	100
Solvent A	Water w/0.01% NH3	Solvent B	Methanol w/0.01% NH3
Solvent Ratio A	70	Solvent Ratio B	30
Compress. A (*10-6/bar)	50	Compress. B (*10-6/bar)	115
Stroke A	Auto	Stroke B	Auto
Contact 1	0		
Contact 2	0		
Contact 3	0		
Contact 4	0		

Pump Time Table

Time	Flow	Pressure	Solv Ratio B
0	0.2	250	30
15	0.2	250	50
15.01	0.2	250	80
21	0.2	250	80
21.1	0.2	250	30
26.9	0.2	250	30
27	0.2	250	30

Thermostated Column Compartment

Name	Column	Model	G1316A
Ordinal #	1	Options	CSV
Stop Time (min)	As Pump	Post Time (min)	Off
Left Temp.	30	Right Temp.	Not Controlled
Left Ready	Off	Right Ready	Off
Valve Position	1		
Contact 1	0		

Section No.	<u>1</u>
Revision No.	<u>3.3</u>
Date	<u>6/2/10</u>
Page	<u>14 of 16</u>

Contact 2 0
Contact 3 0
Contact 4 0

Signals Selected

Description

Temperature of left heat exchanger

Section No.	<u>1</u>
Revision No.	<u>3.3</u>
Date	<u>6/2/10</u>
Page	<u>15 of 16</u>

Table 7 Data QC Flags

<u>Symbol</u>	<u>Meaning</u>	<u>Action</u>
ND	Compound not detected	Replace value with <LOD
NQ	Compound not quantifiable but detected	delete value/censor value cell
NR	Concentration has error	delete value/censor value cell
NS	ISTD Missing	delete value/censor value cell
N<	Conc is < LQL	no action
AI	Area not valid ^ Interference	delete value/censor value cell
N>	Conc is > UQL	no action
AX	Area not valid	delete value/censor value cell
N1	Compound not analyzed for	no action
RY	Recovery outside QA limits or was indeterminate	delete value/censor value cell
MR	Value less than minimum reported level	replace with <LOD
R2	Calibration outside QA limit	delete value/censor value cell
M03	outlier	no action

Section No.	<u>1</u>
Revision No.	<u>3.3</u>
Date	<u>6/2/10</u>
Page	<u>16 of 16</u>

