EXPOSURES TO HOUSEHOLD AIR POLLUTION IN WOMEN ACROSS FOUR DIVERSE COUNTRIES AS PART OF A MULTI-COUNTRY COOKSTOVE INTERVENTION TRIAL

by

KATHERINE ANN KEARNS

(Under the Direction of Luke Peter Naeher)

ABSTRACT

Background: The Household Air Pollution Intervention Network (HAPIN) trial measures pregnant women's exposures to and concentrations of household air pollutants (HAP) in four low- and middle-income countries (LMICs). **Objectives:** 1) To measure nitrogen dioxide (NO_2) in households enrolled in the HAPIN trial, 2) characterize exposures to HAP including household waste burning (fine particulate matter, $PM_{2.5}$; black carbon, BC; polycyclic aromatic hydrocarbons, PAHs), and exposure to plastics (bisphenol A, BPA; phthalates), in adolescent girls in rural Guatemala, 3) compare gravimetric data between three HAPIN laboratories in Guatemala, India, and the U.S., and to highlight the launch of the newly-established Guatemala laboratory that resulted from this work, and 4) explore sources of $PM_{2.5}$ in Guatemala and Rwanda via source apportionment. **Methods:** We used passive sampling to measure 24h concentrations of NO_2 in households in Guatemala (n=151), Peru (n=101), and Rwanda (n=36). In Guatemala, we assessed exposures to HAP and plastics via air monitoring and biomarkers analysis, respectively, in n=56 adolescent girls. As part of capacity building and validation, we used Bland-Altman analyses to compare $PM_{2.5}$ and BC measurements between the three laboratories. Finally, we conducted source apportionment on filter samples from Guatemala (n=64) and Rwanda (n=59). **Results:** Study-wide, we observed significant reductions in NO_2 concentrations in homes with gas stoves compared to homes with biomass stoves. In adolescent girls in Guatemala, exposures to $PM_{2.5}$, BC, and PAHs were significantly lower in gas stove compared to biomass participants. BPA and phthalates were not significantly different between study arm, indicating that exposure was independent of study arm. For capacity building, we observed repeatability in measurements between all three laboratories and launched the Guatemala laboratory for use in later stages of the HAPIN trial. Source apportionment resolved four potential sources of $PM_{2.5}$ in Guatemala and Rwanda including biomass, crustal, agricultural, and gasoline. **Conclusions:** We characterized various HAP-related exposures in susceptible populations: pregnant women, new mothers, and adolescent girls in LMICs, which are disproportionately impacted by HAP. Our results suggest that LPG stoves can help reduce exposures to multiple household air pollutants that are associated with adverse health effects.

INDEX WORDS: Nitrogen dioxide, $PM_{2.5}$, Black carbon, Household garbage burning, Capacity building, Biomonitoring

EXPOSURES TO HOUSEHOLD AIR POLLUTION IN WOMEN ACROSS FOUR DIVERSE COUNTRIES AS PART OF A MULTI-COUNTRY COOKSTOVE INTERVENTION TRIAL

by

KATHERINE ANN KEARNS

BSEH, University of Georgia, 2016

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2022

EXPOSURES TO HOUSEHOLD AIR POLLUTION IN WOMEN ACROSS FOUR DIVERSE COUNTRIES AS PART OF A MULTI-COUNTRY COOKSTOVE INTERVENTION TRIAL

by

KATHERINE ANN KEARNS

Major Professor:	Luke P. Naeher
Committee:	Jia-Sheng Wang
	Stephen L. Rathbun
	John P. McCracken
	Travis C. Glenn

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
August 2022

DEDICATION

“Promise me you’ll always remember you’re braver than you believe, stronger than you seem, and smarter than you think.”

-Winnie the Pooh

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude for my advisor, Dr. Luke Naeher. From the time I met him during my undergraduate studies at the University of Georgia in 2015, I admired his charisma, knowledge, and his deep-rooted passion for human and environmental health. As a graduate student, he has helped me to become a critical, scientific thinker; just as importantly, he has always encouraged me to work really hard, and to work really hard to enjoy life outside of work and to recharge. I have always appreciated that about him and it has been an honor to learn from him in all aspects.

I am thankful for my dedicated committee members who have invested so much into my success. Dr. Jia-Sheng Wang has exemplified incredible leadership to the Department of Environmental Health Science. He is a strong advocate for every student and makes them feel at home within the department. Dr. Stephen Rathbun has been an invaluable voice of reason and has inspired me to think more critically about my data. Dr. John McCraken has been instrumental in my success in many ways: he welcomed me into his home in Guatemala while I worked there under his mentorship, where I also learned from his brilliant wife Anaité Diaz-Artiga, befriended their son Ian (age 9 at the time), and enjoyed delicious family meals. Muchas gracias. Last but not least, I have known Dr. Travis Glenn since he was my undergraduate advisor in 2015. He had baby alligators in his office during more than one of our advisement appointments, and I have thoroughly enjoyed learning from him over the past several years.

I would like to recognize our wonderful administrative associates, Darien Bush and Leah Paustian, who have helped me in countless ways over the years with last-minute international shipments and applying for various grants. I would like to give a special shout-out to Darien for saving me from a terrifying spider cricket that infiltrated our laboratory one time. I would also like to thank our business manager, Haoguang Liu for all you have done for me over the years, and largely behind the scenes. I am so thankful not only for your kindness, but also your immense support.

I would like to acknowledge the Interdisciplinary Toxicology Program, the program that believed in my academic success when I was accepted as a doctoral student in 2017. It has been a pleasure getting to know and learn from all of the ITP faculty and students that come from such diverse backgrounds. Thank you to the former Director of ITP, Dr. Brian Cummings, the current interim director Dr. John Wagner, and the graduate coordinator Dr. Nikolai Filipov for all you have done to support me in my educational pursuits. A special thank you to our administrative specialist Ms. Wanda Darden for all you have done for me behind the scenes and for your quick assistance in times of need.

I am very thankful for the Graduate School, who accepted my application in April 2017 and has been an advocate for my success in many ways over the years. In the summer of 2019, the Graduate School fully funded two trips for my advisor, two other lab mates, and me: In June, we visited Guatemala and in July, we visited Peru, which are both study sites for the HAPIN trial. This trip was invaluable for me, as I was invited back to the Guatemala site to work and experience field work for five months later that year, and my lab mates have since followed suit. Thank you for giving us unforgettable experiences that have shaped our professional successes.

I am deeply grateful for my lab mates in Dr. Naeher's lab over the past five years. Abby Jones and I joined together in 2017 and learned a lot together and from each other. Davis Reardon, Devan Campbell, Jacob Kremer, and Erick Mollinedo and I not only worked incredibly well together for years but we have also formed real friendships with one another. I cannot wait to see what the future holds for all of us.

I would also like to recognize my predecessors in the Naeher lab whom I have leaned on for support over the years: Anna and Femi Adetona, and Ruth Wangia. I would like to give a special thanks to another predecessor, my friend Jona Johnson for all the e-mails, text messages, phone calls, and Zoom sessions; your advice and words of encouragement mean the world to me. I would also like to acknowledge all of the current faculty and students of the EHS department who have offered me support and a great sense of community.

I want to thank all the many HAPIN investigators, many of whom I have been fortunate to get to know on both a personal and professional level. I simply cannot put into words all I have learned from you over the years, whether you have hosted me in your country, city, or home, or even if I shook your hand once at one of our annual meetings and proceeded to correspond with you over e-mail for years. I am eternally grateful to have collaborated with you and to have learned from you. I feel that I have grown up (professionally) in the HAPIN study. I would like to give an extra special thank-you to all of the incredibly hard-working field teams in all four of the HAPIN study sites, especially those at the Guatemala site who hosted me and befriended me during the five months I spent in their beautiful country.

I would like to thank all my friends who have loved me and supported me through this challenging pursuit: Sara Kearns (my sister and my best friend), Rose Lawson, Chelsea Cary, Kelly Seagraves, Emily McCoy, Maddie Street, Sami McCabe, Lizzie Reigelman, Hannah Xu, Lillie Barnett, and Soo Shin for your wonderful friendship. You have seen me through all of the triumphs (and some of the tears…), and I love you all very much.

I would not have been able to complete this degree without the unending support of my family. My parents have instilled in me immense willpower, incredible strength and perseverance, and an insatiable desire to learn. My grandmother, aunt, and uncle have always been my biggest champions for success. The support from my siblings has been unwavering, and being roommates with my sister throughout this adventure has been the biggest blessing. Visiting my parents, grandmother, and my aunt and uncle where they live on the lake has been my place of solace over the years where we have enjoyed many cups of coffee and word puzzles on the back porch, walks down Tranquility Lane, and horseback riding through trails lined with pine trees. I love you all so much.

Last but certainly not least, quisiera expresar el agradecimiento profundo para mi novio Juan Carlos. Siempre has tenido un montón de paciencia conmigo. Gracias por todo tu apoyo, las palabras de ánimo, los consejos, las citas por Zoom, por tantas risas (qué payaso eres), y sobre todo, el amor. Te amo mucho.

It has been a journey, but we made it!

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS...v

LIST OF TABLES xii

LIST OF FIGURES xiii

CHAPTER

1 INTRODUCTION AND LITERATURE REVIEW 1

Introduction to Household Air Pollution and the HAPIN trial 1

Purpose of Research…………………………………………………………..…...5

Outline of Dissertation 6

Literature Review...7

2 COMPARING EXPOSURES TO NITROGEN DIOXIDE IN HOMES WITH BIOMASS AND GAS COOKSTOVES IN THREE LMICS AS PART OF THE HAPIN RANDOMIZED CONTROLLED TRIAL…………………………………..26

Abstract 27

Introduction...28

Methods...30

Results...34

Discussion 37

Conclusion 45

3 EXPOSURES TO HOUSEHOLD AIR POLLUTION AND GARBAGE BURNING VIA AIR MONITORING OF $PM_{2.5}$ AND BC, AND URINARY BIOMARKERS OF EXPOSURE TO PAHS, BISPHENOL A, AND PHTHALATES IN ADOLESCENT GIRLS IN RURAL GUATEMALA………………………………………………....54

Abstract 55

Introduction...56

Methods...60

Results...65

Discussion 69

Conclusion 71

4 $PM_{2.5}$ AND BC DATA COMPARISONS AND CAPACITY BUILDING BETWEEN GRAVIMETRIC LABORATORIES IN THREE DIFFERENT COUNTRIES FROM THE HAPIN RANDOMIZED CONTROLLED TRIAL 82

Abstract 83

Introduction...84

Methods...85

Results...88

Discussion 90

Conclusion 92

5 INVESTIGATING POTENTIAL SOURCES OF $PM_{2.5}$ IN GUATEMALA AND RWANDA VIA X-RAY FLUORESCENCE AND SOURCE APPORTIONMENT ………………………..………………………………104

Abstract 105

Introduction 106

Methods 111

Results and Discussion 115

Conclusion 118

6 SUMMARY AND CONCLUSIONS 121

Summary 121

Conclusions 124

Future Research 125

REFERENCES 128

LIST OF TABLES

Page

Table 2.1. Household and participant characteristics in the HAPIN NO_2 study………..……….46

Table 2.2. Personal and kitchen area NO_2 concentrations (ppb)………………………………...48

Table 2.3. Samples below the unadjusted and altitude-adjusted (site-specific) WHO IT-2 guidelines………………………………………………………………………………...49

Table 3.1. Participant characteristics and personal care product, cosmetic, and plastic use…....72

Table 3.2. Adolescent 24-h exposures to $PM_{2.5}$ and BC, and kitchen concentrations of BC……73

Table 4.1. Range of mass depositions and high attenuation (ATN > 125) of post-sampled HAPIN filters used for the laboratory comparisons……………………………………………94

Table 5.1. $PM_{2.5}$ and BC mass concentrations in Guatemala and Rwanda……………………..119

LIST OF FIGURES

Page

Figure 2.1a. LPG stoves installed in Guatemala, Rwanda, and Peru in the intervention arm…………………………………………………………………………………....50

Figure 2.1b. Traditional biomass stoves installed in Guatemala, Rwanda, and Peru in the intervention arm….……………………………………………………………..…..50

Figure 2.2a. Kitchen area concentrations and personal exposures to NO_2 by study site and overall compared to unadjusted WHO 24-h (red) and annual (blue) IT-2 guidelines…………………………………………………………………………...52

Figure 2.2b. Kitchen area concentrations and personal exposures to NO_2 by study site and overall compared to site-specific WHO 24-h and annual IT-2 guidelines…………………..….52

Figure 2.3. Spearman correlations between personal exposures and kitchen concentrations from the same household presented globally and by study site………………………………..53

Figure 3.1. A field nurse administers a questionnaire to an adolescent participant in the intervention arm……………………………………………………………………….74

Figure 3.2. Box plots of 24-h real-time kitchen BC concentrations, and personal BC and $PM_{2.5}$ exposures in control and intervention adolescent participants…………………………..75

Figure 3.3. Spearman correlation between adolescent and mother $PM_{2.5}$ exposures from the parent HAPIN trial living in the same household……………………………………….76

Figure 3.4. Spearman correlation between adolescent and mother BC exposures from the parent HAPIN trial living in the same household……………………………………………….77

Figure 3.5. Box plot of urinary PAH metabolite concentrations in adolescent girls from the control compared to intervention arm with age- and sex-matched participants from NHANES 2015-2016 as a reference……………………………………………………………………..…...78

Figure 3.6. Correlation between total PAH concentrations and adolescent $PM_{2.5}$ exposures…………………………………………………………………………………79

Figure 3.7. Box plot of urinary phthalate metabolite concentrations in adolescent girls from the control compared to intervention arm with age- and sex-matched participants from NHANES 2017-2018 as a reference……………………………………………………………...........................80

Figure 3.8. Box plot of urinary BPA concentrations in adolescent girls from the control and intervention arms compared to age- and sex- matched participants of NHANES 2011-2012...81

Figure 4.1. Study design and flow of filters and MRS from UGA to SRIHER and UVG………94

Figure 4.2a. Agreement between UGA and SRIHER average pre-weights (n=100 filters)….….95

Figure 4.2b. Agreement between UGA average post-weights and SRIHER average post-weights (n=112 filters)……………………………………………………………………………96

Figure 4.2c. Agreement between UGA and SRIHER average pre-scans for BC (n=100 filters)……………………………………………………………………………97

Figure 4.2d. Agreement between UGA and SRIHER average post-scans for BC (n=112 filters)..97

Figure 4.3a. Agreement between average UGA and UVG pre-weights (N=100 filters)………...98

Figure 4.3b. Agreement between average UGA and UVG post-weights (N=112 filters)……....99

Figure 4.3c. Agreement between average UGA and UVG pre-scans (N=100 filters)…………100

Figure 4.3d. Agreement between average UGA and UVG post-scans (N=112 filters)………...101

Figure 4.4. Agreement between SRIHER and UGA MRS……………………………………..102

Figure 4.5. Agreement between UVG and UGA MRS………………………………………...103

Figure 5.1. Factor fingerprints for personal exposure samples in Guatemala (n=64)………….119

Figure 5.2. Factor fingerprints for personal exposure samples in Rwanda (n=59)…………….120

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Household air pollution (HAP) results from the practice of heating or cooking over an open fire using polluting fuels including wood, agricultural residues, coal, charcoal, kerosene, and animal dung.[1,2] Homes are often poorly ventilated, increasing the concentration of hazardous air pollutants emitted from the cookstoves including particulate matter with aerodynamic diameter of ≤ 2.5 μm ($PM_{2.5}$), black carbon (BC), carbon monoxide (CO), nitrogen dioxide (NO_2), and others.[3–5]

Approximately 3.8 billion people globally are routinely exposed to HAP, and the majority reside in low- and middle-income countries (LMICs).[2] Women are generally at the greatest risk for exposure due to the disproportionate amount of time they spend at home over the fire.[1] Exposure to HAP contributes a large burden of disease globally, accounting for 2.31 million deaths in 2019 and attributing to 91 million years of healthy life lost.[2] To address these health disparities, various cookstove intervention studies have been implemented over the years, a few of which are highlighted here.

The late Dr. Kirk R. Smith began investigating personal exposures to HAP in India in 1981 and worked diligently alongside many investigators for 27 more years to identify research needs and eligible sites for investigation. In 2002, Dr. Smith and colleagues launched the first major randomized controlled trial (RCT) in air pollution history.[6] The Randomized Exposure Study of Pollution Indoors and Respiratory Effects (RESPIRE) took place in the Guatemalan highlands where they investigated the impact of HAP reduction on pneumonia incidence in children 18 months and younger.[6]

In the RESPIRE trial, 48-h personal exposures to CO were assessed in 515 children 0-18 months of age and 532 mothers aged 15-55 years to measure long-term exposures to woodsmoke. In a subset of 77 kitchens, area measurements were also taken.[7] Participants were randomized to either the control arm (continued use of open wood fire) or the intervention arm (wood-fueled stove with a chimney). Substantial reductions in mean CO concentrations were observed in the intervention arm for all measurements (kitchen levels: -90%; mothers: -61%; and children: -52%), indicating that adding a chimney to a biomass stove could meaningfully reduce exposures.

In an exposure-response analysis from RESPIRE, they explored the association between physician-diagnosed pneumonia CO exposures in n=265 intervention infants and n=253 control infants.[6] They found that a 50% exposure reduction was significantly associated with physician-diagnosed pneumonia (RR 0.82, 0.70-0.98), suggesting that even improved biomass stoves with a chimney may not be sufficient to substantially reduce pneumonia in populations impacted by HAP.

In 2009, a randomized controlled trial in rural Mexico investigated the impact of an improved biomass cookstove (the Patsari) compared to traditional biomass cookstoves in 552 women.[8] Although the Patsari stove was associated with a significant reduction of symptoms and an improvement of lung function, compliance to the intervention was found to be low at only 50%. This study highlights an issue known as “stove stacking” (using the traditional stove in addition to the intervention stove) that is a prevailing challenge in many cookstove studies to date, which can lead to exposure misclassification.

In 2010, the Clean Cooking Alliance (CCA; formerly known as the Global Alliance for Clean Cookstoves) was launched with the mission of providing 100 million clean-cooking stoves by 2020. The launch of the CCA was a call to action to mobilize an inclusive network of partners to improve health, reduce climate and environmental impacts of HAP, and to empower women. At the time of the CCA's launch, the RESPIRE study and the Patsari intervention study were the only clinical trials conducted, both of which were meaningful first steps in this novel area of the literature, but neither of which had found reductions in HAP significant enough to improve health.[9]

In 2013, the Cooking and Pneumonia Study (CAPS) compared an improved biomass stove (the Philips HD4012LS) to traditional stoves (primarily open wood fires) and assessed the impact on childhood pneumonia in 1928 children in Malawi in a community-level cluster randomized controlled trial.[10] They measured 48h real-time personal exposures to CO and found that average exposures overall were not high, but high peaks in exposures were frequent. They found that there were many potential sources of exposure in these communities in addition to regular cooking activities including commercial cooking and garbage and agricultural burning. Similar to the conclusions from the RESPIRE trial, the CAPS found that biomass stoves, even with improvements such as combustion efficiency, may not be sufficient to meaningfully reduce exposures to levels that are not associated with adverse health outcomes.

Starting that same year in 2013 was the Ghana Randomized Air Pollution and Health Study (GRAPHS), a three-arm RCT in which 1714 pregnant women were randomized to traditional cookstoves (3-stone fire), an improved biomass (wood) stove, or a natural gas (liquefied petroleum gas; LPG) stove.[11] Personal exposures to CO were measured over two years, and associated outcomes of interest were birth weight and other obstetric outcomes. This study found that there were no significant differences between any of the three study arms, and they found no association with low birth weight. It concluded that more large-scale clean stove interventions may be required in order to see community-level improvements in obstetric health.

A few years later in 2017, the Cardiopulmonary outcomes and Household Air Pollution (CHAP) randomized controlled trial was launched in Puno, Peru.[12] Investigators measured blood pressure, peak expiratory flow, and respiratory symptoms in 180 women aged 25-64 years and compared results between participants in the control arm (biomass stove) and intervention arm (LPG stove). This study did not find any significant differences between the groups for cardiopulmonary outcomes.

In 2018, the Household Air Pollution Intervention Network (HAPIN) trial was launched.[3,4,13] Funded by the Bill and Melinda Gates Foundation and the National Institutes of Health, it is one of the most recent RCTs and is the largest of its kind with 3200 pregnant women enrolled across four countries (800 pregnant women in each): Guatemala, India, Peru, and Rwanda. A subset of non-pregnant, older adult women that live with HAPIN participants were also recruited to assess their exposures to HAP. Half of all study participants were randomized to either the control arm (continued use of traditional cookstove) or the intervention arm, where participants received an LPG stove and enough gas to last the duration of the trial (about 18 months total). Participants wore air sampling monitors in their approximate breathing zones and had the same types of monitors hung in their kitchens near their stoves to assess 24-hour personal exposures to and kitchen area concentrations of $PM_{2.5}$, BC, and CO. The HAPIN trial has recently concluded after the last baby was born in September 2020, and analyses are still underway.

Purpose of Research

The HAPIN study is an extensive global effort with the aim of characterizing personal exposures to major pollutants among people using either biomass or LPG stoves. The major aims of the trial are to determine the effect of the LPG intervention primary outcomes including birth weight, pneumonia incidence, length-for-age of the child, and systolic blood pressure in the older adult women. Secondary outcomes include maternal blood pressure, and incidence of preterm birth. With such a sizable portion of the global population exposed to HAP, many important research questions remain beyond the major aims of the HAPIN study.

My dissertation projects all take place within the HAPIN study and expand upon the primary aims to continue assessing the impact of HAP exposure in susceptible populations. My dissertation research has four major objectives, which are presented in four main chapters:

1) To characterize personal exposures to and kitchen concentrations of nitrogen dioxide (NO_2) in homes of new mothers using biomass stoves and gas stoves in Guatemala, Peru, and Rwanda (Chapter 2),

2) Characterize personal exposures to household air pollution including household waste burning and exposure to plastics in adolescent girls in rural Guatemala (Chapter 3),

3) Compare gravimetric laboratory data between three laboratories in different countries and highlight the capacity building efforts (Chapter 4), and

4) Determine the chemical composition of $PM_{2.5}$ and explore potential sources contributing to household air pollution in Guatemala and Rwanda via source apportionment (Chapter 5).

OUTLINE OF DISSERTATION

- **Chapter 1** introduces household air pollution, provides an outline of this dissertation, and provides a review of the current scientific literature relevant to household air pollution and specific topics related to this dissertation.
- **Chapter 2** is a manuscript that presents findings from a study where we measured personal exposures to and kitchen concentrations of NO_2 in new mothers from the HAPIN trial from the Guatemala, Peru, and Rwanda study sites. We compared exposures and concentrations between the two study arms: control (biomass stoves) and intervention (LPG stoves).
- **Chapter 3** is a manuscript that presents the findings from a pilot project that aimed to characterize personal exposures to HAP including that which results from household waste burning in adolescent girls in rural Jalapa, Guatemala. In Jalapa, household waste burning has been reported as one of the most common methods for waste disposal, and because plastic waste is ubiquitous, we sought to characterize exposures to plastics via biomarkers analysis of phthalates and BPA. We measured personal exposures to $PM_{2.5}$, BC via air monitoring, and polycyclic aromatic hydrocarbons (PAHs), BPA, and phthalates via urinary biomarkers analysis.
- **Chapter 4** presents results from a comparison of gravimetric laboratory data between three HAPIN laboratories in three different countries. It also highlights a successful capacity building effort between these three laboratories. We sought to show that no matter where filters are analyzed (Athens, GA; India; or Guatemala), the laboratory data will show satisfactory agreement upon adherence to standardized protocols.

- **Chapter 5** highlights a pilot study which has led to a much larger effort to characterize potential sources of $PM_{2.5}$ in LMICs. In the pilot study, we analyzed personal exposure samples for $PM_{2.5}$, BC, and a suite of 22 metals to determine elemental composition of the samples. $PM_{2.5}$ and BC were measure via gravimetric analysis and optical transmission, respectively, and the metals were quantified via x-ray fluorescence. We then conducted source apportionment via the Environmental Protection Agency's Positive Matrix Factorization (PMF) to determine source profiles of the samples. We were able to resolve four potential sources of $PM_{2.5}$ at these two HAPIN study sites.
- **Chapter 6** contains a summary of this body of work and concluding remarks.

LITERATURE REVIEW

This literature review summarizes 1) the global burden and impact of HAP, 2) nitrogen dioxide as a byproduct of HAP; 3) household waste burning as a means of garbage disposal, waste disposal practices in LMICs and high income countries (HICs), and implications for health upon exposure; 4) biomarkers of exposure to HAP and plastics; and 5) $PM_{2.5}$ source apportionment and implications for health upon exposure.

The Global Burden and Impact of HAP

Household air pollution (HAP) refers to the pollution that results from the practice of using solid fuels and open fires inside the home for cooking and heating.[1,14] Approximately 3.8 billion people worldwide rely on solid fuels as their primary fuel source[2], with a disproportionate number of people residing in low- and middle-income countries (LMICs).[1,2] Solid fuels including wood, coal, charcoal, dung, and kerosene, while more readily available, are considered highly polluting.[15] Additionally, homes are often poorly ventilated, creating more concentrated levels of health-damaging pollutants.[16–18]

Solid fuel reliance has decreased globally by about 11% since 2010, but nearly 50% of the global population is still exposed to levels of $PM_{2.5}$ many times in exceedance of health-based guidelines.[2] Health impacts associated with HAP are well documented and include childhood pneumonia[19], chronic obstructive pulmonary disease (COPD), cardiovascular effects, respiratory infections, and lung cancer.[20–23] Women and children are most at risk for these health impacts upon HAP exposure due to the amount of time spent at home and around the stove.[1,24,25]

Some of the most commonly studied air pollutants in the context of residential biomass burning are $PM_{2.5}$ and CO due to their well-documented health effects associated with exposure.[26,27] $PM_{2.5}$ is a heterogeneous mixture of nitrates, sulfates, BC, organic chemicals, metals, and soil (crustal) material.[28] Inhalation exposure to $PM_{2.5}$ triggers the overproduction of reactive oxygen species (ROS), which can cause oxidative stress, inflammation, and DNA and cellular damage.[29]

BC is gaining increased attention due to its associations with health effects and implications for climate change.[30,31] In addition to being identified as a climate forcing agent, BC has also shown toxicity upon exposure likely due to its small dimension and adsorptive properties with other air pollutants.[31] Both $PM_{2.5}$ and BC are emitted from the incomplete combustion of biomass and fossil fuels[32] and from burning household waste including plastics.[33] Both $PM_{2.5}$ and BC have been shown to have the ability to cross the blood placental barrier[34,35], underscoring the importance of studying these pollutant exposures in pregnant women and children.

The populations most impacted by HAP represent only 17 countries, 10 of which have more than 97% reliance on solid fuels.[2] Africa and South Asia are the hardest hit by HAP[2] and experience 60% of the global pre-term births each year.[2,15] Exposure to HAP is ranked 9th in the number of attributable global deaths and contributed to nearly 500,000 deaths among infants in their first month of life in the year 2019.[2] The HAPIN trial takes place in four diverse LMICs: Guatemala, India, Peru, and Rwanda. Impacts of HAP in these specific settings are highlighted here.

Just over 50% of the total population and approximately 86% of the rural population of Guatemala relies on polluting fuels and technologies for cooking.[36] The attributable death rate of ambient and HAP is approximately 50 per 100,000 population, about twice what it is in the U.S.[36] In rural Jalapa, smoking is generally uncommon and cooking is mainly done indoors over an open fire or a wood-fueled chimney stove.[3]

The results from the HAPIN pilot study indicated that baseline 24-h $PM_{2.5}$ personal exposures in Jalapa were [Median (IQR)]: 115 (80-265) $\mu g/m^3$.[37] The World Health Organization (WHO) updated the annual air quality guideline (AQG) for $PM_{2.5}$ concentrations in 2021 as well as the interim targets, which can be applied to places where the AQGs may not be readily attainable[38]. The WHO annual AQG is 5 $\mu g/m^3$ and the annual interim target 1 (IT-1) is 35 $\mu g/m^3$, which is three times lower than the median level observed in Jalapa.

The first RCT in air pollution history in normal populations is credited to the RESPIRE trial, mentioned previously.[6,39,40] This study was conducted in a poor, rural community in northwestern Guatemala with the goal of assessing the impact on respiratory health of reduced air pollution from biomass fuel. The RESPIRE trial found that compared to traditional biomass stoves, the improved biomass cookstove (*plancha*) with a chimney did not significantly reduce physician-diagnosed severe pneumonia, indicating more drastic changes in fuel and stove type would be needed in order to obtain tangible health benefits.[6]

In India, 36% of the total population and 52% of the rural population relies on polluting fuels and technologies for cooking.[36] The attributable death rate to ambient and HAP is approximately 141 per 100,000 population, approximately six times higher than it is in the U.S.[36] HAP accounts for approximately 1 million premature deaths and 31 million disability-adjusted life years (DALYs) annually in India.[41]

The HAPIN trial took place in two districts of Tamil Nadu, India: Villupuram and Nagapattinam. Traditional cookstoves constructed from clay and mud are typically fueled by wood and 90% of cooking occurs indoors. Smoking among women is not common in these settings, and smoking indoors by other members of the household was reported in less than 1% of households.[3] The baseline results from the HAPIN trial indicated that mean 24-h personal exposures to $PM_{2.5}$ were 155 µg/m^3,[42] approximately four times higher than the WHO annual IT-1 of 35 µg/m^3.[38]

The HAPIN Peru site took place in the Department of Puno. One of the unique characteristics about this site is that it is located approximately 3825 m above sea level (MASL). Compared to the other study sites, only 17% of the total population of Peru relies on polluting fuels and technologies for cooking, but almost 60% of the rural population relies on biomass fuels.[36] Traditional open or chimney stoves are typically fueled by cow dung or wood. The 24-h median indoor $PM_{2.5}$ concentrations in Puno were found to be 130 µg/m^3[3], approximately four times higher than the WHO annual IT-1.

Approximately 99% of the total population of Rwanda relies on polluting fuels and technologies for cooking. The ambient and HAP attributable death rate is approximately 60 per

100,000 population.[36] HAPIN participants were recruited from the Kayonza district in the Eastern Province, where cooking primarily occurs indoors. Traditional stove types are the three-stone fire, or a simple open stove called the *rondereza* fueled by either wood or charcoal.[3] The mean 24-h personal exposure from the HAPIN pilot work in Rwanda was 329 μg/m^3,[3] nearly ten times higher than the WHO annual IT-1 of 35 μg/m^3.

These are just four examples of the many places in the world that are impacted daily by HAP. Despite the slow and steady decline of reliance on polluting fuels and technologies that has been observed since 2010, currently about 50% of the global population is still at risk of exposures to hazardous air pollutants such as $PM_{2.5}$, BC, and CO. Randomized controlled trials are essential to continue characterizing exposures to HAP and to understanding possible implications to health upon exposure. The HAPIN trial is the largest LPG cookstove intervention trial to date with the aim of addressing the various knowledge gaps that remain and will hopefully bring us closer to answering "how clean is clean enough to see tangible health benefits?"

Nitrogen dioxide as a byproduct of HAP and fuel combustion

Although the majority of HAP studies seem to prioritize exposure assessment for $PM_{2.5}$ and CO due to their well-documented health effects associated with exposure[26,27], and now BC due to its associations with health effects and implications for climate change[30,31], nitrogen dioxide (NO_2) is also a product of fuel combustion, including residential biomass burning and gasoline emissions.[16] Studies measuring personal exposures to NO_2 in the context of residential biomass burning are currently sparse in the literature; therefore, the extent to which NO_2 poses significant health concerns in low-resource HAP settings is not well characterized.[43]

There are seven oxides of nitrogen found in ambient air, but nitrogen monoxide (NO) and nitrogen dioxide (NO_2) are the primary two associated with combustion. NO_2 is formed from the combustion of fuels at high temperatures or from the oxidation of NO in the air.[5] NO_2 is a toxic gas and is a pollutant precursor to tropospheric ozone (O_3).[44] Long-term exposures to NO_2 are associated with various diseases including increased blood pressure, asthma, and COPD[45,46], and it has also been shown to increase susceptibility to viral infections.[44,47] This finding has been of increased importance since the start of the COVID-19 pandemic.

Ecuador was one of the hardest hit countries in the Latin American and Caribbean region terms of deaths per capita due to COVID-19.[44] Guayaquil, the most populated and industrialized city in the country, saw some of the greatest surges in cases, spurring even more stringent lockdowns than before including limiting vehicular travel to only one day per week.[44] Although this caused a temporary though devastating collapse in the national health system and severe social problems, Pacheco et al. (2020) observed that one of the greatest trade-offs was a 23% reduction in atmospheric NO_2 in Guayaquil and a 22% reduction in Quito, the capital of Ecuador. There was a strong correlation ($r = 0.91$, $p < 0.001$) between NO_2 concentrations and the cases/mortality caused by COVID-19, underscoring the intricate link between air pollution and human health.

The vast majority of NO_2 studies are conducted in high income countries (HICs), likely due to motor vehicle emissions being the largest contributor to ambient concentrations, with power plants, industrial facilities, and other forms of transportation in urban areas also being significant contributors to ambient NO_2 concentrations.[48] Additionally, gas appliances are used by over one-third of households in the U.S.[49] and are a common residential energy option in HICs[49–52].

NO_2 studies in LMICs, particularly in places where residential biomass burning and HAP are prevalent, are sparse in the literature. One study of note is Kephart et al. (2021), where they measured 48-h real-time kitchen concentrations of and time-integrated personal exposures to NO_2 in Puno, Peru, which happens to be one of the same study sites in HAPIN. Although substantial reductions in personal exposures and kitchen concentrations were seen in the LPG intervention group compared to the biomass group, authors found that 69% of intervention kitchens were still in exceedance of the WHO indoor annual guideline for NO_2, which at the time was established at approximately 20 ppb.

Another study conducted by Helen et al. was conducted in peri-urban Trujillo, Peru and found that 48-h time-integrated kitchen NO_2 measurements in homes using wood stoves were approximately five times higher than those using gas stoves.[53] In a pooled analysis of various fuel types including wood, gas, coal, kerosene, or some combination of those, NO_2 concentrations for kitchen, personal, and secondary rooms were [Mean(SD)]: 18.4(17.5), 10.4(8.8), and 9.4(8.6) ppb, respectively. NO_2 concentrations measured in this study were substantially lower than those measured in Kephart et al. (2021), although there are many differences between the study designs that affect their comparability to one another.

The existing NO_2 studies in LMICs are somewhat inconsistent, and the vast majority of NO_2 exposure studies are conducted in high-income countries (HICs)[5,48], resulting in a dearth of knowledge about exposures in lower-resource settings where biomass is the primary fuel source. Additionally, studies measuring NO_2 emissions by gas appliances in HICs have shown that even ‘cleaner’ fuels such as LPG may continue contributing exposures to harmful air pollutants, including NO_2.[51,54,55] For these reasons, we aimed to contribute to this area of the literature that is currently lacking by measuring NO_2 personal exposures and kitchen concentrations in three of the four HAPIN study sites.

Household waste burning and waste disposal practices

Aside from normal cooking activities, another source of HAP exposure in LMICs is household garbage burning. In many LMICs, particularly in rural areas, municipal sanitation services are virtually nonexistent.[56–59] Unsustainable waste management practices are much more common in LMICs compared to HICs, increasing the risk of harmful pollutants through water, soil, and air pollution.[60] At the municipal level, waste disposal options include landfilling, open dumping, recycling, composting, anaerobic digestion, and incineration.[61] At the household level, residents are left with few options including tossing waste onto public land or in waterways, burying it, or burning it.

Household garbage burning has been observed in rural Guatemala. Additionally, according to the Guatemala National Population Census (2018), approximately 43% of the total population of Guatemala and 50% of the population of Jalapa burns their household waste on or near their property as a primary means of waste disposal. Plastic waste is ubiquitous in communities including Jalapa, creating the potential for exposures to plasticizers such as phthalates and bisphenol A (BPA). Routes of exposure include the common dermal and ingestion routes of exposure[62], as well as the inhalation route as plastic waste products get added to the household burn pile. Burning products containing BPA at high temperatures such as in the open burning of dumped waste has been shown to cause BPA to leach into the environment, though more studies are needed to understand the fate and associated health impacts upon exposure to BPA and similar environmental chemicals.[63,64]

Phthalates and BPA are chemicals added to plastics to make them more rigid and durable, and both are known endocrine disrupting compounds.[62,65,66] Both are found in personal care products such as soap, shampoo hair spray, and nail polishes[65]; BPA is also found in a number of consumer materials including thermal receipt paper, linings of canned foods, electronics, pipes, coatings, and flame retardants.[67] BPA and phthalates are ubiquitous contaminants in the human body, wildlife, and the environment, but they are still used in numerous consumer products due to the many challenges that come with identifying safter alternatives.[68] Upon entry to the body, BPA and phthalates are excreted in the urine.[62,69,70]

In addition to BPA and phthalates, another class of pollutants associated with both HAP and household garbage burning are polycyclic aromatic hydrocarbons (PAHs).[71–73] PAHs are organic compounds composed of two or more fused benzene rings and are generally categorized as either high-, medium-, or low molecular weight PAHs (HMW, MMW, and LMW, respectively).[74] Many PAHs exist in the particle phase, especially as $PM_{2.5}$, and have been identified as known, probable, or possible human carcinogens (IARC, 2010). Common routes of exposure are inhalation[75] and dermal exposure[76], and through dietary ingestion, particularly through the consumption of grilled, roasted, and charred foods.[76,77]

When humans are exposed to PAHs, the parent compounds undergo biotransformation by cytochrome P450 enzymes to their metabolized derivatives, many of which have carcinogenic and mutagenic properties.[78,79] PAHs are associated with respiratory and cardiovascular diseases, reduced lung capacity, and lung cancer.[76] The environmental fate and toxicity of PAHs is dependent on several factors including stove type, fuel source, combustion factors such as temperature and oxygen supply, and specific properties of the PAHs themselves, such as molecular weight.[79,80] HMW PAHs have a higher affinity to adsorb to fine particles which are of increased interest in terms of human health, given their ability to deposit more deeply into sensitive lung tissue. Moreover, fine particles have the ability to transport PAHs over longer distances, which has implications for environmental health.[81]

The U.S. EPA has designated 16 PAHs between two and six benzene rings as priority pollutants. Among these 16 PAHs are the parent compounds naphthalene (NAP), phenanthrene (PHE), and fluorene (FLU), which are LMW, and pyrene (PYR) which is HMW.[81] Once exposed to these PAHs, they are metabolized to mono-hydroxylated metabolites that can ultimately be excreted through the urine and feces. For this reason, urinary analysis (biomonitoring) for PAH metabolites has become a common technique to characterize exposures.[82]

Biomarkers of exposure: Phthalates, BPA, and PAHs

<u>Phthalates</u>

Phthalates are added to plastic products which can contain up to 50% phthalates by weight. Phthalates are bound non-covalently to the material matrix to which they are added, making them susceptible to leaching into the environment.[70] Di(2-ethylhexyl)phthalate (DEHP) is a HMW plasticizer for polyvinyl chloride (PVC), and is considered the most common phthalate, making it a major component of clothing, upholstery, flooring, carpeting, and roofing.[83] Upon entry to the body, DEHP is rapidly metabolized, first undergoing cleavage into the monoester mono(2-ethylhexyl) phthalate (MEHP), and then being further metabolized to mono(2-ethyl5-hydroxyhexyl) phthalate (MEHHP) and mono(2-ethyl05-oxohexyl) phthalate (MEOHP).[70,83] Other breakdown products of DEHP include mono(2-ethyl-5-carboxypentyl) phthalate (MECPP) and mono(2-carboxymethylhexyl) phthalate (MCMHP).

LMW phthalates include di-n-butyl phthalate (DnBP), diethyl phthalate (DEP), and butyl benzyl phthalate (BBzP), which are commonly used in pharmaceuticals, personal care products, infant care products, and cosmetics.[84] Their breakdown products include mono-butyl phthalate (MBP), mono-ethyl phthalate (MEP), and monobenzyl phthalate (MBzP). All of the metabolites highlighted here are commonly measured as urinary biomarkers of exposure to phthalates.[85,86]

BPA

BPA is ubiquitous in the environment and has been measured in 93% of tested urine samples.[87] It has also been measured in serum, amniotic fluid, follicular fluid, the placenta, and breast milk of pregnant women.[88] Upon exposure to BPA, it is metabolized by cytochrome P450 enzymes into several different breakdown products, most of which have been shown to exert stronger estrogenic effects than the parent compounds. In phase II of metabolism, BPA and its metabolites are conjugated with glucuronide and then they are quickly eliminated from the body through the urine. The major biomarker of exposure to BPA is therefore BPA glucuronide.[89]

PAHs

PAHs are metabolized to mono-hydroxylated compounds in glucuronide conjugated forms to facilitate excretion through urine and feces.[82] The parent compounds of the metabolites measured in our study are part of the 16 priority PAHs established by the EPA. The metabolites we measured are commonly found in urine and include 1- and 2-naphthol (1NAP and 2NAP), 1-, 2- and 3-hydroxyfluorene (1FLU, 2FLU, 3FLU), 1-hydroxypyrene (1PYR), and 2-, 3-, and 4-hydroxyphenanthrene (2PHE, 3PHE, 4PHE).[82,90–92]

Using urinary creatinine concentrations to adjust for sample variation

Creatinine is a nitrogenous waste product from muscle creatine metabolism.[93,94] It is processed by the kidneys, specifically through the functional units of the kidneys called glomeruli, through a process called glomerular filtration (GF). Markers of GF can be endogenous or exogenous and are substances that are filtered by the glomeruli but have no effect on the rate of filtration. The most common endogenous marker of GF is creatinine, as it is produced at a fairly constant rate and excreted freely in the urine.[95]

The major advantage of urinary sample collection for biomonitoring is the non-invasiveness of the sample collection, compared to taking a blood sample. One of the major disadvantages is the variability in water content and the concentrations of endogenous and exogenous chemicals between among individuals and from void to void within individuals.[94] Given the relative consistency in the production and excretion of creatinine, individual creatinine concentrations can be measured in a urine sample, along with the analytes of interest (i.e., metabolites of PAHs, phthalates, or BPA), and can be used to adjust each sample for these variable factors.[94]

Adjusting for creatinine is a simple correction done by dividing the analyte concentration (µg/L urine) by the creatinine concentration (g/L urine) to get final units of µg of analyte per g of creatinine.[94] This adjustment is commonly used to make results more standardized within the sample population.[96,97] Biomonitoring results reported in this dissertation will be adjusted for creatinine concentrations for each of the individual samples.

$PM_{2.5}$ Source Apportionment

"Particulate matter" is a broad term that refers to particles composed of solid or liquid droplets of varying size fractions and chemical compositions.[98,99] $PM_{2.5}$ refers to particles of ≤ 2.5 microns in diameter and has been referred to as the "alveolar fraction" for its ability to deposit deeply in sensitive lung tissue where gas exchange occurs. Beyond the respiratory system, $PM_{2.5}$ can pass into the circulatory system and spread to the whole body, where it exerts toxic effects.[100] Furthermore, its tendency to bind with other toxic compounds including PAHs and metals makes $PM_{2.5}$ of increased health importance.[100,101]

PM is one of the six criteria pollutants for which the EPA sets National Ambient Air Quality Standards (NAAQS) as required by the Clean Air Act. Thousands of epidemiologic, controlled human exposure, and animal toxicity studies are reviewed to inform primary standards to protect public health and secondary standards to protect public welfare, all of which are reassessed every five years.[99] The WHO, among other entities, also establishes air quality guidelines (AQGs) for $PM_{2.5}$, which were updated in September 2021. In addition to the new annual AQG of 5 $\mu g/m^3$, the WHO established four interim targets, which are designed to be incremental steps towards the AQG in areas where the AQG is not as easily attained.[38] The interim target 1 (IT-1) of 35 $\mu g/m^3$ is the guideline most often used in HAP studies due to the high baseline levels of $PM_{2.5}$.

Many studies have reported on household concentrations of $PM_{2.5}$ in the context of residential biomass burning in LMICs. A study in Kigali, Rwanda by Kebera et al. (2020) measured kitchen area concentrations of $PM_{2.5}$ in n=20 homes that use biomass stoves fueled by either wood or charcoal. The air monitors recorded the $PM_{2.5}$ concentration once every minute for 24 h. The household with the lowest 24-h average $PM_{2.5}$ concentration was 66 $\mu g/m^3$; the household with the highest 24-h average was 611 μg/m^3. Although this study was limited by sample size, the averages found in these homes were many times in exceedance the WHO IT-1 of 35 μg/m^3.[102]

Another study in Honduras by Walker et al. (2020) compared 24-h personal exposures to and kitchen concentrations of $PM_{2.5}$ in homes with a traditional biomass stove compared to a *Justa* (improved biomass) stove.[103] Personal exposures in non-pregnant women using the *Justa* stove with the improved combustion chamber and biomass fuels were [Mean (SD)]: 66 (38) μg/m^3. While still approximately twice as high as the WHO IT-1, this was about a 50% reduction in personal exposures in participants using a traditional biomass stove [Mean (SD)]: 125 (76) μg/m^3. Kitchen area concentrations were even higher: [Mean (SD)] 137 (194) and 354 (373) μg/m^3 for *Justa* stoves and traditional stoves, respectively. This supports the conclusion by Smith et al. (2011) that any stove fueled with biomass may not provide sufficient reductions in exposures to attain tangible health benefits.

Katz et al. (2020) reported the results of two sequential randomized trials in rural Nepal and assessed the impact of the intervention on birth outcomes. Trial 1 compared 20-h kitchen $PM_{2.5}$ concentrations in homes using a traditional biomass stove (n=2963) to an improved biomass (chimney) stove (n=2752). Mean kitchen area $PM_{2.5}$ concentrations were reduced from 1380 to 936 μg/m^3 in homes with the improved biomass stove, though adverse birth outcomes were not significantly different by study arm.

Trial 2 compared 20-h kitchen concentrations in homes with vented biomass stoves (n=659) to homes with LPG stoves (n=661) over the duration of a year. They found that the mean 20-h $PM_{2.5}$ concentration was 885 μg/m^3 in homes with vented biomass stoves, compared to 442 μg/m^3 in homes with LPG stoves. While this is a substantial reduction, indoor concentrations of $PM_{2.5}$ are orders of magnitude higher than WHO guidelines. Additionally, the cleaner alternatives (improved biomass in Trial 1 and LPG in Trial 2) did not reduce adverse birth outcomes.

The studies summarized above not only exemplify the continued need to investigate ways to get $PM_{2.5}$ concentrations lower and in accordance with health-based guidelines, but also warrant further investigation into the specific chemical characteristics of $PM_{2.5}$, which may be contributing to differential toxicities. Source apportionment (SA) models are tools that help us to reconstruct the impact of emissions from various sources of atmospheric pollutants including $PM_{2.5}$. A few different SA techniques are available, with one of the more common ones being receptor-based models.[104]

There are many distinct types of receptor models that are used, but two of the most common are chemical mass balance (CMB) and positive matrix factorization (PMF), a multivariate model developed by EPA scientists. The major differences between CMB and PMF is the amount of knowledge required about the pollution sources prior to using the models.[104] Whereas for CMB it is required to have knowledge of the composition of the emissions for all relevant sources[105], PMF does not require *known* source profiles from a particular study site as model inputs, but does require *knowledge* of general source compositions to be able to determine the relationship between factors derived from the model with air pollution sources, which are usually obtained from the literature.[106] The overall goal of PMF is to resolve the identities and contributions of components in an unknown mixture by incorporating the variable uncertainties associated with each $PM_{2.5}$ measurement.[107]

The PMF model as it is used for analyzing $PM_{2.5}$ sample data expresses observations of PM species as the sum of contributions from a number of time-invariant source profiles:

$$x_{ij} = \sum_{k=1}^{p} g_{ik} f_{kj} + e_{ij},$$

where x_{ij} is the concentration of species *j* measured on sample *i*, *p* is the number of factors contributing to the samples, f_{kj} is the concentration of the species *j* in factor profile *k*, g_{ik} is the relative contribution of factor *k* to sample *i*, and e_{ij} is the error of the model for the species *j* measured on sample *i*.

PMF is a free software provided by the EPA in which the user provides the sample concentration data along with the derived uncertainty values for each concentration. Sample concentration data can be determined a number of ways, including gravimetric analysis to determine $PM_{2.5}$ concentrations and x-ray fluorescence (XRF) to determine elemental concentration. Uncertainty values are derived by considering the various sources of variability that may exist in measurements such instrument or technician variability, flow rate of the sampling monitor, and effective area of the sample media. If uncertainty values are not provided as output from laboratory instrumentation, they can be derived using the law of propagation of uncertainty.[108]

While exposure studies to $PM_{2.5}$ are plentiful in the literature, SA studies are less common, particularly in LMICs. One study by Sharma et al. (2016) was conducted in Delhi, India between 2013 and 2014. The annual average $PM_{2.5}$ concentration at an urban site in Delhi was found to be [Mean (SD)]: 122 (94.1) μg/m^3 and seven major $PM_{2.5}$ sources were resolved via PMF including secondary aerosols, soil dust, vehicle emissions, biomass burning, fossil fuel combustion, industrial emissions, and sea salt.

Secondary aerosols (Source 1) were attributed to elevated levels of NO_3^-, SO_4^{2-}, and NH^{4+}. Soil dust (Source 2) was attributed to elevated crustal elements including Al, Si, Ca, Ti, Fe, Pb, Cu, Cr, Ni, Co and Mg. Vehicle exhaust (Source 3) was identified due to elevated levels of Cu, Zn, Mn, Pb, and elemental carbon (EC). Biomass burning (Source 4) was identified by elevated levels of K and S. Fossil fuel combustion (Source 5) was identified by elevated levels of Al, Cl, Fe, Zn, Cr, and SO_4^{2-}. Industrial emissions were identified by elevated levels of Zn, Cu, Mn, Si, Ni, Cd, Fe, Mo, and Cr. Finally, sea salt was attributed to higher concentrations of Na, K, and Cl.

Another study by Zhou et al. (2014) was conducted in three unique study sites in sub-Saharan Africa where biomass burning is common for cooking: in various neighborhoods in Accra, Ghana (n=80 homes), and at a rural (n=154 homes) and an urban site (n=49) in The Gambia.[109] Elemental concentrations for all filter samples were quantified via energy dispersive XRF and source apportionment was conducted via PMF. Average $PM_{2.5}$ concentrations were significantly higher in Gambian homes compared to homes from the more socioeconomically-diverse neighborhoods of Accra. The number of $PM_{2.5}$ source contributions varied between five and six depending on study site but were identified as follows:

The sea salt factor contribution was characterized primarily by Na, Cl, and S. The crustal sources were attributed to elevated levels of Al, Si, Mg, Ti, Mn, and Fe. Biomass smoke was broken into two categories: fresh biomass smoke, which is characterized by strong peaks of K, as well as Cl, S, and BC, and aged biomass smoke, which is characterized specifically by K_2SO_4. Road dust and traffic were characterized by Al, Si, Ca, Fe, Zn, and BC. Solid waste burning was identified by elevated levels of Br. This study highlighted the continued need to explore variations in $PM_{2.5}$ composition by study site.

Lai et al. (2019) measured the chemical composition and conducted source apportionment of ambient, household, and personal exposures to $PM_{2.5}$ in rural China where biomass stove use is common.[110] They analyzed 40 personal exposure, 40 household, and 36 ambient $PM_{2.5}$ samples collected for 48-h across the non-heating and heating seasons. Chemical speciation was measured via inductively-coupled plasma mass spectrometry (ICP-MS) and SA was conducted via CMB. Biomass burning was found to be the largest contributor to household concentrations of and personal exposures to $PM_{2.5}$.

Overall, household concentrations of total $PM_{2.5}$ were highest compared to personal and ambient samples, but levels of both household and personal concentrations were more than doubled in winter compared to summer. Furthermore, the winter household concentrations and personal exposures more than doubled in winter compared to summer (average household: 275 ± 118 μg/m^3 in winter compared to 106 ± 53.4 μg/m^3 in summer; average personal: 202 ± 99.1 μg/m^3 in winter compared to 88.5 ± 54.8 μg/m^3 in summer), with all averages being many times in exceedance of the WHO annual IT-1 of 35 μg/m^3. This study is among the first to publish SA outdoor, indoor, and personal $PM_{2.5}$ concentrations in a rural setting where biomass burning is prevalent.

The studies highlighted here represent a few of the SA studies conducted in LMICs, particularly in rural areas where there is a high reliance on polluting fuels and technologies for cooking and heating. Higher representation of studies are needed in other LMICs, especially given the potentially drastic differences between $PM_{2.5}$ compositions by factors including geography and fuel source. More research overall is needed to try and understand whether differences in chemical compositions may affect their hazardous effects on human health.

Summary

Although $PM_{2.5}$ exposures have seen a steady decline globally since 2010, nearly half of the global population is still receiving exposures many times in exceedance of health-based guidelines.[2] Many factors affect pollutant concentrations and exposures including variability in cook stove use and time-activity patterns, weather conditions, ventilation, fuel type, and instrument error[27], making exposure assessment, especially in these settings, extra challenging. Although great progress has been made in making cleaner alternatives such as LPG more accessible to the populations who need them most, more work is needed to determine how to continue protecting vulnerable populations such as women and children in LMICs.

CHAPTER 2

COMPARING EXPOSURES TO NITROGEN DIOXIDE IN HOMES WITH BIOMASS AND GAS COOKSTOVES IN THREE LMICS AS PART OF THE HAPIN RANDOMIZED CONTROLLED TRIAL[1]

[1] Kearns, Katherine A., Johnson, Michael, Pillarisetti, Ajay, Ryan, P. Barry, Sarnat, Jeremy, Steenland, Kyle, Waller, Lance A., Clark, Maggie, Rosa, Ghislaine, Kirby, Miles, Ndagijimana, Florien, McCracken, John P., Diaz-Artiga, Anaite, Thompson, Lisa M., Balakrishnan, Kalpana, Mollinedo, Erick, Underhill, Lindsay J., Kremer, Jacob, Campbell, Devan, Clasen, Thomas F., Peel, Jennifer L., Checkley, William, Naeher, Luke P., and HAPIN investigators. To be submitted to *Environmental Health Perspectives*.

ABSTRACT

Background: Household air pollution (HAP) creates exposures to harmful air pollutants including fine particulate matter ($PM_{2.5}$), black carbon (BC), carbon monoxide (CO), and nitrogen dioxide (NO_2); however, the extent to which NO_2 creates a health risk in low-resource settings is not well characterized.

Objectives: We sought to characterize personal exposures to and kitchen concentrations of NO_2 in homes with biomass stoves compared to homes with liquefied petroleum gas (LPG) stoves as part of the HAPIN trial.

Methods: We randomly selected a subset of 288 homes across Guatemala, Rwanda, and Peru from the HAPIN trial to measure 24-hour kitchen concentrations of and personal exposures to NO_2 using passive sampling. Households and participants were divided between control (biomass) and intervention (LPG) study arms.

Results: The median (IQR) personal exposure in the control arm was 11.3 (7.4-17.9) ppb and in the intervention arm was 7.4 (7.4-13.1) ppb ($p<0.05$). The median (IQR) concentration in control kitchens was 23.2 (12.6-36.4) ppb and in intervention kitchens was 14.0 (7.4-21.9) ppb ($p<0.001$). Compared to the WHO 24-hour interim target 2 of 16 ppb for NO_2, 69% of control participants and 84% of intervention participants were below the guideline, and 34% of control kitchens and 59% of intervention kitchens were below the guideline. These trends show variation between the study sites.

Discussion: Overall, we observed statistically significant reductions in NO_2 concentrations and exposures in the intervention group compared to control, suggesting that LPG can help reduce exposures to NO_2. We explore covariates and factors potentially affecting site-variable NO_2

trends, including the impact of altitude. This project contributes to NO_2 exposure characterization in low-resource settings, which is currently lacking.

INTRODUCTION

Nearly 50% of the global population relies on biomass such as wood, crop residues, and animal dung for cooking.[2] The resultant household air pollution (HAP) creates exposures to harmful air pollutants including particulate matter with an aerodynamic diameter of ≤2.5 µm ($PM_{2.5}$), black carbon (BC), carbon monoxide (CO), and nitrogen dioxide (NO_2), among others.[3,111]

Exposure to HAP was attributed to 2.31 million deaths in 2019 (Health Effects Institute 2020) and is associated with many adverse health effects including chronic obstructive pulmonary disorder (COPD), childhood pneumonia, adverse birth outcomes, and lung cancer.[21–23] NO_2 is most commonly associated with asthma[50,55] and short term exposures are causally associated with respiratory effects.[38,112]

Many HAP studies prioritize exposure assessment for $PM_{2.5}$ and CO due to their well-documented health effects associated with exposure.[26,27] BC is gaining increased attention due to its associations with health effects and implications for climate change.[30,31] NO_2 is a byproduct of HAP and any type of fuel combustion[5], but studies measuring personal exposures to NO_2 in the context of residential biomass burning are currently sparse in the literature. Therefore, the extent to which NO_2 poses significant health concerns in low-resource HAP settings is not well characterized.[43]

One study in peri-urban Trujillo, Peru by St. Helen et al. (2015) found that 48-hour, time-integrated NO_2 concentrations for kitchen, personal, and secondary rooms were [Mean(SD)]: 18.4(17.5), 10.4(8.8), and 9.4(8.6) ppb, respectively.[53] These averages were based on a pooled analysis of a variety of fuel types including wood, gas, coal, kerosene, or a combination. Stratified analysis of kitchen NO_2 measurements showed that concentrations in homes using wood stoves were approximately five times higher than those using gas stoves.

Another study conducted by Kephart et al. (2021) was nested within the Cardiopulmonary Outcomes and Household Air Pollution (CHAP) Trial in rural Puno, Peru where they measured personal exposures in women and kitchen area concentrations of NO_2.[17] They compared 48h real-time kitchen area concentrations of NO_2 between homes using biomass stoves, primarily fueled by cow dung (n=47), and homes receiving an LPG stove intervention (n=49). The 48h real-time NO_2 kitchen area concentrations in the biomass and LPG groups were [Mean (SD)]: 96 (65) ppb and 49 (26) ppb, respectively.

In a subset of n=9 control and n=16 intervention homes, Kephart et al. (2021) also measured women's personal exposures to and kitchen area concentrations of NO_2 using 48h passive sampling. They found that personal exposures in the control and intervention participants were [Mean (SD)]: 23 (24) and 8 (11), respectively. Kitchen concentrations in the same control and intervention homes were [Mean (SD)]: 185 (162) and 38 (29) ppb, respectively. Although substantial reductions in personal exposures and kitchen concentrations were seen in the LPG intervention group compared to the biomass group, authors found that 69% of intervention kitchens were still in exceedance of the WHO indoor annual guideline for NO_2.[5]

The existing NO_2 studies in LMICs are somewhat inconsistent, and the vast majority of NO_2 exposure studies are conducted in high-income countries (HICs)[5,48], resulting in a dearth of knowledge about exposures in lower-resource settings where biomass is the primary fuel source. Additionally, studies measuring NO_2 emissions by gas appliances in HICs have shown that even 'cleaner' fuels such as LPG may continue contributing exposures to harmful air pollutants, including NO_2.[51,54,55]

In this study, we sought to address these knowledge gaps by leveraging the randomized design of the Household Air Pollution Intervention Network (HAPIN) trial. We measured 24-hour personal exposures to and kitchen concentrations of NO_2 using passive sampling in a subset of homes in Guatemala, Peru, and Rwanda. We report differences in NO_2 concentrations among biomass and LPG stove homes, and place these levels in risk context per the 2021 WHO guidelines for NO_2.[38]

METHODS

Study setting

The HAPIN trial took place in four low- and middle-income countries (LMICs): Guatemala, India, Peru, and Rwanda. HAPIN households (n=3195) were randomized into either the control (continued use of biomass stove; n=1605) or intervention arm (LPG stove and fuel intervention; n=1590). The HAPIN trial measured personal exposures in pregnant women and kitchen concentrations of $PM_{2.5}$, BC, and CO; in this ancillary study, we sought to measure NO_2 in a randomly-selected subset of enrolled households in Guatemala, Peru, and Rwanda (n=288). Participants of this ancillary study were equally representative of control and intervention arms, and NO_2 measurements were taken alongside the other pollutant measurements at the 12-month follow-up visit (post-birth).

Site and participant information

The HAPIN study sites as well as inclusion and exclusion criteria have been described in detail previously[3,13] and are briefly summarized here for Guatemala, Peru, and Rwanda.

The HAPIN Guatemala study site is located in the Jalapa municipality (14.63° N, 89.98° W, 1362 MASL), which is located 150 km east of the capital, Guatemala City. The climate is mild temperate with relatively stable temperatures year-round and two main seasons – the rainy season, which typically spans May to October, and the dry season, which typically spans November to April.[36] This ancillary study took place at the Guatemala site from July to December 2020, with the first half of the study taking place in the rainy season and the second half during the dry season. The majority of Guatemala's rural population (86%), which includes Jalapa, relies primarily on solid fuels, with wood being the primary fuel source.[3]

The HAPIN Peru study site is located in the Department of Puno (15.50° S, 70.01° W, 3825 MASL). The climate is cold and dry with average daytime temperatures of 10°C. The winter months span May to October and the summer months, which also includes the rainy season, span November to April. The NO_2 ancillary study in Puno took place during the winter months of April through June 2020. The primary biomass fuel source in Puno is cow dung.[3]

The HAPIN Rwanda study site is located in the Eastern Province in the Kayonza district (1.78° S, 30.62° E, 1354 MASL). The climate is tropical with a long rainy season from March to May and a short rainy season from September to November. The NO_2 project in Rwanda took place from April to May. The primary cookstoves in this site include the traditional three-stone fire (the Rondereza) fueled with either wood or charcoal.[3]

Exposure assessment and sampling strategy

This NO_2 study is a cross-sectional study where we randomly selected 151 of 800 households from Guatemala, 101 of 798 from Peru, and 36 of 798 from Rwanda. Households were divided between control (biomass) and intervention (LPG). We used passive samplers for NO_2 loaded with triethanolamine-coated collection pads (Ogawa USA, Pompano Beach, FL). In each household, we collected a 24-hour personal (mother) sample and a kitchen area sample. We collected field blanks and duplicate samples in every tenth household.

Field technicians assembled the passive samplers in the field office prior to deployment, ensuring that each of the six badge pieces was cleaned with filtered water and dried before loading it with a collection pad. Samplers were labeled with the household identification number and sampling location (personal or kitchen) and placed in a sealed plastic bag until the moment of deployment.

NO_2 sampling occurred during regularly-scheduled HAPIN visits alongside measurements of $PM_{2.5}$, BC, and CO. The NO_2 sampler was worn in the approximate breathing zone of the participant on her apron containing the other personal monitors for HAPIN, and the kitchen sampler was deployed alongside the other kitchen instruments. At the end of the 24h sampling period, NO_2 samplers were collected by field technicians and were immediately sealed in a plastic bag for transport back to the field office.

At the field office, field technicians disassembled the passive samplers, placing the loaded collection pad into a labeled petri dish and setting aside the sampler pieces for wash and reuse. The petri dishes were sealed, refrigerated at 4°C, and kept out of sunlight to preserve the samples until they were hand-carried by a traveler to Emory University (Atlanta, GA, USA) for analysis.

Laboratory analysis and NO_2 concentration determinations

We used standard colorimetric techniques and spectrophotometry at Emory University to derive NO_2 concentrations from the filter samples. To improve accuracy and precision in our estimates, we used a dual calibration curve. We used the manufacturer's sampling rate of 12.1 mL/min (Ogawa, 2006), and absorbance values were converted to concentration using Microsoft Excel (Microsoft, Redmond, WA) developed by LEADER Laboratory (LEADER Palmes Sampler Protocol Ver 1.1 November 2007).

We pooled field blank samples from the three study sites (n = 40) and calculated the limit of detection (LOD) as the mean plus 3 times the SD among the blanks. We estimated an LOD of 10.5 ppb, and all sample concentrations that fell below the LOD were replaced with LOD/sqrt(2) = 7.4 ppb.

Statistical analysis

All statistical analyses were performed in R (version 4.0, R Foundation for Statistical Computing, 253 Vienna, Austria). We calculated descriptive statistics (mean and SD, and median and IQR) for NO_2 by study site and for all study sites combined (Overall). Differences in NO_2 exposures between assigned treatment arm were evaluated using non-parametric tests (Wilcoxon Rank Sum and Kruskal Wallis). We assessed Spearman correlations between personal and kitchen measurements within the same household.

To put our results in risk context, we evaluated the percentage of samples that were below the WHO IT guidelines for NO_2.[38] To our knowledge, these new WHO guidelines are the first to provide 24-hour guidelines, which are relevant to our study. We also contextualize our results in terms of annual IT guidelines to explore the impact of chronic exposures to NO_2.

Furthermore, given the potential for environmental conditions relating to altitude to impact gases such as NO_2[113], we contextualize our results in terms of site-specific WHO guidelines that are adjusted for temperature and atmospheric pressure, which is the same approach taken by Kephart et al. (2021).

We used simple linear regression to assess the impact of the LPG intervention on the natural log of NO_2 concentrations (given the non-normal distributions of observed concentrations, sample concentrations were natural log-transformed before modeling). Percent reductions in $\ln(NO_2)$ personal exposures and kitchen concentrations between study arms are presented. We then used multivariate regression to explore the impact of covariates on $\ln(NO_2)$ concentrations.

RESULTS

Household and participant characteristics

Household and participant characteristics are given in Table 2.1. Age of the participants was relatively consistent among study sites but there was heterogeneity in highest education level obtained and predominant biomass fuel source (wood in Guatemala and Rwanda, cow dung in Peru). Participants tend to cook indoors, and indoor heating is uncommon in these settings, even during the cold winter months in Puno. Examples of LPG and biomass stoves at each of the study sites are presented in Figures 2.1a and 2.1b.

Personal exposure assessment and kitchen concentrations of NO_2

One personal exposure sample from Guatemala was noted by the field staff as “wet” and was removed from analysis. One household in Peru had repeat personal and kitchen samples on two different visits, meaning four total samples (two personal and two kitchen) were removed from analysis. The final dataset reported here had N = 142 personal control samples, N = 145 personal intervention samples, N = 145 kitchen control samples, and N = 144 kitchen intervention samples, for a total of 576 samples. Site-specific sample sizes and characteristics are provided in Table 2.1.

Personal exposures to and kitchen concentrations of NO_2 are summarized in Table 2.1 and displayed graphically in Figure 2.2a. Globally, there was a significant reduction (Wilcoxon Rank Sum, $p<0.05$) in NO_2 personal exposures in intervention participants (median 12.6 ppb, IQR 9.6 – 17.9) compared to control (median 15.3 ppb, 10.7 – 25.7). Similarly, there was a significant reduction ($p<0.001$) of kitchen concentrations in intervention homes (median 17.9 ppb, IQR 11.7 – 31.0) compared to control homes (median 29.2 ppb, IQR 16.7 – 49.6).

Due to the variation in altitude of our study sites, we also analyzed our results in terms of site-specific WHO guidelines (Fig. 2.2b), which were adjusted for temperature and atmospheric pressure at the three unique study sites.[17,113] The Guatemala and Rwanda sites had similar environmental conditions during the time of our study (atmospheric pressures of 870 hPa and 843 hPa and average temperatures of 17°C and 20°C, respectively), and thus, had similar adjusted guidelines. For the Peru site, we assumed an average temperature of 10°C and atmospheric pressure of 625 hPa, which caused the site-specific guidelines to be inflated by 58% compared to the unadjusted guidelines. Table 2.3 represents the percentage of samples that are below the unadjusted WHO guidelines compared to the percentage of samples that are below the site-specific guidelines adjusted for altitude.

Study-wide correlations (Spearman's ρ) between kitchen and personal samples were moderate at ρ ~ 0.50. These trends varied substantially by study site with the correlations for Guatemala, Peru, and Rwanda being 0.56, 0.37, and 0.15, respectively (Fig. 2.3).

Duplicate samples were taken in all study sites and were pooled for analysis due to relatively small sample size within study site to equal n=50 total pairs. Duplicate samples had a correlation coefficient (R^2) of 0.91 and a root-mean-square-error (RMSE) value of 4.5 ppb.

Modeling Results

Effect of LPG intervention on NO_2 personal exposures and kitchen concentrations

Intervention status was used to predict natural log-transformed NO_2 personal exposures and kitchen concentrations. The model indicated that globally, there was a significant reduction in personal exposures of 16% (95% CI 3-32%, $p<0.05$) associated with the intervention, and a significant reduction in intervention kitchen concentrations of 35% (95% CI 23-62%, $p<0.001$). There was variation in these reductions after adjusting for study site.

The site-specific model for Guatemala indicated a significant reduction of 24% in personal exposures (95% CI 13-41%, $p<0.001$) and a 44% reduction in kitchen concentrations (95% CI 40-77%, $p<0.001$) in the intervention arm. In Peru and Rwanda, the intervention alone was not associated with significant reductions in personal exposures. There was a 44% reduction in kitchen concentrations in Rwanda (95% CI 15-57%, $p < 0.01$), but no significant reduction in kitchens in Peru.

Multivariate regression

To build upon the simple model described above, we added covariates including number of stoves in the home, given that many homes have more than one stove, and number of times per day the primary stove is used. We found no significant impacts on ln(NO_2) concentration when the additional covariates were added to the model.

DISCUSSION

Comparison to other studies in LMICs and in HICs

This ancillary study nested within the parent HAPIN trial resulted in modest study-wide reductions in personal exposures to and kitchen concentrations of NO_2 in homes with the LPG intervention. Controlling for study site, we found that trends were variable, suggesting that multiple different factors may impact measured NO_2 concentrations. Trends also show some variability in the literature in both LMICs and HICs. While our study presents NO_2 concentrations in ppb, other studies present their findings in units of μg/m^3; the conversion factor is approximately μg/m^3÷1.914 = ppb.

LMICs

One of our study sites was Puno, Peru, which is the same study site where Kephart et al. (2021) measured 48-h real-time kitchen concentrations of NO_2. Kitchen area concentrations in biomass and LPG homes were [Mean (SD)]: 96 (65) and 49 (26) ppb, respectively. Kitchen concentrations in our study in Puno were overall lower in biomass and LPG homes [Mean (SD)]: 48.3 (57.9) and 34.5 (28.2); however, we did not see a significant reduction between study arm, whereas Kephart et al. (2021) observed nearly a 49% decrease in the LPG arm compared to biomass.

In addition to the Kephart et al. (2021) study, a few other notable studies have taken place in LMICs to characterize NO_2 concentrations emitted from biomass and gas stoves and are highlighted here.

Kumar et al. (2008) assessed the relationship between respiratory effects and 6-h kitchen concentrations of NO_2 in the homes of over 2000 children in India.[114] Participants in this study represented nine unique study sites. There was a significant reduction in average kitchen NO_2 concentrations in LPG homes compared to biomass homes for only three of the nine study sites, and the study-wide averages showed no significant differences.

The study-wide average NO_2 concentrations were 30.3 and 31.8 μg/m^3 in LPG and biomass kitchens, respectively, or approximately 15.8 and 16.6 ppb, respectively. These levels are similar to our findings in Guatemala (average of 12.3 and 24.9 ppb in LPG and biomass kitchens, respectively), but are generally lower than the rest of our findings. Although indoor pollutant levels including NO_2 were lower on average in LPG homes compared to biomass homes across the various study sites in the Kumar et al. (2008) study, authors suggest that respiratory diseases including asthma, rhinitis and upper respiratory tract infection in children may still be associated with both types of cooking fuels.

Colbeck et al. (2010) carried out two one-week sampling campaigns in Pakistan, where 90% of its rural population and 22% of its urban population relies on biomass fuels for cooking and heating.[16] This study employed passive sampling and took place in three distinct sites – one rural site where biomass fuels are the predominant fuel source, and another rural site and an urban site where natural gas is the predominant fuel source. Mean concentrations were approximately 133.8, 126.4, and 113.9 ppb in rural-biomass, rural-gas, and urban-gas kitchens, respectively. These two-week averages are generally higher than the levels observed in our study, but the trend of non-significant differences between study arm is consistent with our findings in Peru. The Colbeck et al. (2010) study was limited in sample size (n = 20 at each of the rural sites, n = 16 at the urban site), but the conclusions of the study were that fuel selection did not have a significant effect on NO_2 levels.

A more recent study by Mitra et al. (2022) was conducted in three rural villages in West Bengal, India.[115] They measured 8-h kitchen and living room NO_2 concentrations in LPG stove homes (n=60) and biomass stove homes (n=78). They found that average kitchen NO_2 concentrations were approximately 50% lower in LPG kitchens compared to biomass kitchens (33.7 $\mu g/m^3$ compared to 65.9 $\mu g/m^3$, $p<0.001$). These findings are consistent with our global findings and are similar to our individual findings in Guatemala and Rwanda. Overall, after assessing the relationship between multiple pollutants, including NO_2, the findings from Mitra et al. (2022) study such as use biomass fuels, improper ventilation, and smoking in the kitchen were likely the prime factors contributing to health-related ailments.

There are several characteristics between our study and the studies presented here that do not allow for direct comparison, namely differences in sample duration, participant demographics, behavior, geography, seasonality, sampling microenvironment, and sampling method; however, we are able to draw some parallels between our findings and those of other studies in LMICs.

<u>HICs</u>

Studies measuring NO_2 in HICs are plentiful in the literature, likely due to motor vehicle emissions being the largest contributor to ambient concentrations, with power plants, industrial facilities, and other forms of transportation in urban areas also being significant contributors to ambient NO_2 concentrations.[48] Additionally, gas appliances are used by over one-third of households in the U.S.[49] and are a common residential energy option in HICs.[49–52]

In 2008, Hansel et al. recruited 150 children ages 2-6 years to estimate the effect of indoor NO_2 levels from gas stoves and space heaters on asthma morbidity in Baltimore, Maryland.[51] They collected 72-h NO_2 samples in the children's bedrooms at baseline, 3 months, and 6 months. The overall mean (± SD) was 30.0 ± 33.7 ppb (range, 2.9-394.0 ppb). These findings are similar to what we observed in LPG kitchens in Peru. Although higher NO_2 concentrations were not associated with increased healthcare utilization, they were associated with increased asthma symptoms including cough, nocturnal symptoms, and number of days with limited speech. The WHO annual AQG for NO_2, which was updated in 2021 and is applicable to this setting, is 10 $\mu g/m^3$, or approximately 5 ppb.[38]

A 2015 study by Cibella et al. estimated the effect of indoor and outdoor NO_2 concentrations on lung function in 303 adolescents in Southern Italy.[116] A questionnaire was administered to participants to collect information on the presence of gas appliances, household ventilation, self-reported traffic exposure, among others. Indoor and outdoor NO_2 concentrations were measured over one-week periods using passive diffusion. Average indoor NO_2 concentrations were significantly higher compared to outdoor concentrations 28.1 $\mu g/m^3$ and 32.2 $\mu g/m^3$, respectively. These findings are similar to kitchen concentrations we observed in Guatemala. Authors reported approximately 25% of participants had indoor exposure levels in exceedance of the WHO AQG, which at the time of the publication was 40 µg/m^3 (approximately 20 ppb) but was updated in 2021 to 10 µg/m^3 (approximately 5 ppb).

Dedele et al. (2016) explored seasonal variation of indoor and outdoor NO_2 concentrations in homes with gas and electric stoves in Lithuania.[117] NO_2 was measured during each of the four seasons in seven homes with gas stoves and five homes with electric stoves. All 12 homes were reportedly ventilated in the same way with a natural (passive stack) ventilation system. Sampling microenvironments included the kitchen, living room, bedroom, and outside the home for two consecutive weeks during each season using passive sampling.

NO_2 concentrations were highest in the kitchen microenvironment during the winter (median 28.4 µg/m^3, range 8.6-41.5 µg/m^3). NO_2 levels were significantly higher in all microenvironments in homes with gas stoves compared to homes with electric stoves. Additionally, although an exact proportion was not reported, the majority of measurements in the gas stove group appear to be in exceedance of the WHO annual AQG of 10 µg/m^3, regardless of season.

These studies show significant variability in sample size, methodology, duration, and demographics, but importantly, they also highlight the difficulty in attaining indoor NO_2 levels in accordance with the 2021 WHO AQG of 10 µg/m^3, or 5 ppb, in these particular settings.

Our results compared to WHO guidelines

The WHO updated air quality guidelines for select air pollutants in 2021. In addition to the AQGs for each pollutant, the WHO established interim targets for NO_2, which serve as "incremental steps in reduction of air pollution in areas where air pollution is high and the AQG may not be attainable."[38] In addition to the short-term (24-hour) AQG of 13 ppb, two interim targets were established: IT-1 = 63 and IT-2 = 26 ppb. To our knowledge, the WHO is the only agency that has established a 24-hour guideline for exposure to NO_2. Given that IT-1 levels seem to be largely attainable in our study settings, we interpret our results in terms of the 24-hour IT-2 of 50 $\mu g/m^3$, or approximately 26 ppb.

Similarly, in addition to the annual AQG of 5 ppb, three interim targets were established: IT-1 = 20, IT-2 = 16, and IT-3 = 10 ppb. Annual AQGs help to contextualize our results in terms of potential long-term health effects; similar to the 24-hour AQG, we interpret our results in terms of the annual IT-2 of 16 ppb.

Short-term (24-hour) IT-2 guideline

Overall, we saw that the short-term IT-2 guidelines were highly attainable in Guatemala and Rwanda, especially in the intervention arm, though also in control kitchens and personal exposures. More than 50% of kitchens in Peru, regardless of study arm, were in exceedance of these guidelines, and approximately 20% of control and intervention participants received 24-h exposures in exceedance of these guidelines.

Annual IT-2 guideline

The annual IT-2 guideline, which helps to contextualize results in terms of chronic exposures, is 16 ppb. This level was highly attainable in our Guatemala site but less attainable in Peru and Rwanda. Study-wide, we found that 16% of intervention participants and 41% of intervention kitchens had concentrations in exceedance of these guidelines.

Potential impact of altitude on NO_2 and the WHO guidelines

The WHO guidelines are calculated at standard conditions (20°C and 1013 hPa). The altitudes of our study are highly variable, with the Guatemala and Rwanda sites located at approximately 1300 MASL and the Puno site in Peru located at 3825 MASL.

According to the EPA, the mass of a gaseous pollutant per unit volume of air varies with pressure, and pressure decreases as altitude increases. Furthermore, the mass per unit volume of air decreases with increasing altitude.[113] The atmospheric pressure in Puno is nearly 62% lower compared to that of sea level. The EPA suggests that the effective mass per unit volume changes that occur at different altitudes should be considered when interpreting data taken from different altitudes. Rather than adjusting the individual sample data, health-based guidelines such as those established by the WHO at standard conditions (20°C and 1013 hPa) can be adjusted for temperature and pressure, which is the approach that was taken by Kephart et al. (2021) for their NO_2 measurements taken in Puno and is the approach taken here.

Adjusting the original 24-hour and annual guidelines for ambient temperature and pressure at the individual sites, we saw inflations to the guidelines of approximately 15%, 58%, and 19% in Guatemala, Peru, and Rwanda, respectively. Despite adjusting the WHO guidelines, the overall interpretation of our results does not change compared to leaving the guidelines unadjusted.

Stove stacking

The differences we observed between study site may be attributed to multiple factors including behavior, ventilation patterns, and number of cookstoves in the home. One common challenge common in cookstove intervention studies is a practice known as “stove stacking,” where intervention homes may continue using their traditional biomass stoves, despite efforts by investigators to encourage exclusive uptake of the intervention stove.[118,119] Stove stacking has been observed to some degree at all HAPIN study sites and may have played a role in our study.

Study limitations

While this study stems from one of the largest cookstove intervention studies to date, offering robust sample sizes and diverse study settings, we were still met with some major challenges and limitations.

This ancillary study was slated to begin in the early months of 2020 but was delayed for several months due to the onset of the global COVID-19 pandemic in early March 2020. While the target sample sizes were ultimately achieved in Guatemala and Peru, sample size was affected at the Rwanda site due to the delays, resulting in less power. Nevertheless, we saw similar trends in our results at the Rwanda site compared to the Guatemala site.

In this study, we collected NO_2 samples from the 12-month follow-up visit of the HAPIN trial, which is the third and final exposure visit. Given that the HAPIN trial was still ongoing after the NO_2 data had been processed and analyzed, the data for the other pollutants ($PM_{2.5}$, BC, and CO) in the same households and at the same visit were still undergoing quality assurance and post-processing by HAPIN data management. Thus, we were unable to explore relationships between NO_2 and the other pollutants measured in the parent study. This study was also limited by single household visits instead of repeat measurements, preventing us from exploring day-to-day and/or seasonal variation within and between sites.

The major aim of this study was to assess personal exposures to and area concentrations of NO_2 for a 24-h period. This study was a single snapshot of those exposures and concentrations, and may not reflect daily exposures in these participants or in these populations. Additionally, it was beyond the scope of this particular study to explore health outcomes in association with NO_2 exposure, though it will be important for future studies.

CONCLUSIONS

Our findings suggest that transitioning to LPG stoves and fuels can result in modest reductions in personal exposures to and kitchen concentrations of NO_2, though not equally in all populations. Once we controlled for study site, we observed significant reductions in personal exposures only in Guatemala. In terms of kitchen area concentrations, we observed significant reductions in both Guatemala and Rwanda, while we did not see significant reductions in households or personal exposures in Peru. This may be attributed to differences in behavior and ventilation patterns, which we were not able to fully explore in this study. The WHO annual AQG for NO_2 was updated in 2021 to 10 $\mu g/m^3$, a drastic reduction from the 2010 annual AQG of 40 $\mu g/m^3$. More research is needed to investigate how to continue working towards these health-based guidelines, particularly in areas where these levels are difficult to achieve.

Table 2.1. Household and participant characteristics in the HAPIN NO_2 study.

	Guatemala		Peru		Rwanda		OVERALL	
	Control N (%) or Median (IQR)	**Intervention N (%) or Median (IQR)**	**Control N (%) or Median (IQR)**	**Intervention N (%) or Median (IQR)**	**Control N (%) or Median (IQR)**	**Intervention N (%) or Median (IQR)**	**Control N (%) or Median (IQR)**	**Intervention N (%) or Median (IQR)**
PARTICIPANT CHARACTERISTICS								
Number of participants	74	77	52	48	16	20	142	145
Age in years	24.6 (21.8-28.6)	24.5 (20.6-27.4)	25.3 (21.2-29.8)	25.0 (21.1-28.0)	26.3 (21.4-29.1)	30.1 (24.5-32.9)	24.9 (21.6-28.9)	25.0 (21.2-28.7)
Smokes tobacco								
Yes	0	5 (6)	0	0	1 (6)	0	1 (<1)	5 (3)
Highest education								
Primary or less	56 (76)	55 (71)	8 (15)	6 (13)	9 (56)	8 (40)	73 (51)	69 (48)
Secondary	20 (27)	22 (29)	45 (87)	41 (85)	7 (44)	12 (60)	72 (51)	75 (52)
HOUSEHOLD CHARACTERISTICS								
# Household members	4 (3-5)	4 (3-7)	5 (3-5)	4 (3-6)	3 (3-4)	4 (3-5)	4 (3-5)	4 (3-7)
Kitchen description								
Enclosed	72 (97)	75 (97)	45 (87)	45 (94)	11 (69)	16 (80)	128 (90)	136 (94)
Mesh around room	2 (3)	0	5 (10)	2 (4)	3 (19)	0	10 (7)	2 (1)
Unfinished wall between rooms	1 (1)	2 (3)	3 (6)	0	2 (13)	4 (20)	6 (4)	6 (4)
Roof type								
Corrugated metal	72 (97)	74 (96)	43 (83)	43 (90)	16 (100)	20 (100)	131 (92)	137 (94)
Other	3 (4)	3 (4)	10 (19)	4 (8)	0	0	13 (9)	7 (5)
Wall type								
Mudbrick	70 (95)	66 (86)	34 (65)	31 (65)	16 (100)	20 (100)	120 (85)	117 (81)
Concrete	2 (3)	4 (5)	18 (35)	13 (27)	13 (81)	10 (50)	33 (23)	27 (19)
Other	3 (4)	7 (9)	1 (2)	3 (6)	10 (63)	14 (70)	14 (10)	24 (17)
Floor type								
Mud and concrete	11 (15)	14 (18)	9 (17)	5 (10)	0	0	20 (14)	19 (13)

Mud only	59 (80)	54 (70)	14 (27)	19 (40)	11 (69)	9 (45)	84 (59)	82 (57)
Concrete only	4 (5)	9 (12)	24 (46)	20 (42)	5 (31)	10 (50)	33 (23)	39 (27)
Other	0	0	5 (10)	3 (6)	0	1 (1)	5 (4)	4 (3)
STOVE USE AND CHARACTERISTICS								
Cook times/day								
1-2	4 (5)	1 (1)	23 (44)	16 (33)	16 (100)	15 (75)	43 (30)	32 (22)
3	67 (91)	70 (91)	27 (52)	31 (48)	0	5 (25)	94 (66)	106 (73)
4-5	4 (5)	6 (8)	2 (4)	0	0	0	6 (4)	6 (4)
# Stoves at baseline								
1	28 (38)	22 (29)	18 (35)	13 (27)	12 (75)	11 (55)	58 (41)	46 (32)
2	42 (57)	49 (64)	35 (67)	34 (71)	4 (25)	9 (45)	81 (57)	92 (63)
3	5 (7)	5 (6)	0	0	0	0	5 (4)	5 (3)
4+	0	1 (1)	0	0	0	0	0	1 (<1)
Stove 1 type at baseline								
Open/3 stone	57 (77)	65 (84)	47 (90)	44 (92)	12 (75)	5 (25)	116 (82)	114 (79)
Biomass	18 (24)	12 (16)	6 (12)	3 (6)	2 (13)	8 (40)	26 (18)	23 (16)
Rondereza	NA	NA	NA	NA	2 (13)	5 (25)	2 (1)	5 (3)
Portable wood stove	0	0	0	0	0	2 (10)	0	2 (1)
Stove 1 fuel type								
Wood	74 (100)	77 (100)	12 (23)	4 (8)	14 (88)	11 (55)	100 (70)	92 (63)
Cow dung	0	0	40 (77)	43 (90)	0	0	40 (28)	43 (30)
Charcoal	0	0	0	0	2 (13)	9 (45)	2 (1)	9 (6)
Stove 1 chimney: Yes	18 (24)	14 (18)	20 (38)	18 (38)	0	0	38 (27)	32 (22)
Stove 1 location								
Separate building	12 (16)	14 (18)	28 (54)	23 (48)	13 (81)	11 (55)	53 (37)	48 (33)
Room adjacent to bedroom	40 (54)	36 (47)	5 (10)	3 (6)	0	0	45 (32)	39 (27)

Separated from bedroom, inside	17 (23)	23 (30)	6 (12)	11 (23)	0	0	23 (16)	34 (23)
In participant's bedroom	4 (5)	2 (3)	0	0	0	0	4 (3)	2 (1)
Outside the house	2 (3)	2 (3)	13 (25)	10 (21)	3 (19)	9 (45)	18 (13)	21 (14)
Stove 1 use frequency								
Daily	74 (100)	76 (99)	34 (65)	37 (77)	15 (94)	19 (95)	123 (87)	132 (91)
3x/week	0	1 (1)	4 (8)	4 (8)	0	0	4 (3)	5 (3)
4x/week	0	0	6 (12)	1 (2)	1 (6)	0	7 (5)	1 (<1)
5x/week	0	0	6 (12)	3 (6)	0	1 (5)	6 (4)	4 (3)
6x/week	0	0	2 (4)	3 (6)	0	0	2 (1)	3 (2)

Table 2.2. Personal and kitchen area NO_2 concentrations (ppb).

	Personal exposure			**Kitchen area**		
	N	**Mean (SD)**	**Median (IQR)**	**N**	**Mean (SD)**	**Median (IQR)**
Guatemala						
Control	74	15.6 (10.2)	12.2 (7.4-19.3)	75	29.2 (23.1)	22.5 (14.5-35.1)
Intervention	77	10.6 (3.1)	11.2 (7.4-12.7)	76	14.5 (9.8)	13.2 (7.4-17.1)
Peru						
Control	52	31.1 (36.4)	23.0 (15.3-31.0)	54	75.9 (91.2)	47.7 (20.6-79.4)
Intervention	48	31.0 (38.7)	21.0 (14.6-30.3)	48	55.3 (45.0)	43.5 (29.0-68.5)
Rwanda						
Control	16	12.5 (6.1)	11.7 (7.4-12.7)	16	36.2 (27.3)	29.5 (17.8-48.9)
Intervention	20	13.3 (6.5)	11.2 (7.4-18.4)	20	18.6 (9.2)	19.4 (10.2-25.7)
OVERALL						
Control	143	20.8 (24.4)	15.3 (10.7-25.3)	145	47.4 (62.5)	29.2 (16.7-49.8)
Intervention	145	17.7 (24.3)	12.5 (7.4-17.9)	144	28.7 (32.9)	17.4 (11.7-30.9)

Table 2.3. Samples below the unadjusted and altitude-adjusted (site-specific) WHO IT-2 guidelines.

		N (%) below AQG (ppb)			
Site	**N**	**Unadjusted 24-h IT-2**	**Site-specific 24-h IT-2**	**Unadjusted annual IT-2**	**Site-specific annual IT-2**
Guatemala	**302**	**26**	**30**	**16**	**18**
Control Personal	74	69 (93)	70 (95)	54 (73)	59 (80)
Intervention Personal	77	77 (100)	77 (100)	77 (100)	77 (100)
Control Kitchen	75	75 (100)	75 (100)	75 (100)	75 (100)
Intervention Kitchen	76	74 (97)	75 (99)	64 (84)	68 (89)
Peru	**202**	**26**	**41**	**16**	**25**
Control Personal	52	41 (79)	47 (90)	27 (52)	39 (75)
Intervention Personal	48	39 (81)	45 (94)	30 (63)	39 (81)
Control Kitchen	54	25(46)	35 (65)	15 (27)	23 (42)
Intervention Kitchen	48	23 (48)	35 (73)	11 (23)	23 (48)
Rwanda	**72**	**26**	**31**	**16**	**19**
Control Personal	16	16 (100)	16 (100)	14 (88)	14 (88)
Intervention Personal	20	20 (100)	20 (100)	15 (75)	16 (80)
Control Kitchen	16	8 (50)	10 (63)	5 (31)	7 (44)
Intervention Kitchen	20	19 (95)	20 (100)	10 (50)	12 (60)
OVERALL	**576**	**26**		**16**	
Control Personal	142	129 (91)		98 (69)	
Intervention Personal	145	136 (94)		122 (84)	
Control Kitchen	145	83 (57)		49 (34)	
Intervention Kitchen	144	117 (81)		85 (59)	

Figure 2.1a. LPG stoves installed in Guatemala (A), Rwanda (B), and Peru (C) in the intervention arm.

Figure 2.1b. Traditional biomass stoves installed in Guatemala (A), Rwanda (B), and Peru (C) in the intervention arm.

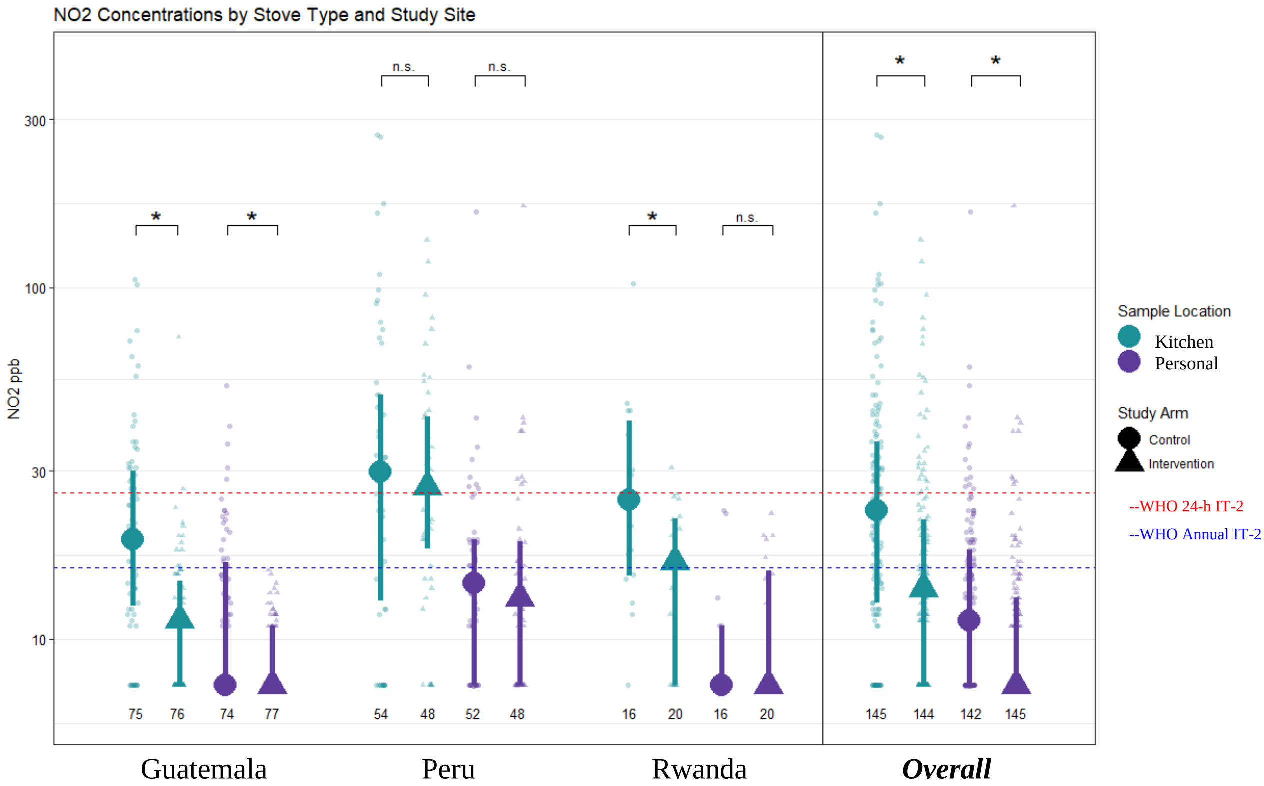

Figure 2.2a. Kitchen area concentrations and personal exposures to NO_2 by study site and pooled (Overall) compared to unadjusted WHO 24-h Interim Target 2 (IT-2; red) and annual IT-2 (blue) guidelines.[38] Sample sizes are given for each category. Significance ($p<0.05$) is indicated (*). Non-significant findings are indicated by “n.s.”

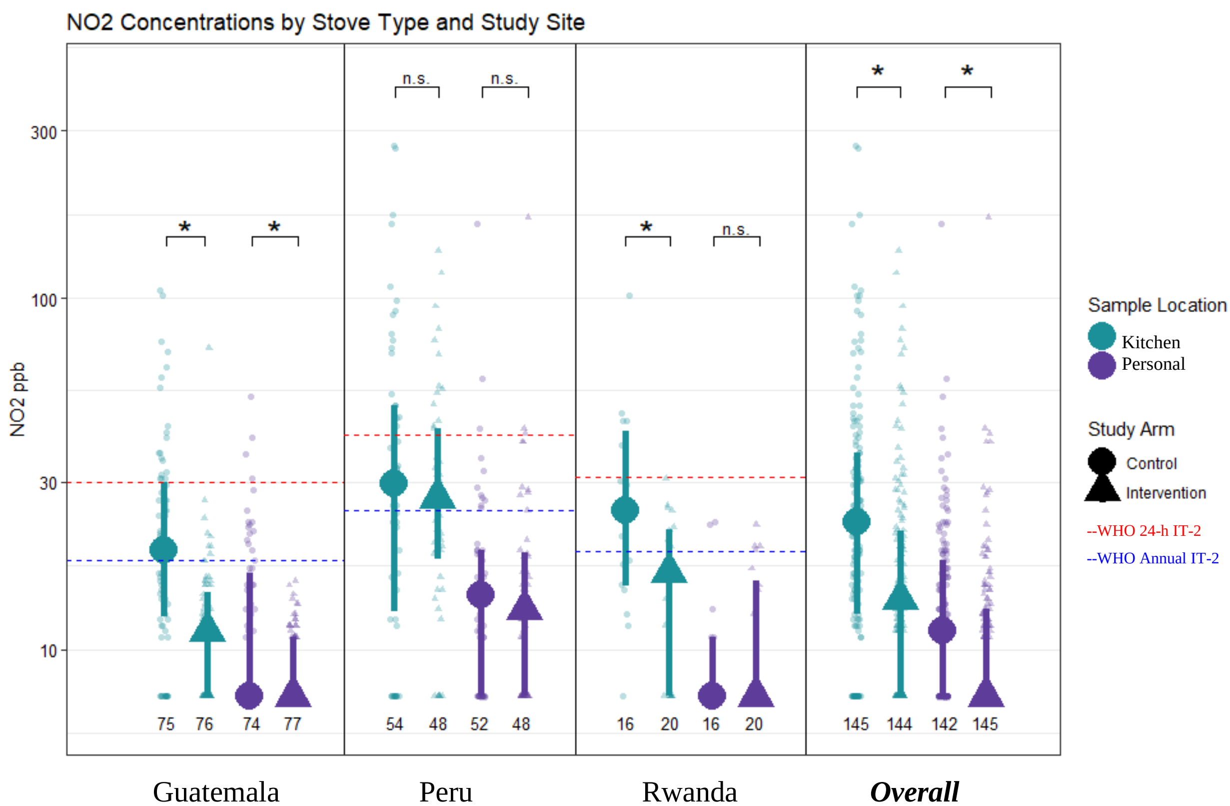

Figure 2.2b. Kitchen area concentrations and personal exposures to NO_2 by study site and pooled (Overall) compared to unadjusted WHO 24-h Interim Target 2 (IT-2; red) and annual IT-2 (blue) guidelines.[38] Sample sizes are given for each category. Significance ($p<0.05$) is indicated (*). Non-significant findings are indicated by “n.s.”

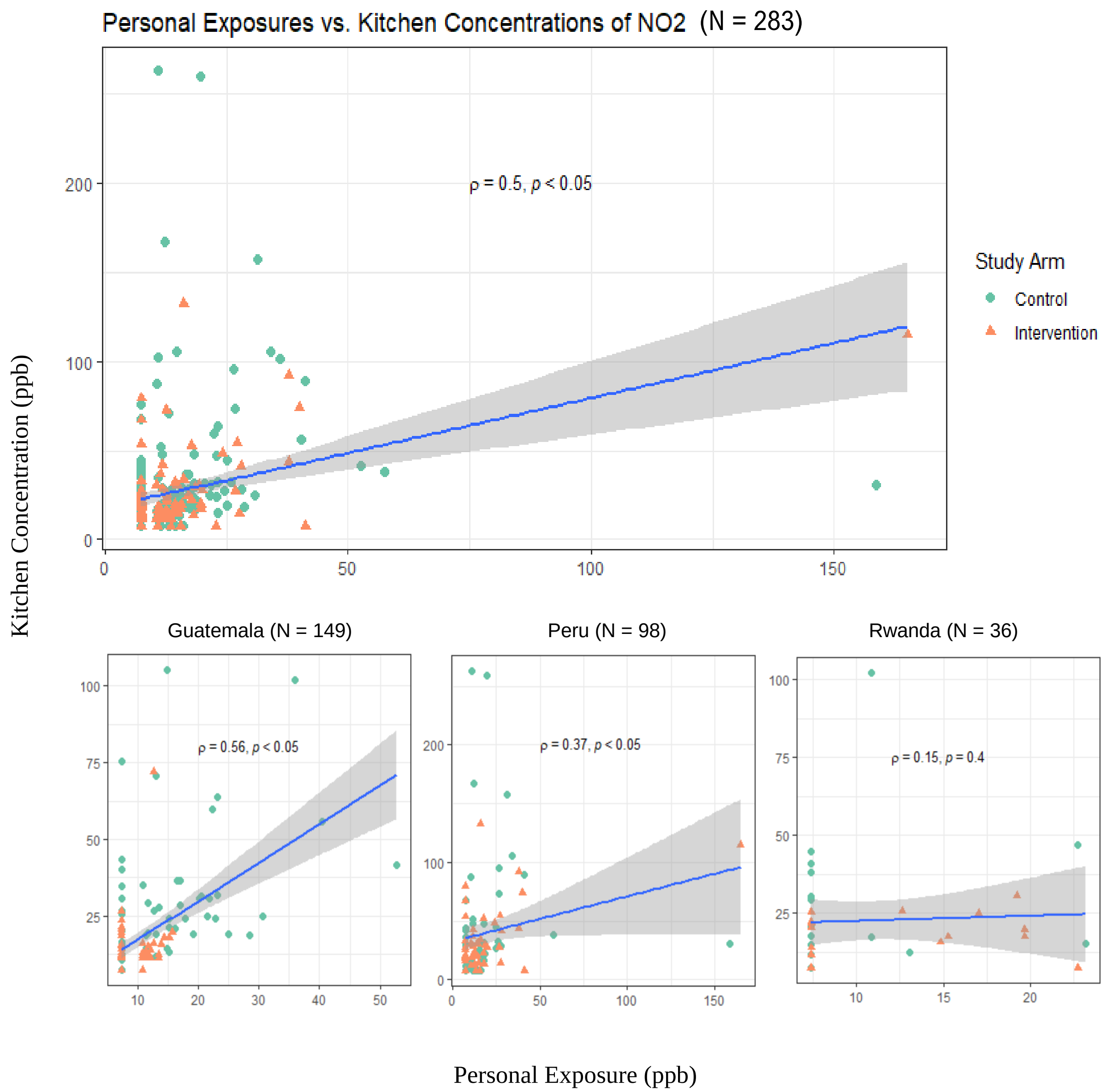

Figure 2.3. Correlations between personal exposures and kitchen concentrations from the same household presented globally (top) and by study site (bottom).

CHAPTER 3

EXPOSURES TO HOUSEHOLD AIR POLLUTION AND GARBAGE BURNING VIA AIR MONITORING OF $PM_{2.5}$ AND BC, AND URINARY BIOMARKERS OF EXPOSURE TO PAHS, BISPHENOL A, AND PHTHALATES IN ADOLESCENT GIRLS IN RURAL GUATEMALA[2]

[2] Kearns, Katherine A., Naeher, Luke P., McCracken, John P., Barr, Dana Boyd, Saikawa, E., Hengstermann, Mayari, Mollinedo, Erick, Panuwet, Parinya, Yakimavets, Volha, Lee, Grace E., Thompson, Lisa M. To be submitted to *Science of the Total Environment.*

ABSTRACT

Background: Household air pollution (HAP) from these sources includes fine particulate matter ($PM_{2.5}$), black carbon (BC), and polycyclic aromatic hydrocarbons (PAHs). Waste sanitation services are uncommon in many rural areas of LMICs such as Jalapa, Guatemala, causing accumulation of waste, including plastic waste, until it is either burned or dumped, both of which usually occur in or near households. In addition to HAP resulting from cooking activities, household garbage burning has been observed in Jalapa, including the burning of plastic waste. Adolescent girls often help with these cooking and household tasks, but little is known about their exposures to the resultant HAP and exposure to plastics.

Objectives: In this pilot study, we sought to characterize HAP exposures, including exposures to plastic waste burning in adolescent girls in Jalapa using personal air sampling and urinary biomarker analysis.

Methods: We recruited 60 adolescent girls between 13-17 years of age who lived with participants of the Household Air Pollution Intervention Network (HAPIN) trial, a randomized controlled trial that assessed the effect of a liquefied petroleum gas (LPG) stove and fuel intervention compared to biomass stove use. Adolescents in this study were recruited if they helped with cooking activities, with n=30 in the biomass (wood-burning stove) arm and n=30 in the LPG arm. We measured 24-h personal exposures to $PM_{2.5}$ and BC via air monitoring, and collected urine samples at the end of the sampling period to measure biomarkers exposure to PAHs and plasticizers including phthalates and bisphenol A (BPA). We also measured real-time kitchen concentrations of BC in 20 homes (33%).

Results: $PM_{2.5}$ and BC personal exposures were measured in n=21 control and n=19 intervention participants. participants. Median concentrations were 87% lower for personal $PM_{2.5}$, 80% lower

for personal BC, and 85% lower for kitchen area BC for intervention compared to control homes. Concentrations of hydroxylated PAH metabolites were significantly lower ($p<0.001$) for all nine metabolites in intervention (n=26) compared to control participants (n=28). We did not observe any differences between study arm for phthalates and BPA, but some metabolites were elevated compared to age- and sex-matched participants of a U.S.-based survey.

Discussion: This study contributes to HAP exposure assessment of adolescent girls, an area that is currently understudied. We observed statistically significant reductions in personal exposures and kitchen concentrations in LPG compared to biomass homes for $PM_{2.5}$, BC, and PAHs, suggesting that LPG stoves can help reduce exposures to HAP among adolescent girls. We did not observe statistically significant differences between study arm for phthalates and BPA, suggesting that exposure was independent of the study arm the participants were randomized to.

INTRODUCTION

Approximately 3.8 billion people worldwide rely on solid or biomass fuels, such as coal, charcoal, wood, animal dung, and agricultural crop residues for cooking and heating (Health Effects Institute, 2020). Homes are often minimally ventilated, and the resultant smoke from the incomplete combustion of biomass fuels is a heterogeneous mixture of health-damaging pollutants such as fine particulate matter ($PM_{2.5}$), black carbon (BC), and polycyclic aromatic hydrocarbons (PAHs).[80,121]

The vast majority of people affected by HAP reside in lower- and middle-income countries (LMICs), and women and children are at greatest risk of exposure due to time spent at home and around the fire.[1] Exposure to household air pollution (HAP) is associated with adverse health effects including decreased lung function[21], hypertension[122], acute respiratory infection[123], and other acute and chronic diseases.[1]

In addition to normal cooking activities, HAP exposure is also attributed to household waste burning, especially in areas where municipal sanitation services are nonexistent.[56–59] In LMICs, unsustainable waste management practices are more common compared to high income countries (HICs), which increases the risk of harmful exposures through water, soil, and air pollution.[60] Waste disposal options include landfilling, open dumping, recycling, composting, anaerobic digestion, and incineration.[68]

Approximately 43% of the total population of Guatemala and 50% of the population of Jalapa burns their household waste in or near their property as a primary means of waste disposal, according to the Guatemala Population and Housing Census of 2018. Additionally, plastic waste is ubiquitous in communities including Jalapa, creating the potential for exposure to plasticizers such as phthalates and bisphenol A (BPA).[86]

In Guatemala, plastic comprises 17.3% of the total waste stream, with up to 500,000 metric tons of plastic waste currently classified as mismanaged waste. Phthalates and BPA are chemicals added to plastic resins to make them more rigid and durable, and both are known endocrine disrupting compounds.[62,65] Both BPA and phthalates are found in personal care products such as soap, shampoo hair spray, and nail polishes.[65] BPA is also found in a number of consumer materials including thermal receipt paper, linings of canned foods, electronics, pipes, coatings, and flame retardants.[67]

Long-term exposure to phthalates has been shown to impact pregnancy[65,124], child growth and development, and reproductive systems in young children and adolescents.[65,125] BPA interacts with various biological receptors including estrogen receptors and has been associated with breast cancer development.[126] Both BPA and phthalates have been proposed in the etiology of polycystic ovarian syndrome (PCOS) in adolescent girls.[127]

Common routes of exposure to phthalates and BPA are dermally through the use of personal care products, through ingestion of food or drinks that have been packaged in or come into contact with BPA and phthalates, and through the inhalation of airborne particles containing phthalates and BPA.[62,124] Burning products containing BPA at high temperatures such as in the open burning of waste has been shown to cause BPA to leach into the environment, though more studies are needed to understand the fate and associated health impacts upon exposure to BPA and similar environmental chemicals.[63,64]

The most commonly studied air pollutants in the context of residential biomass burning are $PM_{2.5}$ and CO due to their well-documented health effects associated with exposure.[26,27] $PM_{2.5}$ is a heterogeneous mixture of nitrates, sulfates, BC, organic chemicals, metals, and soil (crustal) material.[28] Inhalation exposure to $PM_{2.5}$ triggers the overproduction of reactive oxygen species (ROS), which can cause oxidative stress, inflammation, and DNA and cellular damage.[29]

BC is gaining increased attention due to its associations with health effects and implications for climate change.[30,31] In addition to being identified as a climate forcing agent, BC has also shown toxicity upon exposure likely due to its small dimension and adsorptive properties with other air pollutants.[31] Both $PM_{2.5}$ and BC are emitted from the incomplete combustion of biomass and fossil fuels[32] and from burning household waste including plastics.[33]

Included in the mixture of pollutants released from residential biomass burning and burning of household waste are PAHs.[128,129] When humans are exposed to PAHs, the parent compounds undergo biotransformation by cytochrome P450 enzymes to their metabolized derivatives, many of which have carcinogenic and mutagenic properties.[78,79] PAHs are associated with respiratory and cardiovascular diseases, reduced lung capacity, and lung cancer.[76]

Many PAHs exist in the particle phase, especially in $PM_{2.5}$, and have been identified as known, probable, or possible human carcinogens.[130] Common routes of exposure are inhalation[75] and dermal exposure[76], and through dietary ingestion, particularly through the consumption of grilled, roasted, and charred foods.[76,77]

HAP exposure assessment studies are plentiful in the literature[131], though few are randomized controlled trials of cleaner cookstove interventions like LPG.[132] Women and children are the populations of most interest in HAP studies due to the disproportionate amount of time they spend at home and around the stove.[1] There is limited evidence available on HAP exposure assessment in young adults or adolescents[133–136], despite the knowledge that adolescent girls often help with household tasks including cooking and garbage burning. HAP exposure assessment in adolescent girls in LMICs, where residential biomass and garbage burning are prevalent, is even less common.[133]

In this pilot study, we sought to characterize personal exposures to HAP in adolescent girls between the ages of 13-17 years in Jalapa, Guatemala, where domestic waste burning is common, including the burning of plastic waste in household fires. We conducted 24-h personal exposure assessment to $PM_{2.5}$ and BC, collected real-time kitchen area BC concentrations, and analyzed urinary biomarkers of exposure to hydroxylated PAHs, which are markers for HAP and/or waste burning, and biomarkers of phthalates and BPA to assess exposures to plastics. We also collected survey data to assess other potential dietary, dermal, and inhalation exposures to measured pollutants. This study aims to address important knowledge gaps in personal exposure assessment to HAP in adolescent girls in rural areas of LMICs.

METHODS

Study setting

This pilot study was nested within the Household Air Pollution Intervention Network (HAPIN) randomized controlled trial. The HAPIN trial is described in detail elsewhere[13,137] and summarized briefly here. The HAPIN trial took place in four LMICs: Guatemala, India, Peru, and Rwanda. HAPIN households (n=3195) were randomized into either the control (continued use of biomass stove; n=1605) or intervention arm (LPG stove and fuel intervention; n=1590). The HAPIN trial measured 24-h personal exposures in pregnant women and kitchen concentrations of $PM_{2.5}$ and BC pre- and post-intervention. For this pilot study, we enrolled 60 adolescent girls living with HAPIN participants in Guatemala.

Inclusion and exclusion criteria

Participants evenly represented the control and intervention study arms to explore differential exposures between biomass and LPG stove use. Inclusion criteria were female, ages 13-17 years, residence in households participating in the HAPIN trial, and daily participation in cooking meals at home. Exclusion criteria were known pregnancy, self-reported current cigarette smoker, or plans to move temporarily or permanently outside the study area during the time of the study.

Questionnaire

In total, 56 of the 60 adolescent girls met eligibility criteria and provided their consent to participate in the study; written consent was obtained from a parent or guardian. All participants were informed of study objectives, time commitment, benefits, and risks of the study. Local, trained fieldworkers verbally administered a baseline survey questionnaire in Spanish on sociodemographic information, household and stove characteristics, perception of waste generation and disposal in the community, health effects related to HAP exposure, and sources of HAP in and outside the home. Another survey was administered at sample collection the next day to determine potential sources of exposure over during the 24 hours before the first visit and 24 hours during the monitoring period. Responses were recorded on tablets and saved on RedCap.

Exposure assessment and sampling strategy

Personal exposure assessment and laboratory analysis of $PM_{2.5}$

Personal exposure to $PM_{2.5}$ was assessed on all participants using the Triplex Personal Sampling Cyclone (Mesa Labs) and Casella Tuff Pro pump (Casella, Buffalo, NY, USA). Pre-weighed Teflon filters with a diameter of 37mm (Pall Corporation, Port Washington, NY, USA) were loaded into a plastic cassette (SKC Limited, United Kingdom) and affixed to the aluminum cyclone. The cyclone and filter apparatus were connected to the pump via tubing. The pump/cyclone system weighs about 450 g total and was calibrated at a flow rate of 1.5 L/min. The instruments were calibrated before each deployment, and the flow rate was recorded at the field station before and after the sampling period to determine the average sample flow rate.

At the start of the sample period, trained field staff turned on the air sampling equipment and placed the pump into a small backpack. The tube and cyclone were affixed along the strap of the backpack such that the filter was placed in the participant’s approximate breathing zone

(Figure 3.1). The participant was asked to wear the backpack at all times, unless she were to participate in an activity which might damage the equipment such as bathing or sleeping, in which case she was asked to keep the backpack within a meter of her person.

The instruments were stopped by a field technician when they returned after 24 h to collect the equipment. The sampling equipment was carried back to the field office and filters were removed from the cyclones in a clean laboratory at the field office. Filters were placed in labeled petri dishes and kept refrigerated at 4°C at the field office until they were hand-carried by a traveler to the University of Georgia (UGA, Athens, GA, USA) for analysis.

Laboratory technicians at UGA weighed the filters before and after sampling ("pre-weighed" and "post-weighed," respectively) on a microbalance with a sensitivity of 1 μg (Sartorius Cubis MSU, Gottingen, Germany) to determine the mass deposition of $PM_{2.5}$. Each filter was pre-weighed and post-weighed twice, once each by two laboratory technicians in distinct weighing sessions. If the difference between weights 1 and 2 was greater than ±5 μg for any filter, a third weight was taken and the average of the two closest weights was used as the average pre- or post-weight. Mass deposition was calculated by subtracting average pre-weight from average post-weight. Mass deposition was converted to $PM_{2.5}$ concentration (μg/m^3) by considering the flow rate of the instrument for each filter and the total sampling duration.

<u>Personal exposure assessment and laboratory analysis of BC</u>

BC was measured on the same filters used for personal exposure assessment to $PM_{2.5}$ using the SootScan Model OT21 Optical Transmissometer (Magee Scientific, Berkeley, CA) before and after sampling. Similar to $PM_{2.5}$ gravimetric analysis, each filter was scanned twice, once each by two technicians in distinct scan sessions. The attenuation of BC through the filter

(ATN) was converted to concentration (μg/m^3) using a standard, σ[138], the effective sampling area of the 37-mm filter (30 mm), and other outputs provided by the SootScan.

Real-time kitchen area BC concentrations

Due to availability of equipment, we measured 24h real-time BC concentrations in the kitchens of 20 participants (36%) using the Model AE51 microAeth Black Carbon aerosol monitor (Aethlabs, Inc., range: 0-1 mg/m^3, resolution: 0.001 mg/m^3, flow rate: 50 mL/min) in n=10 control homes and n=10 intervention homes. The microAeth was deployed in the kitchen at 0.5 m above the ground near the stove and was loaded with a Teflon-coated glass fiber filter strip prior to deployment (Pallflex Fiberfilm T60A20, Pall Life Sciences, MI, USA). The monitor draws air into an inlet 3 mm in diameter with a built-in pump and measures the rate of light absorption as aerosols deposit on the filter at a time interval ("timebase") pre-set by the user. The flow rate and timebase used for this study were 50 mL/min and 60 seconds, respectively. The absorbance of the sample area of the filter is therefore measured once every 60 seconds relative to a reference portion of the filter paper. These parameters were chosen based on manufacturer's instructions in order to optimize performance of the microAeth in this particular setting.

The BC data was post-processed using an Optimized Noise-reduction Averaging (ONA) algorithm as recommended by the manufacturer.

Urinary biomarkers for hydroxylated PAHs, phthalate metabolites, and BPA

Urine samples were collected from all participants at the end of the 24-h sample period when the technicians returned to pick up equipment and samples. Samples were transported from the field to the field laboratory in coolers with blue ice and were kept in the freezer at -20°C until shipment to Emory University (Atlanta, GA, USA).

All urine samples were randomized using a Fisher-Yates shuffling algorithm prior to analysis to reduce any potential batch effects. A 0.5-mL aliquot of urine was spiked with isotopically labeled analogues of the target phthalates and phenols and then was subjected to an enzyme hydrolysis to liberate glucuronide-bound conjugates. The hydrolysate was extracted using an ABS Elut-NEXUS solid phase extraction column, eluting with acetonitrile and ethyl acetate. The extract was concentrated to dryness and reconstituted in mobile phase for analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS) using two separate injections and acquisition methods.

Analyte concentrations were calculated using isotope dilution calibration. Two quality control materials (one high and one low) and one blank sample were analyzed concurrently with each set of 28 unknown samples. Further quality assurance measures were included in the sample analyses including the analysis of NIST SRM 3672 and 3673 (one of each per 50 samples), and bi-annual participation in the German External Quality Assessment Scheme (G-EQUAS). Specific gravity was measured using a refractometer.

All metabolite concentrations were adjusted for measured creatinine concentrations to account for variability in the volume of urine and the concentrations of endogenous and exogenous chemicals from void to void.[94]

Statistical analysis

All statistical analyses were performed in R (version 4.0, R Foundation for Statistical Computing, 253 Vienna, Austria). We calculated descriptive statistics for the pollutants by study arm. Differences in exposures between assigned treatment arm were evaluated using non-parametric tests (Wilcoxon Rank Sum), given that that the data violated assumptions of normality. We assessed Spearman correlations between personal and kitchen measurements of BC within the same household. To put our results in risk context, we evaluated the percentage of $PM_{2.5}$ samples that were below the WHO Annual IT-1 of 35 µg/m^3[38], and compared metabolite concentrations of PAHs, phthalates, and BPA to age- and sex-matched participants of the National Health and Nutrition Examination Survey (NHANES). We also assessed the correlation between the mothers' personal exposures from the parent HAPIN trial compared to the adolescent participants living in the same household for data that was available.

RESULTS

Household and participant characteristics

Household and participant characteristics are provided in Table 3.1, in addition to personal care product, cosmetic, and plastic use. Ninety-eight percent (55/56) of participants reported feeling that their community has a waste disposal issue. Participants observed the most trash on the main road in their respective communities, compared to their own yards, church, school, and public land. The most frequently observed type of trash reported by participants were plastic bottles and snack wrappers. Seventy-seven percent (43/56) of participants reported that they typically burn the plastic trash that they generate; the second-most popular response was to bury it. Forty-eight percent of control participants (13/28) and 23% of intervention participants (6/26) said their household burns plastic trash at least twice per week. Eighty-nine percent of all participants (50/56) said that plastic affects health in some way.

After the 24-h sample period when the field staff returned to collect exposure monitors and urine samples, participants were asked via questionnaire about their activities over the previous 48 hours (Table 3.1). All 28 control (100%) and 9 intervention (35%) participants reported they had been near an open fire within the previous 48 hours. Of these participants, 11 control and 1 intervention participant reported they had burned garbage within that timeframe. Seven of the control participants and the same intervention participant stated that the garbage burned included plastic. Sixteen control (57%) and 13 intervention participants (50%) reported having eaten burned or charred tortillas within the past 48 hours. Four control (14%) and 3 intervention participants (12%) reported having eaten grilled food within the same timeframe.

Personal exposure assessment for $PM_{2.5}$ and BC

We analyzed personal $PM_{2.5}$ and BC exposures from 41 participants (n=21 control, n=20 intervention). Fifteen filters (27%) were removed from analysis due to instrument failure and thus, inability to determine total sample duration and final concentration. Our findings are summarized in Table 3.2 and displayed graphically in Figure 3.2. There was a significant reduction (Wilcoxon Rank Sum, $p < 0.001$) in personal $PM_{2.5}$ exposures in intervention participants (median 17.9 $\mu g/m^3$, IQR 10.0–50.0) compared to control participants (median 138.2 $\mu g/m^3$, IQR 42.4–231.7). Similarly, there was a significant reduction (Wilcoxon Rank Sum, p

<0.05) in personal BC exposures in intervention (median 2.2 μg/m^3, IQR 1.5–3.3) compared to control participants (median 10.1 μg/m^3, IQR 4.2–14.7).

Compared to the WHO Annual IT-1 of 35 μg/m^3 for $PM_{2.5}$, 16 control (76%) and 7 intervention participants (35%) had exposures in exceedance of these guidelines. We assessed the correlation between mothers' personal exposures from the main HAPIN trial and adolescent personal exposures from the present sub-study. $PM_{2.5}$ data was available for 35 mothers (n=18 control, n=17 intervention) from the main trial and showed moderate correlation (Spearman's $\rho \sim 0.49$, $p < 0.05$) with the adolescents in their respective households (Figure 3.2). Personal BC data was available for 14 mothers from the main trial and also showed moderate correlation (Spearman's $\rho \sim 0.70$, $p< 0.05$) with the adolescents in their respective households (Figure 3.4).

<u>Kitchen area BC concentrations</u>

Due to instrument availability, real-time BC was measured in 20 kitchens (36%). Kitchen area BC concentrations were about 5 times lower on average in intervention (n=10) compared to control kitchens (n=10). Results are summarized in Table 3.2 and shown graphically in Figure 3.2.

<u>Biomarkers of exposure</u>

Biomarkers of exposure to PAHs, phthalates, and BPA were measured in n=28 control and n=26 intervention participants. Two participants refused to provide a urine sample. Questionnaire data pertaining to household and participant characteristics, HAP and PAH exposure, personal care product use, and plastic use are presented in Table 3.1.

PAHs

Nine hydroxylated PAH metabolites were analyzed, and results are shown in Figure 3.7. Due to the inability to isometrically separate some of the metabolites, 2- and 3-hydroxyfluroene and 2- and 3-hydroxyphenanthrene were combined to equal seven total metabolites. Concentrations were significantly lower ($p < 0.001$) for each of the seven metabolites in intervention compared to control participants. Similarly, metabolite concentrations were significantly higher in our participants regardless of study arm compared to age- and sex-matched participants of NHANES, with the exception of 2-naphthol, where only intervention and NHANES concentrations were not significantly different. Total PAH concentrations were moderately correlated (Spearman's $\rho \sim 0.65$, $p < 0.05$) with $PM_{2.5}$ concentrations (Figure 3.8).

Phthalates

Nine phthalate metabolites were analyzed in urine samples and results are shown graphically in Figure 3.9. We observed non-significant differences ($p>0.05$) between control and intervention for all nine metabolites. Compared to age- and sex-matched NHANES participants (n=113), we observed significantly higher ($p<0.001$) metabolite concentrations in our participants for MBP, MECPP, MEHHP, MEHP, and MEOHP, regardless of study arm. We observed nonsignificant differences between our participants and NHANES participants for MiBP. For MEP, there was not a statistically significant difference ($p > 0.05$) between control and intervention, but NHANES was significantly higher ($p < 0.05$) compared to the control group. Similarly, for MBzP, there was no significant difference ($p > 0.05$) between control and intervention, but NHANES was significantly higher ($p < 0.001$) than both study arms.

BPA

We observed non-statistically significant differences ($p>0.05$) in BPA concentrations between study arm, and BPA concentrations were significantly higher ($p<0.001$) for age- and sex-matched NHANES participants compared to our study participants (Figure 3.10).

DISCUSSION

We found that intervention participants had significantly reduced exposures to $PM_{2.5}$, BC, and PAHs compared to biomass participants, suggesting that LPG can reduce exposures to air pollutants that are harmful to health. We also measured detectable levels of phthalate and BPA metabolites via biomonitoring, some of which were higher than our reference NHANES population.

We observed substantial reductions (-86%) in median $PM_{2.5}$ and BC exposures in intervention compared to control participants. These findings are consistent with similar studies comparing exposures in women primarily using biomass stoves compared to LPG stoves.[132,139,140] We also found that total PAH concentrations were moderately correlated with $PM_{2.5}$ ($\rho = 0.65$, $p < 0.05$), which is consistent with a similar study by Weinstein et al. (2020).[139]

In addition to inhalation exposure after combustion, exposure to PAHs can occur through the diet, specifically from eating grilled or charred foods.[77] Tortillas are a staple food in Guatemala, and over half of control participants and exactly half of intervention participants reported having eaten charred or burned tortillas within the previous 48h of the urine sample collection. While this may account for some PAH exposure in our participants, exposures were more likely attributed to stove type given the significant reductions in median urinary concentrations (-72%) we observed in LPG participants compared to biomass.

These findings are even more drastic compared to those of Weinstein et al. (2020), which found that the LPG stove caused a 37% reduction in median total PAH metabolite concentrations in pregnant women.[139] This study measured only four hydroxylated PAH metabolites, but had the advantage of being a crossover study design where they took a urine sample in the same participant before and after receiving the intervention. That allowed for each participant to serve as their own control, in contrast to our cross-sectional study where measurements were all taken post-randomization.

Plastic packaging is a global problem, widely used in food containers and water bottles are widely used for potable drinking water. BPA is a common plasticizer and highly detectable in media including urine, though data on exposure levels in LMICs is lacking.[141] In this study, we did not see significant differences between study arm, suggesting that BPA exposure was not necessarily dependent on study arm. Furthermore, levels in our participants were lower compared to NHANES participants.

For all nine urinary phthalate metabolites analyzed, we did not see any significant differences between intervention and control participants. This suggests that phthalate exposure is independent of study arm; however, for five of the metabolites, our participants had significantly higher concentrations compared to the reference NHANES population. Four of those five metabolites – MEHHP, MEOHP, MEHP, and MECPP – are all breakdown products of bis(d-ethylhexyl)phthalate, or DEHP, which is the most common member of the chemical class of phthalates and is widely used in plastic products.[70] The fifth metabolite, MBP, is commonly used in cosmetics including nail polish, and nearly half of all participants reported routine cosmetic use. These results illustrate the prevalence of plastics and their breakdown products in the environment, especially in places like Jalapa where municipal sanitation services are largely not available.

To our knowledge, this is the most comprehensive HAP and plastics exposure assessment in adolescent girls in a setting where biomass burning is prevalent. Women and children are considered most at-risk for exposures to HAP due to time spent at home and tending to the stove[1]; however, little is known about adolescents' exposures, who often help with cooking and household tasks. We observed that adolescents in this study experienced similar, oftentimes higher, exposures to $PM_{2.5}$ and BC compared to the participants of the parent HAPIN study, emphasizing the importance of characterizing exposures in adolescent girls.

CONCLUSION

This pilot study characterized exposures to HAP and plastics in adolescent girls via air monitoring of $PM_{2.5}$ and BC and urinary biomarkers analysis of PAHs, phthalates, and BPA. We observed exposure reductions to PAHs, $PM_{2.5}$, and BC in adolescents living in homes where primarily LPG stoves are used compared to biomass stoves. We observed elevated levels of urinary phthalates for five of the nine metabolites compared to participants of NHANES, but our participants had lower levels of BPA compared to NHANES. This study contributes to an area of the literature that is currently lacking, and paves the way for future work in Jalapa and other areas where biomass and domestic waste burning are prevalent.

Table 3.1. Participant characteristics and personal care product, cosmetic, and plastic use.

		Control N (%) or Median (IQR)	Intervention N (%) or Median (IQR)
PARTICIPANT CHARACTERISTICS			
Number of participants		28	26
Age in years		15 (14-16)	14 (14-15)
Currently in school: Yes		18 (64)	19 (73)
Exclusive LPG use: Yes		N/A	21 (81)
Smokes tobacco: Yes		0 (0)	0 (0)
PERSONAL CARE PRODUCT USE AND FREQUENCY			
Cosmetic use: Yes		12 (43)	14 (54)
	Foundation	1 (4)	4 (15)
	Powder	2 (7)	5 (19)
	Blush	2 (7)	2 (8)
	Eyeliner	5 (18)	4 (15)
	Eyeshadow	0 (0)	1 (4)
	Mascara	9 (32)	9 (35)
	Lipstick	8 (29)	10 (38)
	Nail polish	8 (29)	12 (46)
Perfume use: Yes		20 (71)	21 (81)
	Daily	6 (30)	5 (24)
	Occasionally	14 (70)	16 (76)
Body lotion use: Yes		23 (82)	22 (85)
	Daily	6 (26)	11 (50)
	Occasionally	17 (74)	11 (50)
Hair products: Yes		22 (79)	23 (88)
	Daily	10 (45)	6 (26)
	Occasionally	12 (55)	17 (74)
Insect repellant use: Yes		3 (11)	3 (12)
	Daily	1 (33)	0 (0)
	Occasionally	2 (66)	3 (100)
Sunscreen use: Yes		1 (4)	3 (12)
	Daily	0 (0)	0 (0)
	Occasionally	1 (100)	3 (100)
Deodorant: Yes		20 (71)	17 (65)
	Daily	10 (50)	10 (59)

Occasionally	10 (50)	7 (41)
Baby powder: Yes	2 (7)	1 (4)
Daily	0 (0)	0 (0)
Occasionally	2 (100)	1 (100)
HAP AND PAH EXPOSURE WITHIN PAST 48 H†		
Have you been near an open fire within past 48h: Yes	28 (100)	9 (35)
Burned trash in past 48h: Yes	11 (39)	2 (8)
Plastics were burned in open fire: Yes	7 (25)	2 (8)
Have you eaten burnt or charred tortillas in past 48h: Yes	16 (57)	13 (50)
Have you eaten grilled or seared food in past 48h: Yes	4 (14)	3 (12)
PLASTIC USE WITHIN PAST 48 H†		
Plastic plates to eat or reheat food: Yes	16 (57)	15 (58)
Plastic cups: Yes	16 (57)	16 (62)
Plastic utensils: Yes	4 (25)	4 (15)
Drank water packaged in plastic bag: Yes	5 (18)	2 (8)
Ate or drank something packaged in a can: Yes	2 (7)	1 (4)
Drank water, juice, or soda from plastic bottle: Yes	7 (25)	4 (15)

†Participants were asked about their exposures or behaviors during the 24-h sampling period and the previous 24h.

Table 3.2. Adolescent 24-h exposures to $PM_{2.5}$ and BC and kitchen concentrations of BC.

	Control			**Intervention**		
Pollutant (μg/m³)	**N**	**Median**	**IQR**	**N**	**Median**	**IQR**
$PM_{2.5}$	21	130.7	32.0 – 199.4	20	18.5	11.1 – 53.0
BC						
Personal	21	10.0	2.8 – 14.8	20	2.2	1.5 – 3.5
Kitchen, real-time	10	10.5	9.1 – 19.1	10	1.7	1.4 – 2.4

Figure 3.1. A field nurse administers a questionnaire to an adolescent participant in the intervention arm.

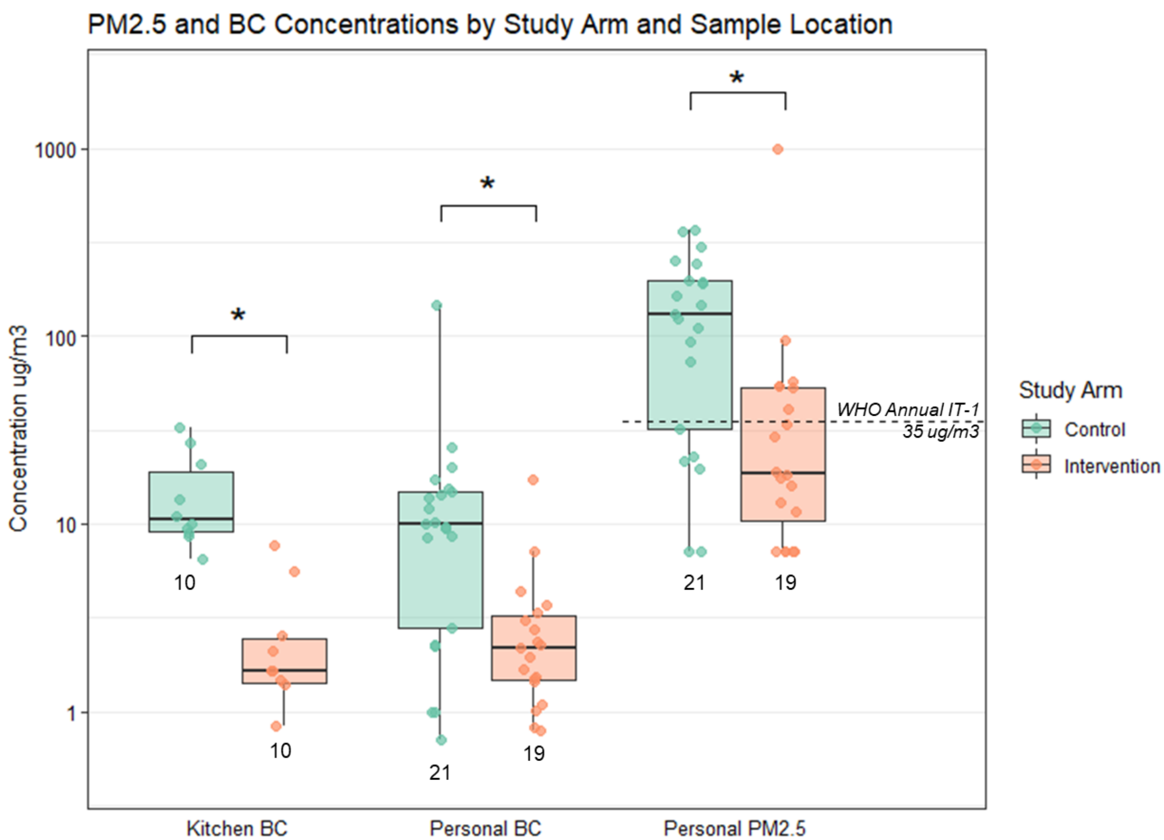

Figure 3.2. Box plots of 24-h real-time kitchen BC concentrations, and personal BC and $PM_{2.5}$ exposures in control (green) and intervention (orange) adolescent participants. The dashed line represents the WHO Annual IT-1 for $PM_{2.5}$.[38] Sample sizes are given below each box. Statistical significance is indicated at $p<0.05$.

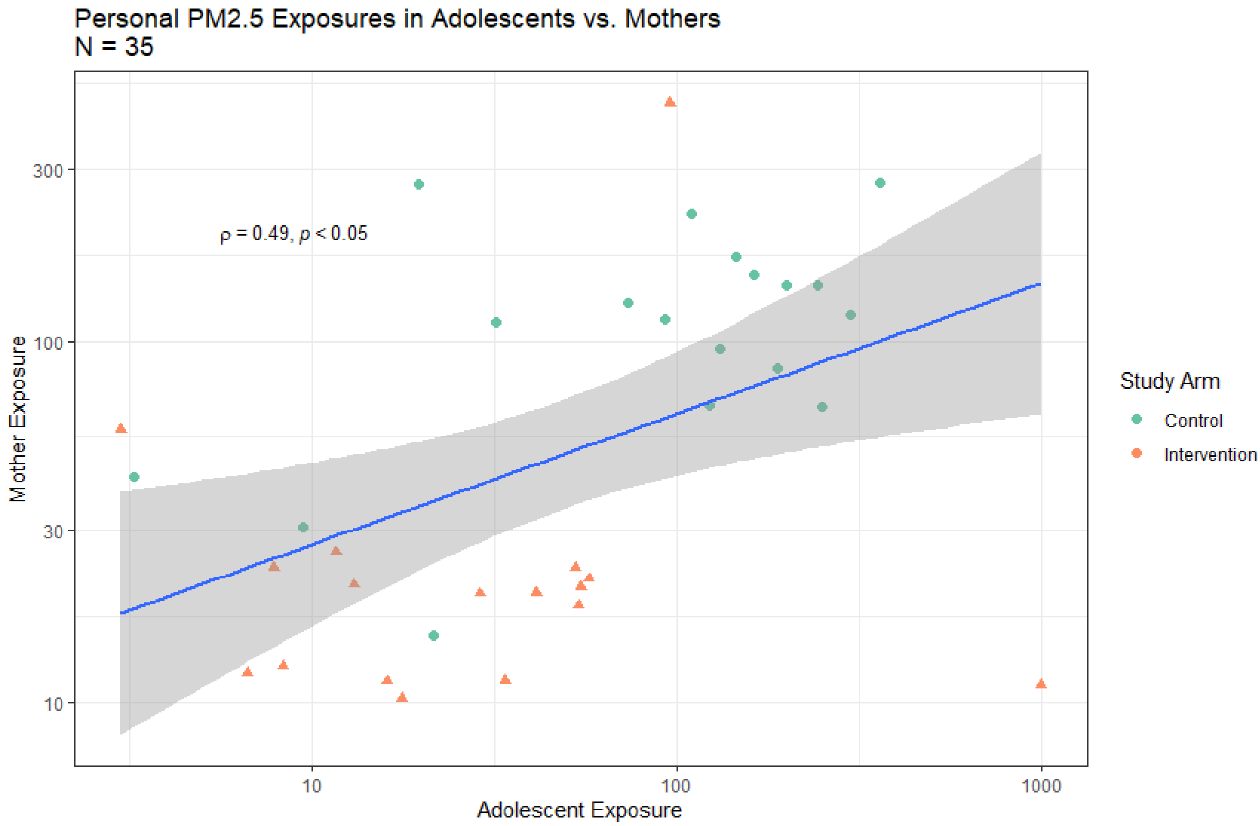

Figure 3.3. Correlation between adolescent and mother $PM_{2.5}$ exposures from the parent HAPIN trial living in the same household. Data were available for n=35 mothers.

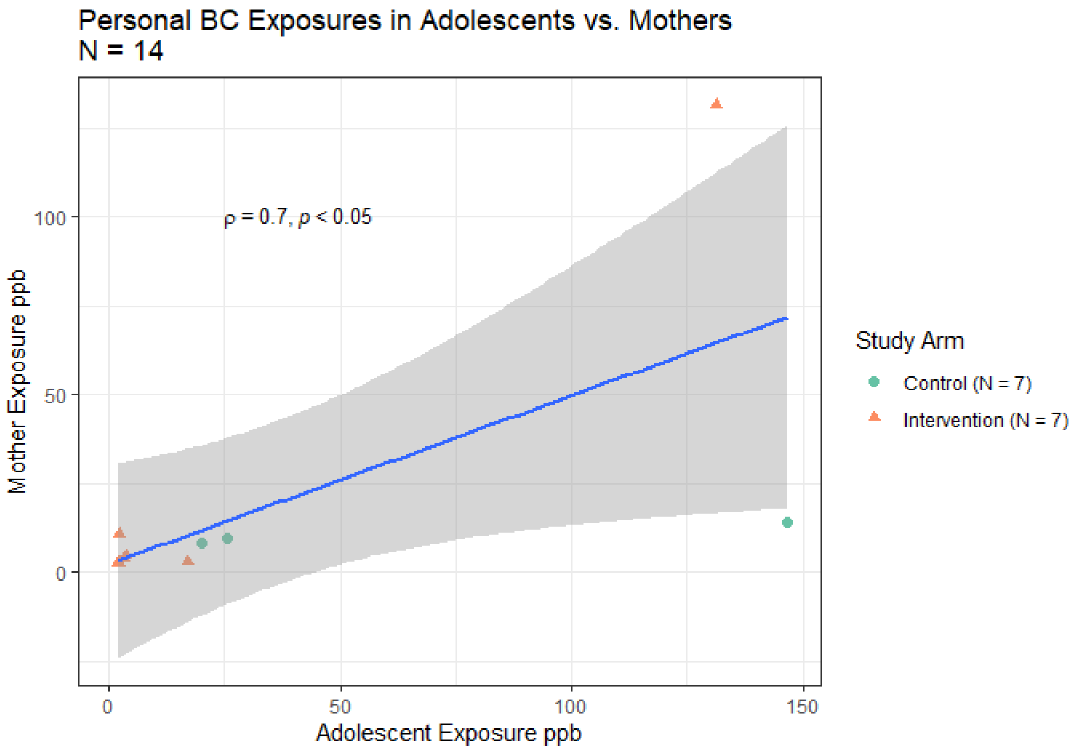

Figure 3.4. Correlation between adolescent and mother BC exposures from the parent HAPIN trial living in the same household. Data were available for n=14 mothers.

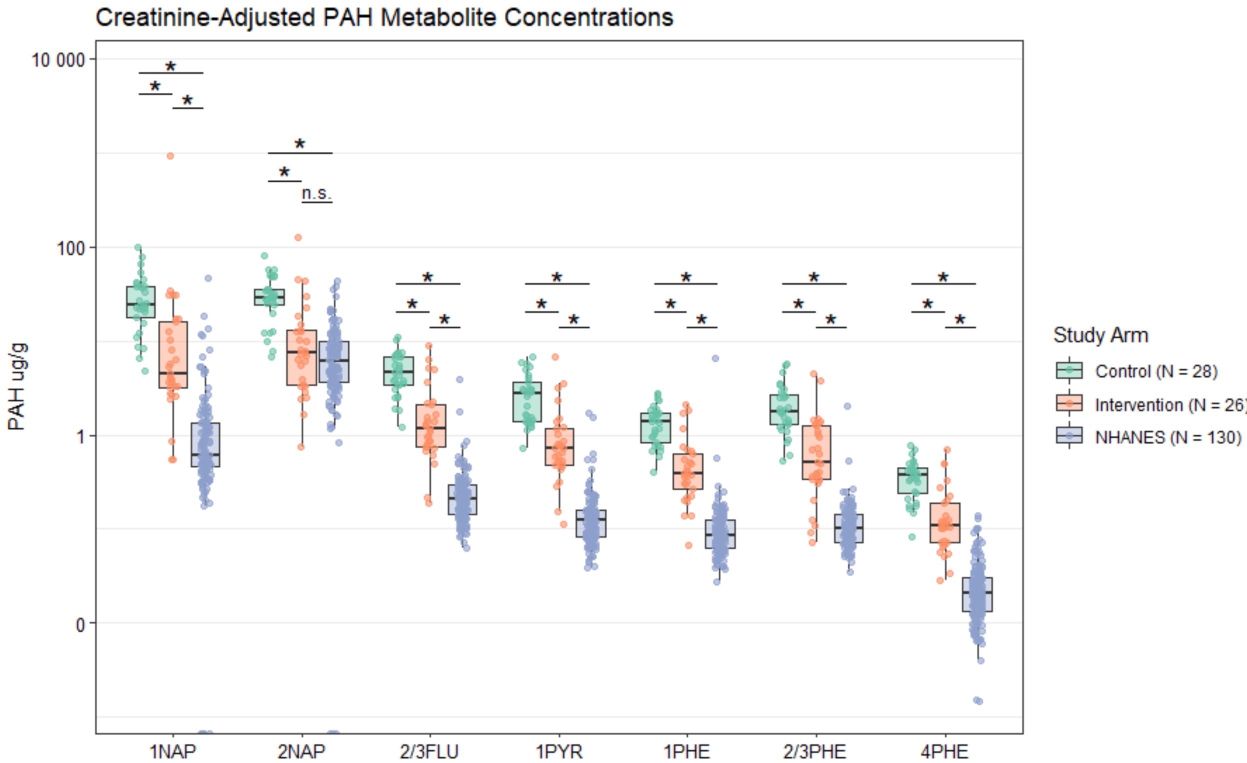

Figure 3.5. Box plot of urinary PAH metabolite concentrations in adolescent girls from the control arm (green) compared to intervention arm (orange) compared to age- and sex-matched participants from NHANES 2015-2016 (blue). Participant and NHANES concentrations are adjusted for creatinine. Statistical significance is indicated (*) at $p<0.05$. 1NAP = 1-naphthol; 2NAP = 2-naphthol; 2/3FLU = 2- and 3-hydroxyfluorene; 1PYR = 1-hydroxypyrene; 1PHE = 1-hydroxyfluorene; 2/3PHE = 2- and 3-hydroxyphenanthrene; 4PHE = 4-hydroxyphenanthrene.

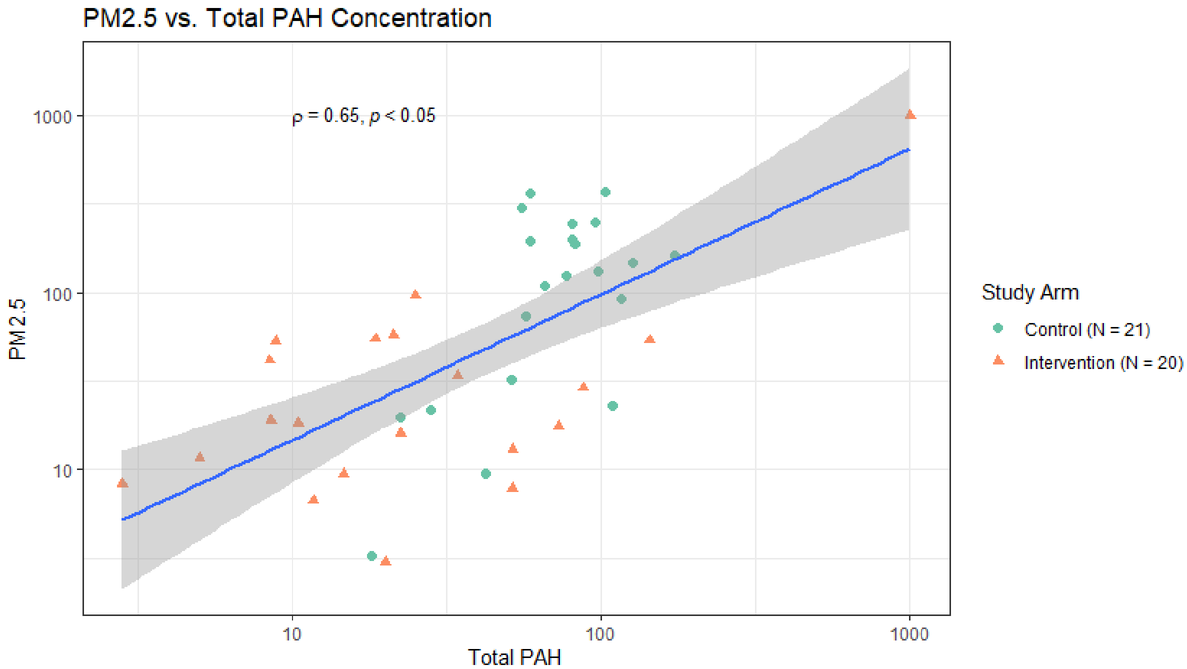

Figure 3.6. Correlation between total PAH concentrations and adolescent $PM_{2.5}$ exposures.

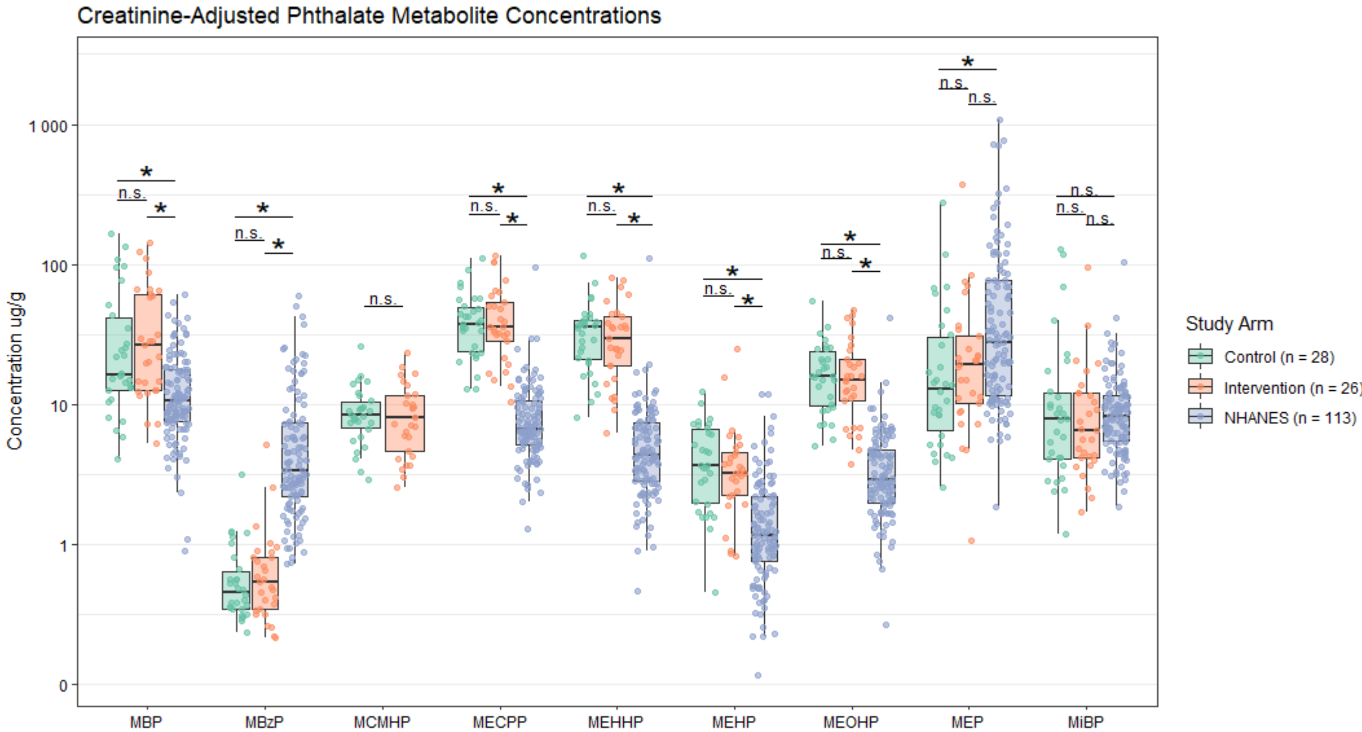

Figure 3.7. Box plot of urinary phthalate metabolite concentrations in adolescent girls from the control arm (green) compared to intervention arm (orange) compared to age- and sex-matched participants from NHANES 2017-2018 (blue). Participant and NHANES concentrations are adjusted for individual creatinine concentrations. Statistical significance is indicated (*) at $p<0.05$ and non-significance is denoted as "n.s." MBP = Mono-butyl phthalate; MBzP = Monobenzyl phthalate; MCMHP = Mono(2-carboxymethylhexyl)phthalate; MECPP = Mono(2-ethyl-5-carboxypentyl)phthalate; MEHHP = Mono-(2-ethyl-5-hydroxyhexyl)phthalate; MEHP = Mono-(2-ethylhexyl)phthalate; MEOHP = Mono-(2-ethyl-5-oxohexyl)phthalate; MEP = Mono-ethyl phthalate; MiBP = Mono-isobutyl phthalate.

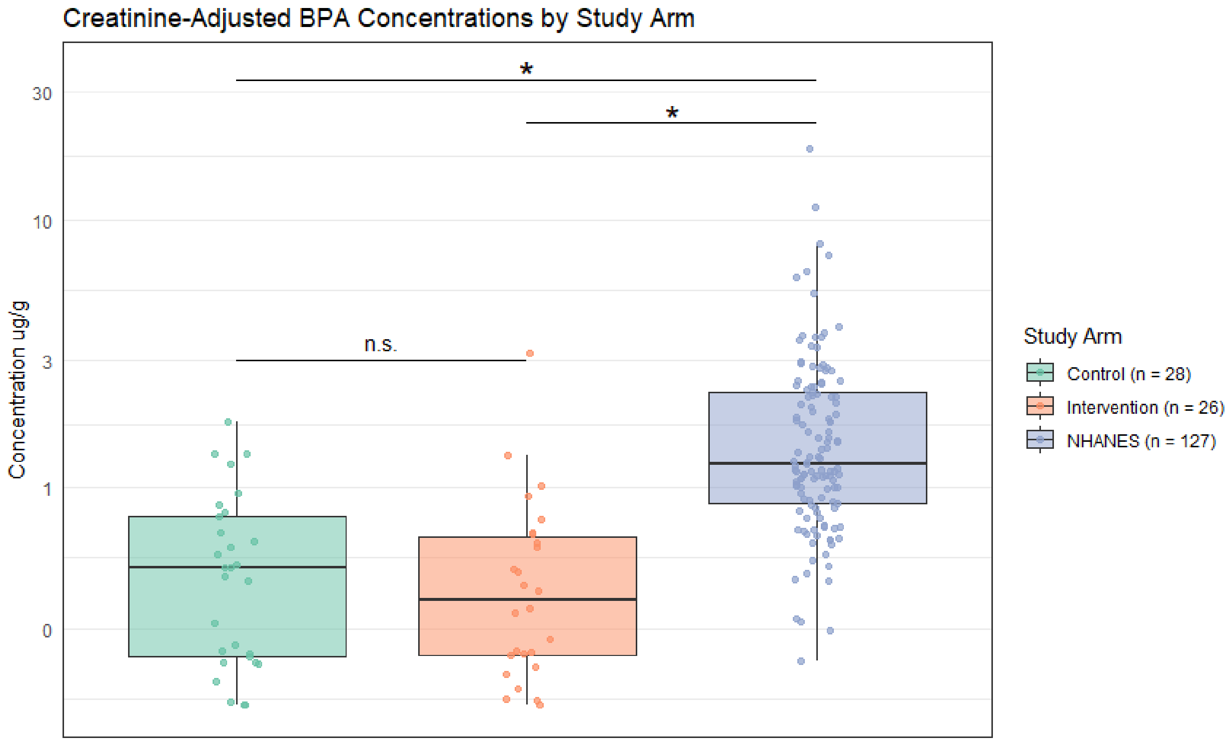

Figure 3.8. Box plot of urinary BPA concentrations in adolescent girls from the control arm (green) and intervention arm (orange) compared to age- and sex- matched participants of NHANES 2011-2012 (blue). Statistical significance is indicated (*) at p<0.05 and non-significance is denoted as “n.s.”

CHAPTER 4

$PM_{2.5}$ AND BC DATA COMPARISONS AND CAPACITY BUILDING BETWEEN GRAVIMETRIC LABORATORIES IN THREE DIFFERENT COUNTRIES FROM THE HAPIN RANDOMIZED CONTROLLED TRIAL[3]

[3] Kearns, Katherine A., Johnson, Michael, Pillarisetti, Ajay, Piedrahita, Ricardo, McCracken, John P., Diaz-Artiga, Anaite, Balakrishnan, Kalpana, Campbell, Devan, Kremer, Jacob, Mollinedo, Erick, Naeher, Luke P. To be submitted to *Journal of Exposure Science and Environmental Epidemiology*.

ABSTRACT

Background: Household air pollution (HAP) resulting from the burning of solid or biomass fuels such as wood, agricultural crop residues, and animal dung is a global health issue that impacts nearly half of the world's population. Exposure to HAP is often estimated using air sampling monitors containing small air filters that collect fine particulate matter ($PM_{2.5}$) and black carbon (BC), both of which are associated with various negative health effects. Laboratory analyses of the filters quantify the mass deposition needed for calculating the concentration of the air pollutants so they can be interpreted in the context of health-based guidelines.

Objectives: The Household Air Pollution Intervention Network (HAPIN) study is a multi-country cookstove intervention trial with filter-processing ("gravimetric") laboratories at the University of Georgia (UGA; Athens, GA, USA) and Sri Ramachandra Institute of Higher Education and Research (SRIHER; Porur, Chennai, India). As part of a capacity building effort within the HAPIN study, a gravimetric laboratory was established at Universidad del Valle de Guatemala (UVG; Guatemala City, Guatemala) and to our knowledge, it is the only gravimetric laboratory in the country. In this work, we compared gravimetric ($PM_{2.5}$) and optical transmission (BC) filter data between the three HAPIN filter laboratories using a total of 200 unused filters, 224 used filters, and 2 sets of 12 mass reference standards (MRS).

Methods: We compared data within laboratory and between laboratory primarily utilizing the Bland-Altman method and descriptive statistics.

Conclusion: We found that agreement can be achieved in laboratories in different parts of the world when there is close adherence to a standardized protocol. We also highlight the successful capacity building effort to launch the gravimetric laboratory at UVG, making it the first laboratory of its kind in the country, to the authors' knowledge.

INTRODUCTION

Household air pollution (HAP) that results from the incomplete combustion of solid ("biomass") fuels such as wood, agricultural crop residues, and animal dung impacts nearly half of the world's population.[120] Women and children in lower- and middle-income countries (LMICs) tend to receive the highest exposures to HAP due to the amount of time spent at home and around the fire.[1] There are many global efforts underway to try to mitigate this global health issue, one of which is the HAPIN study, a multi-center cookstove intervention trial with 4 study sites: India, Rwanda, Guatemala, and Peru.[37,137] The HAPIN study characterized kitchen and ambient concentrations of and personal exposures to major HAPs, namely $PM_{2.5}$ and BC (the most light-absorbing fraction of $PM_{2.5}$) due to their well-established associations with adverse health effects.[142–144]

Traditional gold standard methods for measuring personal exposures to HAP involve using personal air sampling monitors that have a small Teflon filter installed that collects particulate matter. Gravimetric analysis of these filters before and after sampling allows us to measure the net mass of $PM_{2.5}$ deposited on the filters, and optical transmission allows for the determination of the BC content. The concentrations of $PM_{2.5}$ and BC are calculated using Equations 1 and 2, respectively, which allows us to contextualize the concentrations in terms of health-based standards.

UGA has historically processed filters for three of the four HAPIN study sites – Rwanda, Guatemala, and Peru – whereas the India team has had the capacity to analyze their own filters in the gravimetric laboratory at SRIHER. As part of a capacity building effort within the HAPIN study, the Guatemala team established their own gravimetric laboratory at UVG in 2019. This work summarizes the gravimetric laboratory data comparisons between UGA and SRIHER and

UGA and UVG, as well as showcases the successful capacity building effort at UVG by showing that its data compared to the well-established gravimetric laboratory at UGA.

METHODS

Sample selection and preparation at UGA

This laboratory comparison project took place between October 2020 and February 2021. Due to international travel restrictions imposed by the COVID-19 pandemic at the time of this project, the laboratory comparisons were made between UGA and SRIHER, and UGA and UVG, as opposed to a "round robin" comparison where the same filters would circulate between all three laboratories. Three laboratory technicians at UGA prepared n=100 unused filters and n=112 used filters each for SRIHER and UVG using the basic procedure as described below and as shown graphically in Figure 4.1.

The filters used in the HAPIN study and in this project were polytetrafluoroethylene (PTFE) filters 15mm in diameter (Measurement Technology Laboratories, Minneapolis, MN). At UGA, each new/unused filter was scanned twice ("pre-scanned") for BC, once by two technicians in two distinct weigh sessions using the SootScan Model OT21 Optical Transmissometer (Magee Scientific, Berkeley, CA). Each filter was placed into its own petri dish labeled with a unique identifier.

After both technicians pre-scanned all filters with the SootScan, the filters were taken to the climate-controlled portion of the laboratory where they were laid flat on the countertop with the lid of the petri dishes propped slightly ajar to allow for air movement but not for dust settling or contamination on the filters. The filters were conditioned in the gravimetric laboratory for 48 h prior to being weighed to allow for acclimation to the controlled climate conditions. Per the

Environmental Protection Agency's Standard Operating Procedure (SOP) for Particulate Matter (PM) Gravimetric Analysis (https://www3.epa.gov/ttnamti1/files/ambient/pm25/spec/RTIGravMassSOPFINAL.pdf), the climate-controlled laboratory should be maintained at a temperature between 20-23°C with a standard deviation less than 2°C over the previous 24 h, and a relative humidity (RH) between 30-40% with a standard deviation of less than 5% over the previous 24 h. The laboratory must also be cleaned on a regular basis to avoid contamination; latex gloves, laboratory coats, and shoe covers should also be worn while in the lab, and static elimination devices are used during the weighing process to ensure accurate mass measurements.

Similar to the scanning procedure, all filters were weighed in duplicate, once by two technicians in two distinct weigh sessions on a microbalance with a sensitivity of 1 μg (Sartorius Cubis MSU, Gottingen, Germany). If the first and second weight on any filter differed by more than 5 μg, a third weight was taken and the average of the two closest weights was used. After each set of 10 filters was weighed, the temperature and RH are recorded on the data spreadsheet, and a 200 mg working standard and three laboratory blanks were weighed for continuous quality assurance. Post-sampled filters are typically processed in the reverse order as the pre-sampled filters so that conditioning and post-weighing occur before post-scanning for the reasons outlined below.

The used filters that were sent to both UVG and SRIHER were archived HAPIN filters that had been deployed in Guatemala and were already post-analyzed by the team at UGA. A total of 224 archived filters were selected in a manner that accounted for a range of mass loadings and higher attenuation (ATN) of BC. Mass-loaded filters tend to present more analytical challenges compared to unused filters due to volatilization of the particulate matter and increased static, among other factors, which can affect the stability of the weights.[145–147] An additional category was made for filters with a higher ATN of BC since the relationship between attenuated light and BC loses linearity after 125 ATN units.[138] This is depicted in Table 4.1. The UVG and SRIHER labs received approximately half of the filters from each of the mass deposition and high ATN categories to ensure that they received 112 used filters with varying mass depositions and attenuation, in addition to 100 unused filters.

Sample transport and laboratory analyses at SRIHER and UVG

Filters were prepared at UGA and sent on blue ice to travelers going from the United States to either India or Guatemala. Filters were hand-carried by the travelers and were maintained on cold transport (4°C) to ensure smoother transport conditions and better preservation of the samples. For purposes of this comparison, the SRIHER and UVG laboratories conditioned and weighed all filters (unused and used) before scanning since the scanning process is believed to be a potentially more disruptive process for the filters compared to weighing. This was a modification to the standard HAPIN filter-processing protocol in an effort to best preserve the true mass of the samples for this comparison, especially for used/post-sampled filters. The basic procedure for the laboratory comparison is presented in Figure 4.2.

Converting Depositions of $PM_{2.5}$ and BC to Concentrations

The primary air sampler used in the HAPIN study to measure personal exposures to $PM_{2.5}$ and BC is the Enhanced Children's MicroPEM (ECM, RTI International). It is calibrated with a flow rate of 0.3 L/min and the sampling period for the study is 24 h. This project aimed to compare the differences (μg) in the laboratory measurements of $PM_{2.5}$ and BC produced at UGA compared to each of the other two laboratories. Using a nominal flow rate of 0.3 L/min and a sampling time of 24 h (1440 min), we converted the mass difference of $PM_{2.5}$ between UGA and the other laboratory to units of concentration (μg/m^3), as shown in Equation 1 below.

$$[PM_{2.5}]\ \mu g/m^3 = flow\ rate\ \frac{L}{min} * \frac{sample\ duration\ (min)}{1000\ m^3} * mass\ difference, \mu g \qquad \text{(Eq-1)}$$

To convert BC mass depositions to units of concentration, we used Eq 2, where σ is a standard equal to 137,000 μg/m^2 that was derived in a process described by Garland et al. (2017).[138] The actual sampling area of the 15 mm filters used in this study is 10 mm, and *I* is the infrared (IR) output provided by the SootScan, where I_0 is the blank IR wavelength (nm) and I_F is the sample IR wavelength. The denominator of Eq 2 uses the same inputs as described for Eq 1.

$$[\mathrm{BC}]\ \mu g/m^3 = \frac{\sigma\left(\frac{\mu g}{m^2}\right) * filter\ area\ (m^2) * \log(I_0/I_F)}{flow\ rate\left(\frac{L}{min}\right) * sample\ duration\ (min) * 0.001\ \left(\frac{m^3}{L}\right)} \qquad \text{(Eq-2)}$$

RESULTS

Gravimetric and Optical Transmission

For each of the points of comparison (gravimetric, optical transmission, and MRS), the subtraction order for calculating the differences between laboratories was always SRIHER or UVG minus UGA. This was done to logically observe what happened to the filter mass or optical transmission by the time it reached the second laboratory for comparison (e.g., if the mass difference is negative between laboratories, that indicates that the filter lost mass after being weighed at UGA and before being weighed at the next laboratory).

Bland-Altman analyses are used to assess the agreement between two methods. In this case, the two methods are measurements taken at UGA, and measurements taken at the other laboratory (either SRIHER or UVG). The bias is the average difference between the measurements taken at UGA and the other laboratory, and the limits of agreement are calculated as the average difference ± 1.96 standard deviation of the difference, which indicate how much random variation may be influencing the ratings.[148]

Between SRIHER and UGA, the bias in $PM_{2.5}$ concentration for the pre-sampled and post-sampled filters was [Mean (SD)]: -10.2 (2.1) $\mu g/m^3$ and -20.8 (6.9) $\mu g/m^3$ (6.9), respectively (Figures 4.3a and 4.3b). The bias in BC concentration between SRIHER and UGA for the pre-sampled and post-sampled filters was [Mean (SD)]: 0.59 (0.23) $\mu g/m^3$ and 0.06 (0.46) $\mu g/m^3$, respectively (Figures 4.3c and 4.3d).

Between UVG and UGA, the bias in $PM_{2.5}$ for the pre-sampled and post-sampled filters was [Mean (SD)]: 0.95 (2.08) $\mu g/m^3$ and -4.60 (4.64) $\mu g/m^3$, respectively (Figures 4.4a and 4.4b). The bias difference in BC concentration for the pre-sampled and post-sampled filters was [Mean (SD)]: -0.15 (0.09) $\mu g/m^3$ and -0.16 (0.33) $\mu g/m^3$, respectively (Figures 4.4c and 4.4d).

<u>Mass Reference Standards</u>

In addition to the 224 filters sent to SRIHER and UVG, UGA weighed and sent a set of 12 stainless steel mass reference standards ranging from 1-500 mg in mass (MRS; Mettler Toledo, Columbus, OH, USA) to the other two laboratories. The two gravimetric technicians at UGA weighed each set of MRS twice before sending to the other laboratories.

Between SRIHER and UGA, the bias for all of the weights was [Mean (SD)]: -6 (7) μg (Figure 4.5) and between UVG and UGA it was 1 (2) μg (Figure 4.6). In general, the biases increased as each MRS increased in mass.

DISCUSSION

For this project, we aimed to show that regardless of which gravimetric laboratory filters are processed in, as long as they adhere to EPA's specifications and a standard operating procedure is closely followed, the data between the laboratories show relatively low bias differences. Many factors could have affected the mass and therefore the comparability of data between laboratories including transport and filter handling among various technicians, which can potentially dislodge particles from the filter surface. Static impacts the stability of the filter itself and causes inaccurate measurements, and RH, temperature, transport, and ambient climate or weather conditions can also impact the weight of the filter.[145–147]

Despite all of these factors, measures were taken to minimize sample loss and to ensure measurement accuracy and comparability, not only between-laboratory but within-laboratory as well. Aside from maintaining the laboratory within EPA's specifications for RH and temperature, laboratory technicians all underwent rigorous training and endured multiple practice sessions to ensure proper filter handling technique, protocol adherence, microbalance training, and data entry under the supervision of a more senior technician. Laboratory blanks and standards were used in each training session to ensure long-term stability and performance of the microbalance and of the laboratory, and static eliminators were used during the weighing process to ensure stability in repeat measurements. Although all HAPIN gravimetric laboratories follow the same standardized protocol, slight modifications exist to account for local conditions, individual preferences, and/or availability of resources including personnel.

The HAPIN laboratories have variation in availability of technicians or personnel. While the UGA laboratory has two weighing technicians and two different scanning technicians for a total of four technicians, the SRIHER and UVG laboratories each have one primary technician to fill both roles of filter weighing and scanning. As such, the daily output varies by laboratory with UGA completing the pre-weights, post-weights, pre-scans, and post-scans on one day each; meanwhile, the SRIHER and UVG laboratories completed the measurements across various weigh sessions and days. This may have caused day-to-day variation in measurements and laboratory conditions.

All gravimetric laboratories displayed satisfactory temperature and RH throughout the duration of this project in reference to EPA guidelines. The average temperature (°C) at UGA, SRIHER, and UVG was [Mean (SD)]: 20.0 (0.0), 21.8 (0.8), and 22.1 (0.5), respectively. The average RH (%) at UGA, SRIHER, and UVG was [Mean (SD)]: 39.7 (1.4), 41.7 (2.6), and 40.7 (1.6), respectively.

The EPA has four gravimetric laboratories that routinely participate in a round robin, interlaboratory comparison study for mass-loaded filters. Although no acceptance criteria has been established for this type of interlaboratory comparison study, the EPA's $PM_{2.5}$ Performance Evaluation Program (PEP) has set standards for pre- and post-mass differences for laboratory and field blanks and mass reference standards at 15, 30, and 3 µg, respectively.[149] Comparing these standards to our results, the largest bias differences occur between SRIHER and UGA at -10.2 µg/m^3 and -20.8 (6.9) µg/m^3 for the pre- and post-sampled filters, respectively. Converting these numbers to the same units as the PEP standards (µg), the bias differences were |4.4| µg and

|9.0| μg for pre- and post-sampled filters for SRIHER-UGA, respectively, which are both well below the PEP standards for both laboratory and field blanks.

Another important achievement that resulted from this project was the successful capacity building effort at UVG. Due to the relatively low bias difference observed between UVG and UGA, which is an established gravimetric laboratory that has been used in similar studies for the past couple of decades, HAPIN leadership approved of the future use of the UVG laboratory for measurements in the HAPIN study. The gravimetric laboratory at UVG, which is the first of its kind in the country to the authors' knowledge, can now be used in later phases of the HAPIN study and beyond. Since travel restrictions previously imposed by COVID-19 have lifted, there have been opportunities for continued capacity building between UGA and UVG, where technicians from each institution have been able to visit the other. Similar capacity building efforts between UGA and SRIHER are expected to continue in 2022.

A clear limitation of this study was the inability to complete a round robin comparison, where the same filters would be processed at all three of the laboratories. This is a future study of interest and would allow further investigation of the extent to which transport and other factors may impact filter mass. Replicates of this study where measurements occur across several days would also inform more on day-to-day variability observed within and potentially between laboratories.

CONCLUSION

Through this relatively simple yet thoughtful study design, we observed relatively low bias differences between each of the HAPIN filter laboratories that seem to satisfy the PEP standards, which are the only standards that exist for a study of this kind, to the authors' knowledge. Each of the laboratories follows similar protocols, though minor variations exist between labs. In spite of these variations in protocol and in the challenges in comparing mass-loaded filters, we showed that it is possible to transport air sampling filters – with and without particulate mass deposition – across the world and still observe relatively low bias differences between laboratories.

Table 4.1. Range of mass depositions and high attenuation (ATN > 125) of post-sampled HAPIN filters used for the laboratory comparisons.

$PM_{2.5}$ mass depositions, µg	**$PM_{2.5}$ mass concentrations, µg/m^3**	**% Avg filter mass**	**N (% of total)**
20-40	46-92		76 (34)
41-60	93-140		48 (21)
61-80	141-185		29 (13)
81-100	186-232		19 (8)
>100	233-793		29 (13)
High BC ATN (>125 units)	111-250		23 (10)

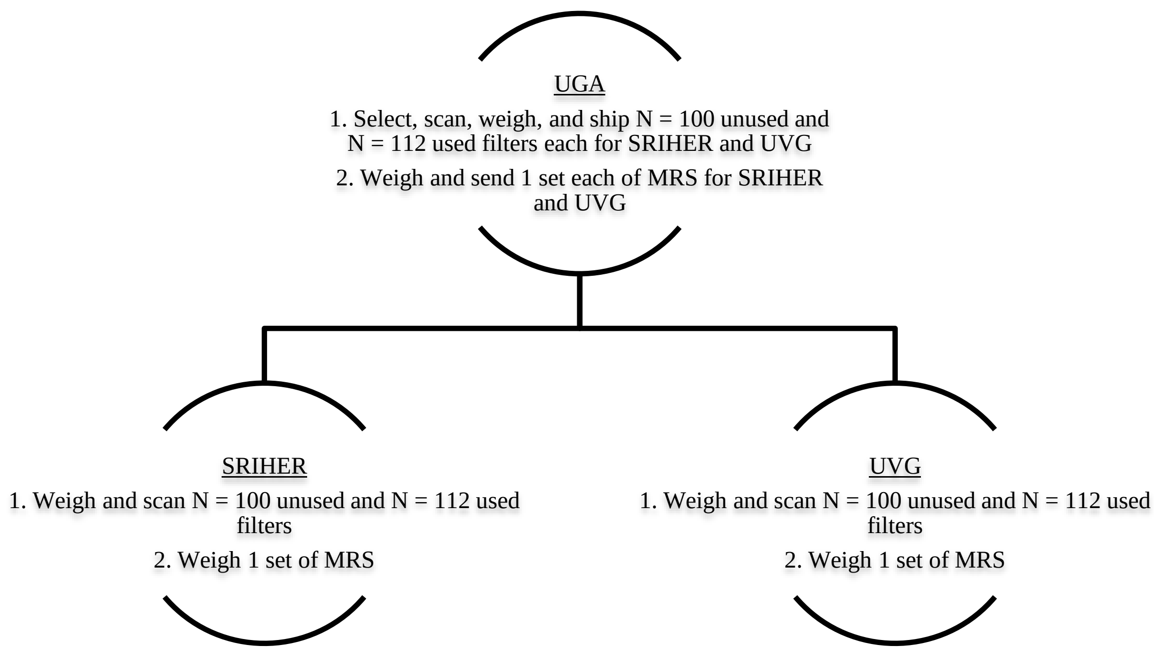

Figure 4.1. Study design and flow of filters and MRS from UGA to SRIHER and UVG.

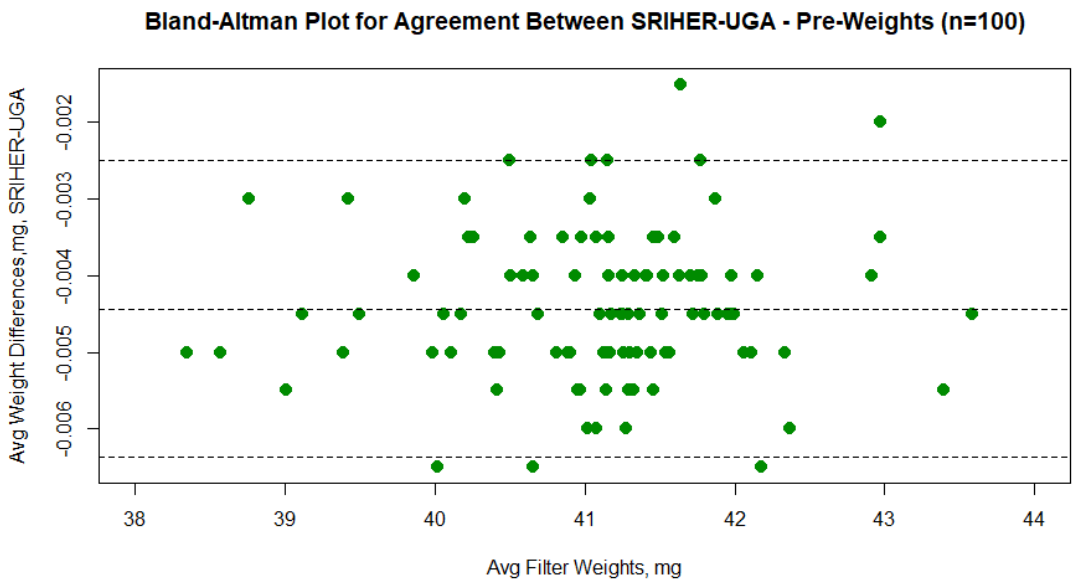

Figure 4.2a. Agreement between UGA and SRIHER average pre-weights (n=100 filters). "Bias difference" is depicted as the middle dashed line; upper and lower limits of agreement are depicted as the top and bottom dashed lines, respectively. Bias difference is shown at -4.4 µg, which converts to a nominal concentration of -10.2 µg/m^3.

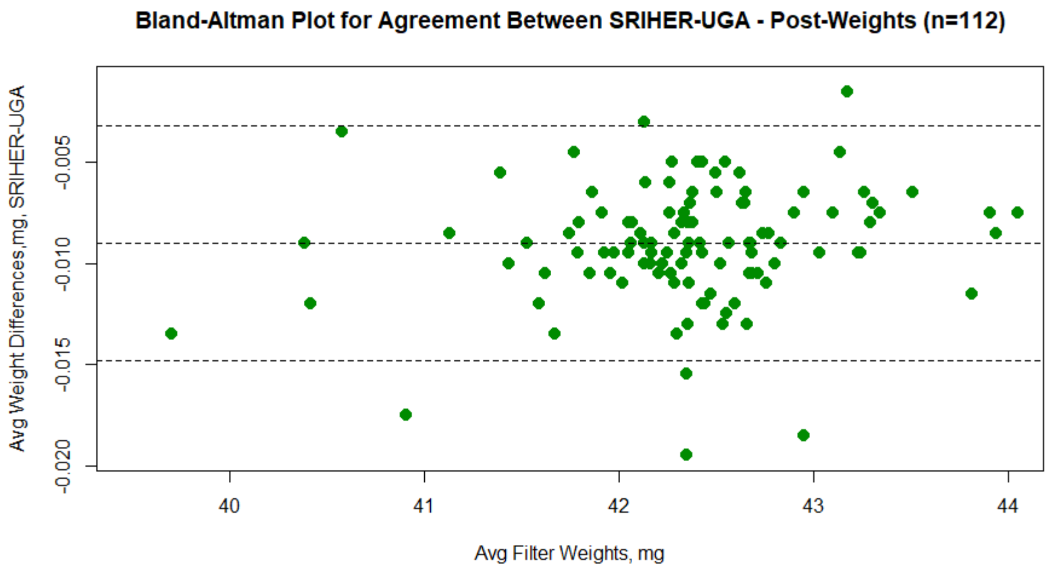

Figure 4.2b. Agreement between UGA average post-weights and SRIHER average post-weights (n=112 filters). Bias difference" is depicted as the middle dashed line; upper and lower limits of agreement are depicted as the top and bottom dashed lines (respectively). Bias difference is shown at -9 µg, which converts to a nominal concentration of -20.8 µg/m^3.

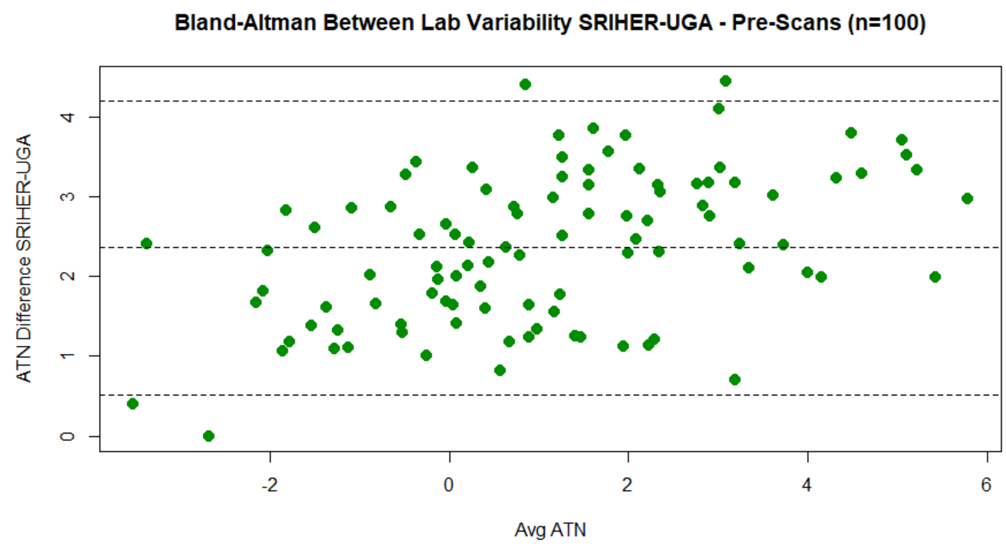

Figure 4.2c. Agreement between UGA and SRIHER average pre-scans for BC (n=100 filters). Bias difference is shown as 2.4 ATN, which converts to a nominal concentration of 0.6 µg/m^3.

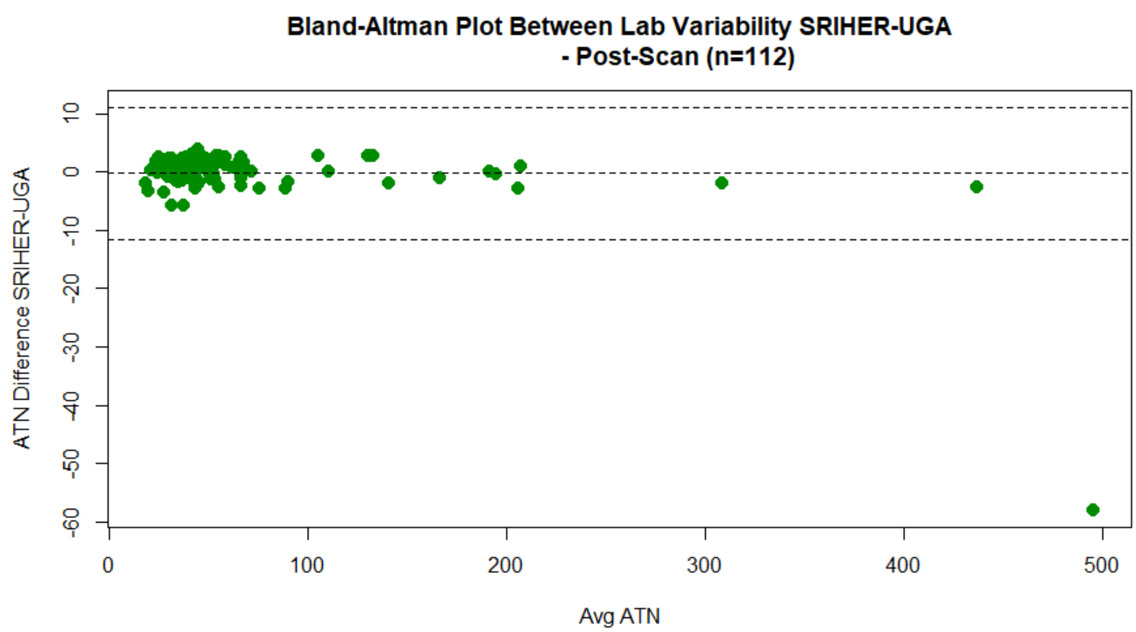

Figure 4.2d. Agreement between UGA and SRIHER average post-scans for BC (N=112 filters). Bias difference is shown -0.3 ATN, which converts to a nominal concentration of 0.07 µg/m^3.

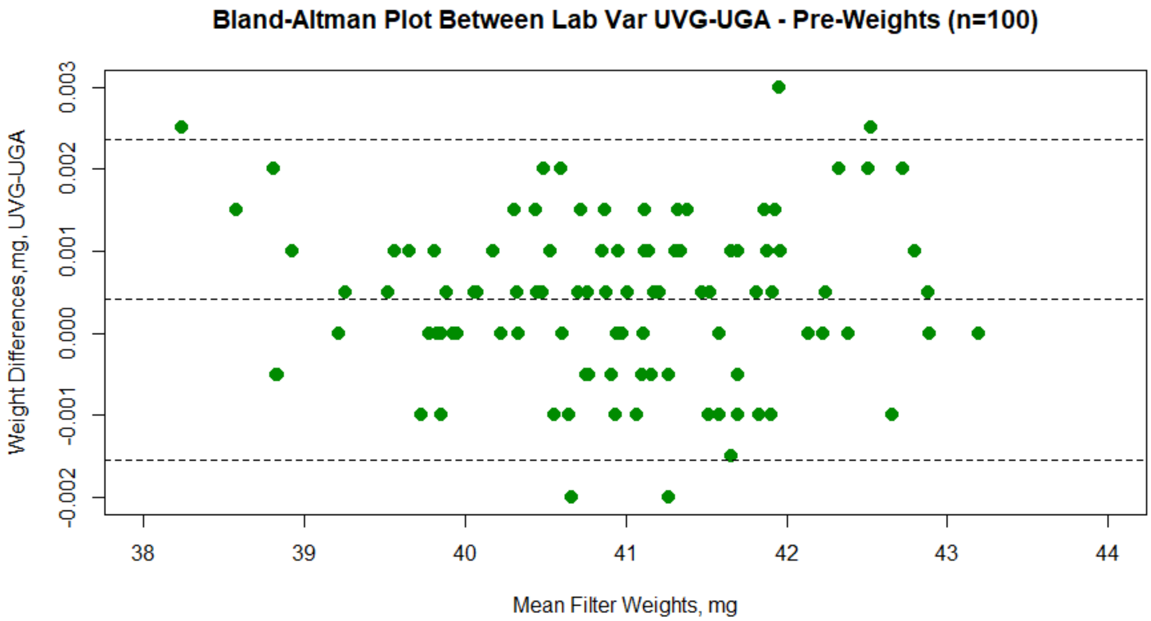

Figure 4.3a. Agreement between average UGA and UVG pre-weights (N=100 filters). Bias difference is shown as 0.0004 mg (0.4 µg), which converts to a nominal concentration of approximately 1 µg/m^3.

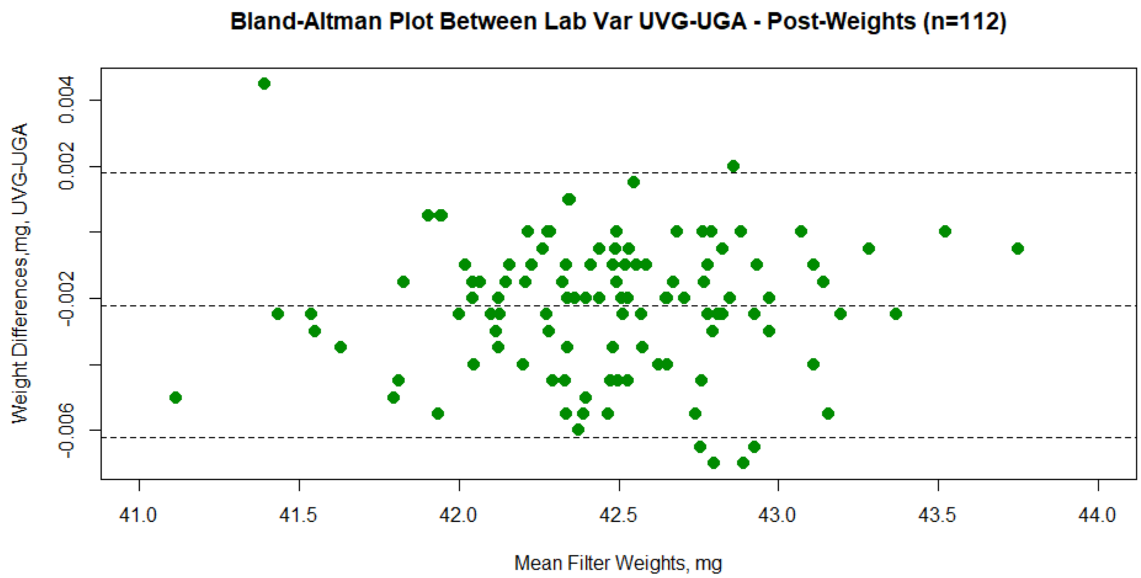

Figure 4.3b. Agreement between average UGA and UVG post-weights (N=112 filters). Bias difference is shown as approximately -2.0 µg, which converts to a nominal concentration of -4.6 µg/m^3.

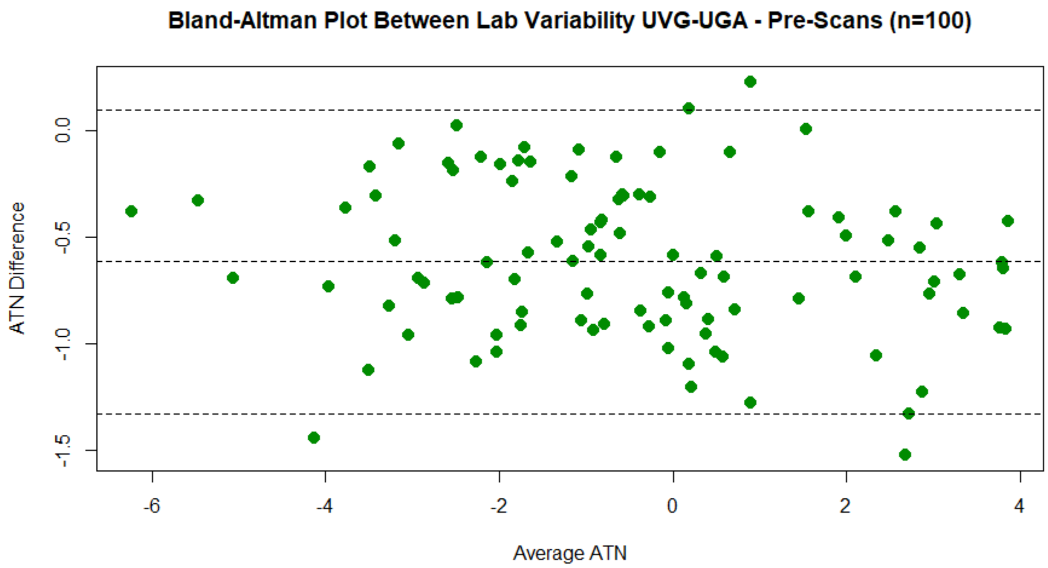

Figure 4.3c. Agreement between average UGA and UVG pre-scans (N=100 filters). Bias difference is shown as approximately -0.6 µg, which converts to a nominal concentration of -0.2 µg/m^3.

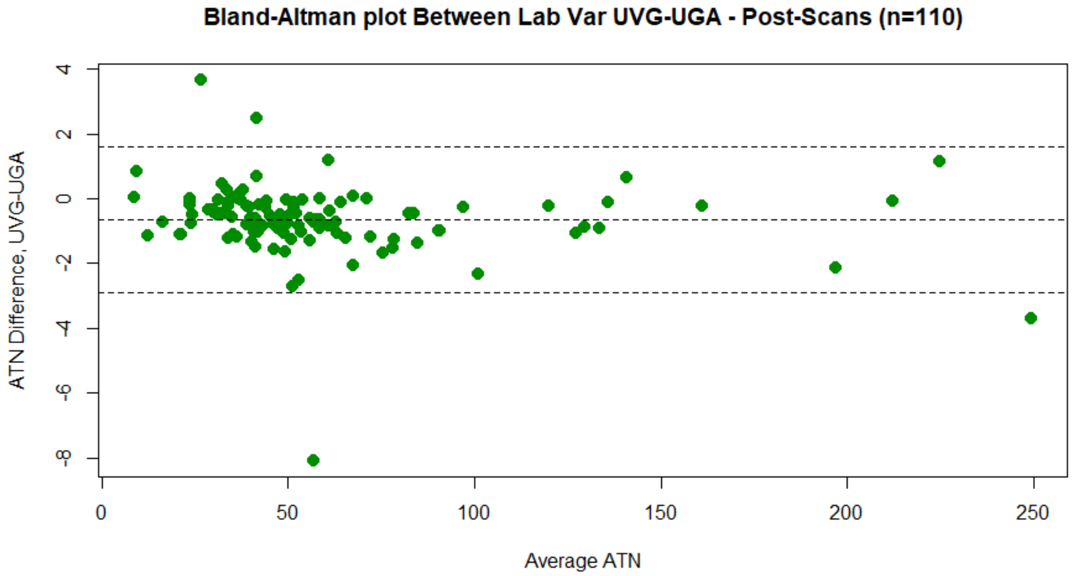

Figure 4.3d. Agreement between average UGA and UVG post-scans (N=112 filters). Bias difference is shown as approximately -0.7 µg, which converts to a nominal concentration of -0.2 µg/m^3.

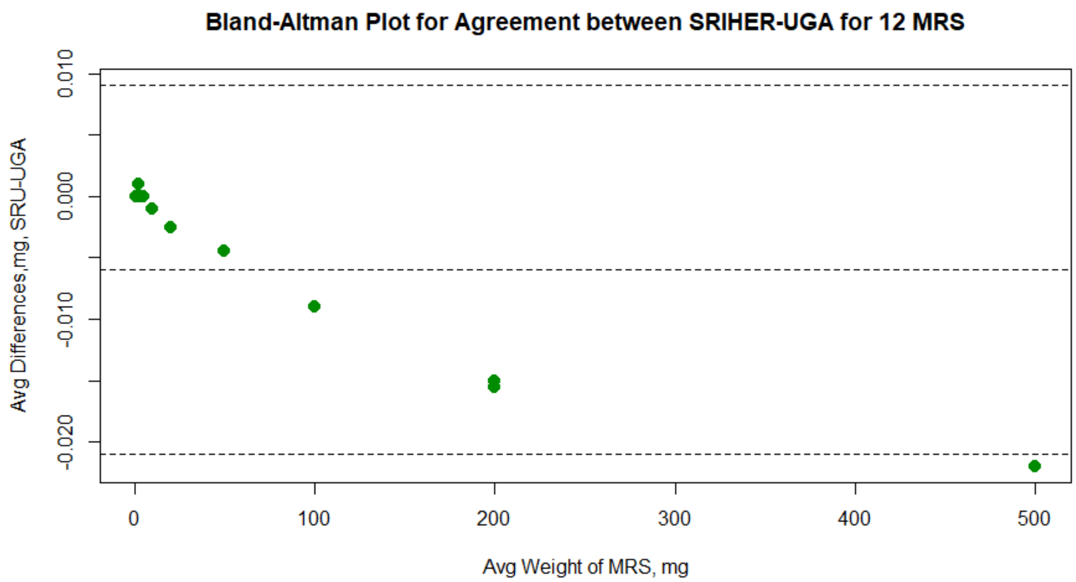

Figure 4.4. Agreement between SRIHER and UGA MRS. The MRS were weighed twice by one technician at SRIHER in a single weigh session and once each by two technicians at UGA in two distinct weigh sessions. N=12 weights ranging from 1-500 mg. Bias difference is shown at -6 µg.

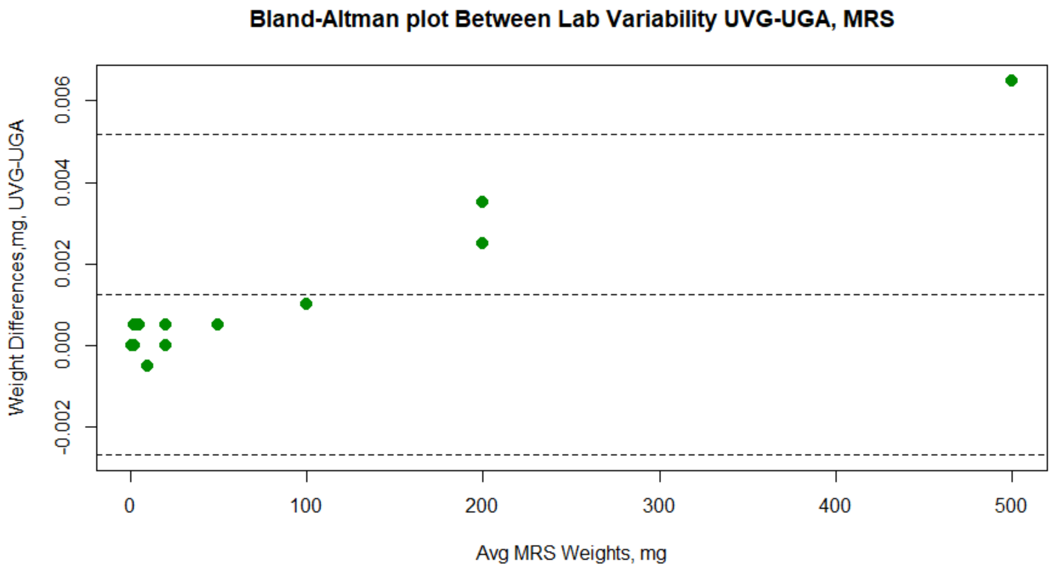

Figure 4.5. Agreement between UVG and UGA MRS. The MRS were weighed twice by one technician at UVG in a single weigh session and once each by two technicians at UGA in two distinct weigh sessions. N=12 weights ranging from 1-500 mg. Bias difference is shown at 1 µg.

CHAPTER 5

INVESTIGATING POTENTIAL SOURCES OF $PM_{2.5}$ IN GUATEMALA AND RWANDA VIA X-RAY FLUORESCENCE AND SOURCE APPORTIONMENT[4]

---

[4]Kearns, Katherine A., Johnson, Michael, Piedrahita, Ricardo, Pillarisetti, Ajay, Waller, Lance M., Chang, Howard, Sarnat, Jeremy, L'Orange, Christian, Clark, Maggie, Russell, Ted, Rosa, Ghislaine, McCracken, John P., Diaz-Artiga, Anaité, Thompson, Lisa M., Clasen, Thomas F., Peel, Jennifer L., Checkley, William, Naeher, Luke P., and HAPIN investigators. To be submitted to *Environmental Science and Technology*.

ABSTRACT

Background: The incomplete combustion of biomass fuels such as wood, dung, and agricultural crop residues results in household air pollution (HAP) and creates exposures to harmful air pollutants such as fine particulate matter ($PM_{2.5}$) and black carbon (BC). The composition of $PM_{2.5}$ is heterogeneous and varies depending on factors such as emission source, season, geography. Characterizing sources of $PM_{2.5}$ is important for exposure assessment and for investigating differential toxicities upon exposure, but source apportionment studies in low-resource settings is currently limited.

Objectives: We sought to characterize potential sources of $PM_{2.5}$ in Guatemala and Rwanda, two study sites of the Household Air Pollution Intervention Network (HAPIN) trial.

Methods: Archived personal exposure filter samples from the HAPIN trial were selected for Guatemala (n = 64) and Rwanda (n = 59). Filters were previously analyzed for $PM_{2.5}$ and BC. Filters were subsequently analyzed for chemical composition via x-ray fluorescence (XRF) and then underwent source apportionment using the Environmental Protection Agency's Positive Matrix Factorization (EPA PMF 5.0).

Results: We analyzed for 22 chemical species and 12 were found in detectable levels in our samples. We resolved four potential sources of $PM_{2.5}$ in Guatemala including biomass smoke, a crustal source, an agricultural source, and gas emissions. We identified three potential sources of $PM_{2.5}$ in Rwanda including biomass smoke, a crustal source, and an agricultural source.

Discussion: This pilot study was a hypothesis-driven study to explore potential $PM_{2.5}$ source contributions in two LMICs, an area that is currently understudied. These findings paved the way for a larger source apportionment study within the HAPIN trial to continue exploring sources of $PM_{2.5}$ in these settings.

INTRODUCTION

Primary reliance on solid fuels for cooking and heating homes is a widespread issue, particularly in low- and middle-income countries (LMICs).[14] Solid fuels, or "biomass" fuels such as wood, animal dung, and agricultural crop residues are burned in inefficient cookstoves and often in poorly ventilated areas, resulting in considerable concentrations of and exposures to household air pollution (HAP).[109,150,151] Major pollutants of interest include fine particulate matter ($PM_{2.5}$; particulate matter with aerodynamic diameter ≤ 2 micrometers) and black carbon (BC), the most light-absorbing fraction of $PM_{2.5}$.[137] Populations that are most impacted by this form of HAP are women and children, as they are the ones that typically spend the most time at home and around the fire.[1]

Exposure to HAP was attributed to 2.31 million deaths in 2019[2] and is associated with many adverse health effects including chronic obstructive pulmonary disorder (COPD), childhood pneumonia, adverse birth outcomes, and lung cancer.[21–23] While $PM_{2.5}$ is a major pollutant of interest especially in these resource-poor HAP settings, there is not one single source of $PM_{2.5}$.[151]

The toxicity of $PM_{2.5}$ at the cellular and gene level is highly variable due to its heterogeneity in composition and due to different emissions sources.[152] $PM_{2.5}$ is a heterogeneous mixture of nitrates, sulfates, BC, organic chemicals, metals, and soil (crustal) material.[28] Inhalation exposure to $PM_{2.5}$ triggers the overproduction of reactive oxygen species (ROS), which can cause oxidative stress, inflammation, and DNA and cellular damage.[29] BC, in addition to being identified as a climate forcing agent, also has shown toxicity upon exposure likely due to its small dimension and adsorptive properties with other air pollutants.[31] Both $PM_{2.5}$ and BC are emitted from the incomplete combustion of biomass and fossil fuels[32] and have been associated with cardiovascular effects.[28]

$PM_{2.5}$ receives the most attention in HAP settings due to its well-documented health effects associated with exposure.[26,27] Average $PM_{2.5}$ levels in households using biomass fuels have also been found to be orders of magnitude higher than levels in most urban air pollution studies.[31,153] BC is gaining increased attention due to potential co-benefits of improving health effects and combating climate change.[30,31]

Source apportionment (SA) is a tool that helps us to reconstruct the impact of emissions from different sources of atmospheric pollutants including $PM_{2.5}$. A few different SA techniques are available, with one of the more common ones being receptor-based models.[104] There are many different types of receptor models that are used, but one of the most common ones is positive matrix factorization (PMF), a multivariate model developed by EPA scientists.

The overall goal of PMF is to resolve the identities and contributions of components in an unknown mixture by incorporating the variable uncertainties associated with each $PM_{2.5}$ measurement.[107] PMF does not require known source profiles as model inputs but does require knowledge of source profiles to be able to determine the relationship between factors derived from the model with air pollution sources, which are usually obtained from the literature.[106]

The PMF model as it is used for analyzing $PM_{2.5}$ sample data expresses observations of PM species as the sum of contributions from a number of time-invariant source profiles:

$$x_{ij} = \sum_{k=1}^{p} g_{ik} f_{kj} + e_{ij},$$

where x_{ij} is the concentration of species *j* measured on sample *i*, *p* is the number of factors contributing to the samples, f_{kj} is the concentration of the species *j* in factor profile *k*, g_{ik} is the relative contribution of factor k to sample i, and e_{ij} is the error of the model for the species j measured on sample i.

PMF is a free software provided by the EPA in which the user provides the sample concentration data along with the derived uncertainty values for each concentration. Sample concentration data can be determined a number of ways, including gravimetric analysis to determine $PM_{2.5}$ concentrations and x-ray fluorescence (XRF) to determine elemental concentration. Uncertainty values are derived by considering the various sources of variability that may exist in measurements such instrument or technician variability, flow rate of the sampling monitor, and effective area of the sample media. If uncertainty values are not provided as output from laboratory instrumentation, they can be derived using the law of propagation of uncertainty.[108]

There are many cookstove intervention studies in LMICs that measure personal exposures to and concentrations of $PM_{2.5}$, but few studies have investigated the chemical compositions of $PM_{2.5}$, which is imperative for better understanding health effects upon exposure.[109,154,155] Furthermore, source apportionment offers policymakers important tools for quantifying sources of air pollution and can better inform effective policy measures to reduce air pollution in accordance with health-based standards.[155]

One study by Sharma et al. (2016) was carried out in Delhi, India between 2013 and 2014. The annual average $PM_{2.5}$ concentration at an urban site in Delhi was found to be [Mean (SD)]: 122 (94.1) µg/m^3 and seven major $PM_{2.5}$ sources were resolved via PMF including secondary aerosols, soil dust, vehicle emissions, biomass burning, fossil fuel combustion, industrial emissions, and sea salt.

Secondary aerosols (Source 1) were attributed to elevated levels of NO_3^-, SO_4^{2-}, and NH^{4+}. Soil dust (Source 2) was attributed to elevated crustal elements including Al, Si, Ca, Ti, Fe, Pb, Cu, Cr, Ni, Co and Mg. Vehicle exhaust (Source 3) was identified due to elevated levels of Cu, Zn, Mn, Pb, and elemental carbon (EC). Biomass burning (Source 4) was identified by elevated levels of K and S. Fossil fuel combustion (Source 5) was identified by elevated levels of Al, Cl, Fe, Zn, Cr, and SO_4^{2-}. Industrial emissions were identified by elevated levels of Zn, Cu, Mn, Si, Ni, Cd, Fe, Mo, and Cr. Finally, sea salt was attributed to higher concentrations of Na, K, and Cl.

Another study by Zhou et al. (2014) took place in three sites in rural, peri-urban, and urban West Africa. They analyzed the chemical composition of $PM_{2.5}$ samples (n=283) from the household cooking areas of multiple neighborhoods. Filter samples were analyzed for $PM_{2.5}$ and BC via gravimetric analysis and reflectometry, respectively, and chemical composition was determined via energy-dispersive x-ray fluorescence (ED-XRF). They measured 17 elements including sodium (Na), magnesium (Mg), aluminum (Al), silicon (Si), sulfur (S), chlorine (Cl), potassium (K), calcium (Ca), titanium (Ti), vanadium (V), chromium (Cr), nickel (Ni), manganese (Mn), iron (Fe), zinc (Zn), bromine (Br), and lead (Pb).

Authors resolved six sources attributed to $PM_{2.5}$ including sea salt (Na, Cl, S), crustal sources (Al, Si, Mg, Ti, Mn, and Fe), biomass smoke (K, Cl, S, and BC), road dust and traffic emissions (Al, Si, Ca, Fe, Zn, and BC), and solid waste burning (Br). They observed some heterogeneity between study sites. This study highlights the continued need to characterize $PM_{2.5}$ source contributions, particularly in LMICs, to determine potential impacts on human health and differential toxicities.

Ofosu et al. (2013) conducted a study in Navrongo, Ghana, an area where biomass burning and bush burning is common.[156] $PM_{2.5}$ and BC concentrations were determined, and ED-XRF was used to determine chemical composition of samples. Source apportionment was conducted via PMF, and six major sources were resolved including two stroke engines, gasoline emissions, soil dust, diesel emissions, biomass burning, and resuspended soil dust. Biomass combustion was identified as the second most important source in this area.

Lai et al. (2019) measured the chemical composition and conducted source apportionment of ambient, household, and personal exposures to $PM_{2.5}$ in rural China where biomass stove use is common.[110] They analyzed 40 personal exposure, 40 household, and 36 ambient $PM_{2.5}$ samples collected for 48 h across the non-heating and heating seasons. Chemical speciation was measured via inductively-coupled plasma mass spectrometry (ICP-MS) and SA was conducted via Chemical Mass Balance (CMB), another type of receptor model similar to PMF. Biomass burning was found to be the largest contributor to household concentrations of and personal exposures to $PM_{2.5}$.

Overall, household concentrations of total $PM_{2.5}$ were highest compared to personal and ambient samples, but both household and personal concentrations more than doubled in winter compared to summer. Furthermore, the winter household concentrations and personal exposures more than doubled in winter compared to summer (average household: 275 ± 118 μg/m^3 in winter compared to 106 ± 53.4 μg/m^3 in summer; average personal: 202 ± 99.1 μg/m^3 in winter compared to 88.5 ± 54.8 μg/m^3 in summer), with all averages being many times in exceedance of the WHO annual IT-1 of 35 μg/m^3. This study is among the first to publish SA outdoor, indoor, and personal $PM_{2.5}$ concentrations in a rural setting where biomass burning is prevalent.

The studies highlighted here represent a few of the SA studies conducted in LMICs, particularly in rural areas where there is a high reliance on polluting fuels and technologies for cooking and heating. Higher representation of studies are needed in other LMICs such as Latin America and South Asia, especially given the potentially drastic differences between $PM_{2.5}$ compositions by factors including geography and fuel source.

Our pilot study takes place within the larger Household Air Pollution Intervention Network (HAPIN) trial, a study that assessed personal exposure in pregnant women and kitchen and ambient concentrations of HAP in 3200 pregnant women across four LMICs: Guatemala, India, Peru, and Rwanda. We identified a portion of archived filter samples from two of the study sites, Guatemala and Rwanda, to determine potential source contributions to $PM_{2.5}$. This study contribute to source apportionment in LMICs, and paved the way for larger source apportionment studies within the HAPIN trial.

METHODS

Study settings

The HAPIN trial took place in four low- and middle-income countries (LMICs): Guatemala, India, Peru, and Rwanda. HAPIN households (n=3195) were randomized into either the control (continued use of biomass stove; n=1605) or intervention arm (LPG stove and fuel intervention; n=1590). The HAPIN trial measured personal exposures in pregnant women and kitchen concentrations of $PM_{2.5}$, BC, and CO. This pilot study draws from the pilot phase of the HAPIN trial, and aimed to characterize potential source contributions of $PM_{2.5}$ in Guatemala and

Rwanda. Site characteristics and inclusion and exclusion criteria have been described in detail previously[37,137] and are summarized briefly here for Guatemala and Rwanda.

The HAPIN Guatemala study site is located in the Jalapa municipality, which is located 150 km east of the capital, Guatemala City (14.63° N, 89.98° W, 1362 MASL). The climate is mild temperate with relatively stable temperatures year-round. The majority of Guatemala's rural population (86%), which includes Jalapa, relies primarily on solid fuels, with wood being the primary fuel source.[137]

The HAPIN Rwanda study site is located in the Eastern Province in the Kayonza district (1.78° S, 30.62° E, 1354 MASL). The climate is tropical with a long rainy season from March to May and a short rainy season from September to November. The primary cookstoves in this site include the traditional three-stone fire (the Rondereza) fueled with either wood or charcoal.[3]

Personal exposure assessment and laboratory analysis of $PM_{2.5}$

Archived personal exposure filter samples from the HAPIN trial were selected for Guatemala (n=64) and Rwanda (n=59). Personal exposure to $PM_{2.5}$ was assessed on participants using either the RTI Enhanced Children's MicroPEM (ECM, RTI International, Research Triangle Park, USA), or the Triplex Personal Sampling Cyclone (Mesa Labs) and Casella Tuff Pro pump (Casella, Buffalo, NY, USA). Both instruments use a 2.5 micron size-cut impactor. The ECM and weighs approximately 150 g, was loaded with Teflon filters 15 mm in diameter (Measurement Technology Laboratories, Bloomington, MN, USA), and was calibrated at a flow rate of 0.3 liters per minute before each deployment. The pump and cyclone system weighs approximately 450 g, is loaded with a filter 37 mm in diameter (Pall Corporation, Port

Washington, NY, USA), and was calibrated at a flow rate of 1.5 L/min before each deployment. Flow rates were recorded at the field station before and after the sampling period to determine the average sample flow rate.

Trained field staff turned on the equipment at the start of the sampling period and placed the monitor in the approximate breathing zone in her apron. The participant was asked to wear the monitor at all times, unless she were to participate in an activity which might damage the equipment such as bathing or sleeping, in which case she was asked to keep the apron with the monitor within a meter of her person. The instruments were stopped by a field technician when they returned after 24 h to collect the equipment. The sampling equipment was carried back to the field office and filters were removed from the monitors in a clean laboratory at the field office. Filters were placed in labeled petri dishes and kept refrigerated at 4°C at the field office until they were hand-carried by a traveler or shipped to the University of Georgia (UGA, Athens, GA, USA) for analysis.

Laboratory technicians at UGA weighed the filters before and after sampling (“pre-weighed” and “post-weighed,” respectively) on a microbalance with a sensitivity of 1 μg (Sartorius Cubis MSU, Gottingen, Germany) to determine the mass deposition of $PM_{2.5}$. Each filter was pre-weighed and post-weighed twice, once each by two laboratory technicians in distinct weighing sessions. If the difference between weights 1 and 2 was greater than ±5 μg for any filter, a third weight was taken and the average of the two closest weights was used as the average pre- or post-weight. Mass deposition was calculated by subtracting average pre-weight from average post-weight and then was converted to $PM_{2.5}$ concentration (μg/m^3) by considering the flow rate of the instrument for each filter and the total sampling duration, as shown in Equation 1 below.

$$[PM_{2.5}]\ \mu g/m^3 = flow\ rate\ \frac{L}{min} * \frac{sample\ duration\ (min)}{1000\ m^3} * mass\ difference, \mu g. \qquad \text{(Eq-1)}$$

Personal exposure assessment and laboratory analysis of BC

BC was measured on the same filters used for personal exposure assessment to $PM_{2.5}$ using the SootScan Model OT21 Optical Transmissometer (Magee Scientific, Berkeley, CA) before and after sampling. Similar to $PM_{2.5}$ gravimetric analysis, each filter was scanned twice, once each by two technicians in distinct scan sessions. The attenuation of BC through the filter (ATN) was converted to concentration (μg/m^3) using a standard, σ [138], the effective sampling area of a 15-mm filter (10 mm) or a 37-mm filter (30 mm), and other outputs provided by the SootScan. BC is converted from mass deposition to concentration as shown in Equation 2 below.

$$[BC]\ \mu g/m^3 = \frac{\sigma\left(\frac{\mu g}{m^2}\right) * filter\ area\ (m^2) * \log(I_0/I_F)}{flow\ rate\left(\frac{L}{min}\right) * sample\ duration\ (min) * 0.001\ (\frac{m^3}{L})} \qquad \text{(Eq-2)}$$

Laboratory analysis for elemental composition

Elemental composition of filters was determined via x-ray fluorescence (XRF) in the Environmental and Radiological Science Department at Colorado State University (CSU, Fort Collins, CO, USA). XRF is a non-destructive analytical method used for quantifying elemental concentrations.[109] Elements analyzed in our study were Mg, Al, Si, S, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Ga, As, Se, Cd, In, Sn, Te, I, and Pb. Species that had >50% of samples below the limit of detection (LOD) were initially removed from analysis. This resulted in a final elemental analysis of Mg, Al, Si, S, K, Ca, Ti, Mn, Fe. Three additional species were deemed of interest by investigators and had narrowly missed the LOD cut-off of 50% of samples, so they were added back to the analysis (Cr, Cu, and Zn), for a total of 12 elemental species.

Statistical methods for source characterization

Positive Matrix Factorization (PMF 5.0) is the mathematical receptor model that we used to identify and quantify potential sources of $PM_{2.5}$ that our participants in Guatemala and Rwanda were exposed to. Source apportionment is based on the fundamental principle that mass conservation can be assumed and mass balanced can be used to identify and apportion sources of airborne particulate matter in the atmosphere.[157]

The software relies on two input files: the chemical compositions and the estimated uncertainty of each of the concentrations, which are both outputs of XRF, and thus, allows each data point to be individually weighted. Given the potential differences in source contributions at our two unique study sites, we ran separate models for the Guatemala and Rwanda samples.

RESULTS AND DISCUSSION

Total $PM_{2.5}$ and BC concentrations

In Guatemala, $PM_{2.5}$ mass concentrations were calculated for 64 personal samples, with 22 samples from the control arm and 42 samples from the intervention arm. On average, $PM_{2.5}$ concentrations [mean (SD)] were 86% lower in the intervention arm controlled to the control arm: 319 (395) and 46 (100) μg/m^3, respectively.

In Rwanda, $PM_{2.5}$ mass concentrations were calculated for 59 personal samples, with 32 samples from the intervention arm and 27 samples from the control arm. On average, $PM_{2.5}$

concentrations [mean (SD)] were 62% lower in the intervention arm compared to the control arm: 222 (134) and 84 (130) μg/m^3, respectively.

Trends are similar for BC mass concentrations. Results and other summary statistics are presented in Table 5.1.

Chemical composition of samples and source contributions

We analyzed for 22 chemical species, but the same 12 chemical species were detected at both study sites in comparable levels.

Guatemala. We resolved four potential sources of $PM_{2.5}$ in Guatemala. The first factor exhibited strong signals of S, K, and BC, which are indicative of a biomass source. Factor two identified exhibited strong signals of Al, Si, Ca, Ti, Mn, and Fe, which point to a crustal source. The third factor was high in Mg, Ca, Mn, Cu, and other trace elements, which may be indicative of an agricultural source, or possibly resuspended street dust. The fourth factor included signals of Zn, Cu, Cr, with traces of other elements, which may be indicative of gasoline emissions. Factor fingerprints are presented in Figure 5.1.

Rwanda. We resolved the same four factor fingerprints for $PM_{2.5}$ in Rwanda, as presented in Figure 5.2. Factor one exhibited elevated levels of K, along with S, Cu, and Zn, which are indicative of a biomass signal. Factor two exhibited elevated levels of Al, Si, Ti, Cr, and Fe, which are indicative of a crustal signal. Factor three exhibited elevated levels of Mg, Ca, and Mg, which is likely an agricultural source. Factor four exhibited elevated levels of S and Cu, with traces of Mn, Zn, and Cr, which may be indicative of gas or traffic emissions.

<u>Implications of findings and limitations</u>

These filter samples were archived samples from the pilot phase of the HAPIN trial, which takes place in four diverse LMICs. This pilot studied allowed for the exploration of potential source contributions of $PM_{2.5}$ in Guatemala and Rwanda. Filters from both of these sites were pre- and post-analyzed at UGA, and it was noted by laboratory technicians that there were visual differences in some of the $PM_{2.5}$ samples between the two sites. Specifically, it was noted that some of more highly exposed samples from Rwanda had a reddish hue, which was not something observed in any highly-exposed Guatemala samples. For this reason, and for other reasons outlined previously pertaining to exploring differential toxicities, we sought to investigate potential sources of $PM_{2.5}$ in two unique LMICs.

We resolved four potential sources in both Guatemala and Rwanda, which had similar chemical compositions. In general, the sources that we identified coincide with the factor profiles described in Zhou et al. (2014). One of the more notable differences we observed within this study is in the chemical composition of the crustal sources (Factor 2) we identified for the two sites. In Rwanda, 80% of Fe measured was found in Factor 2, compared to approximately 50% in Guatemala. This supports the hypothesis that the soil in Rwanda is more iron-rich and thus, redder in color compared to the soil in Guatemala, which may have implications for health.
We were limited in this study due to sample size, which hindered our ability to resolve sources more confidently. Larger sample sizes are needed in order to better represent the study sites, as well as the different treatment arms, and to continue investigating differential toxicities that may exist depending on specific source contributions.

Future directions

This pilot study informed future work that has been funded and will be conducted within the HAPIN study where we will analyze approximately 700 filters from the Guatemala site. With this more robust sample size, we hope to obtain clearer resolution of potential source contributions to $PM_{2.5}$. We have pulled samples from across different timepoints in the HAPIN study (during pregnancy and post-birth of the child). We intend to explore seasonality in our samples, and we have added antimony (Sb) to the suite of chemicals that will be analyzed via XRF. Sb is a marker for garbage burning[158,159], which is the primary means of waste disposal in 50% of the population of Jalapa, Guatemala (Guatemala Population and Housing Census, 2018), which is the HAPIN study site. In addition to personal exposure samples, we will also analyze ambient samples that have been taken a local health post in Jalapa over the course of the trial. In the future, we hope to conduct source apportionment on samples from more of the HAPIN study sites.

CONCLUSIONS

In the pilot study, we conducted $PM_{2.5}$ source apportionment in two diverse LMICs, an area in the literature that is currently lacking. Despite small sample sizes, we were able to identify four potential sources at the Guatemala and Rwanda sites: biomass, crustal, agricultural or resuspended soil, and gasoline. Future studies will allow for more robust characterization of $PM_{2.5}$ sources and continued exploration of potential health impacts upon exposure.

Table 5.1. $PM_{2.5}$ and BC mass concentrations in Guatemala and Rwanda.

		Guatemala		Rwanda	
	N	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**
$PM_{2.5}$					
Control	22	319 (395)	162 (79-361)	222 (134)	189 (134-297)
Intervention	42	46 (100)	20 (8-40)	84 (130)	39 (28-81)
BC					
Control	32	21 (17)	17 (12-20)	29 (47)	12 (10-20)
Intervention	27	7 (7)	5 (3-8)	7 (3)	7 (6-8)

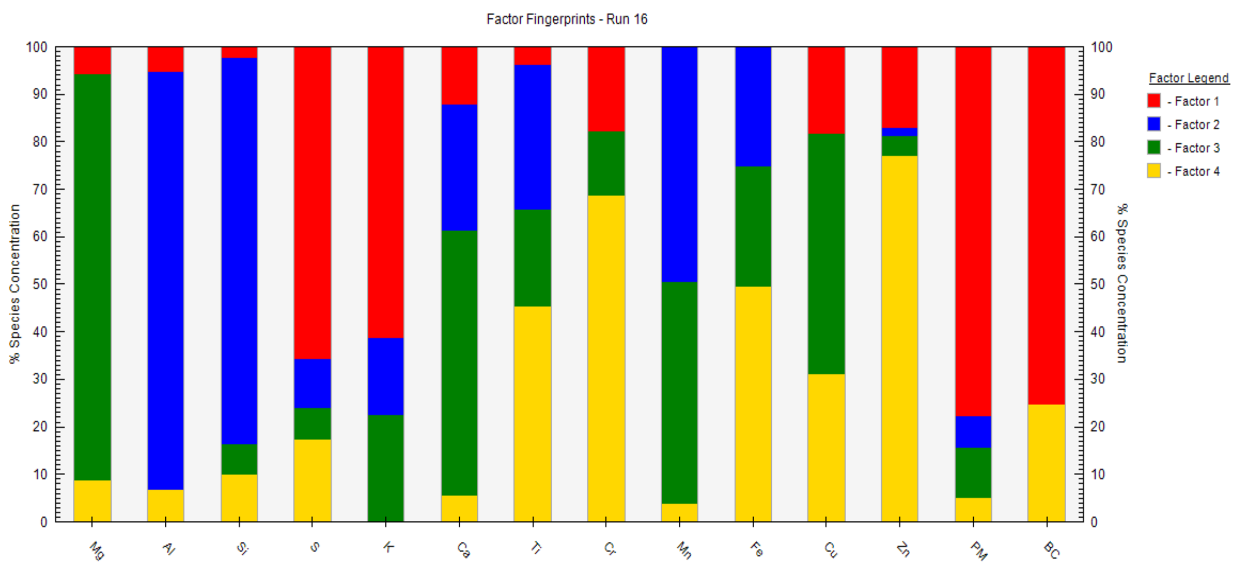

Figure 5.1. Factor fingerprints for personal exposure samples in Guatemala (n=64). Factor 1 (red) is the probable biomass source. Factor 2 (blue) is the probable crustal source. Factor 3 (green) is the possible agricultural source or resuspended street dust. Factor 4 (yellow) is the possible gasoline source.

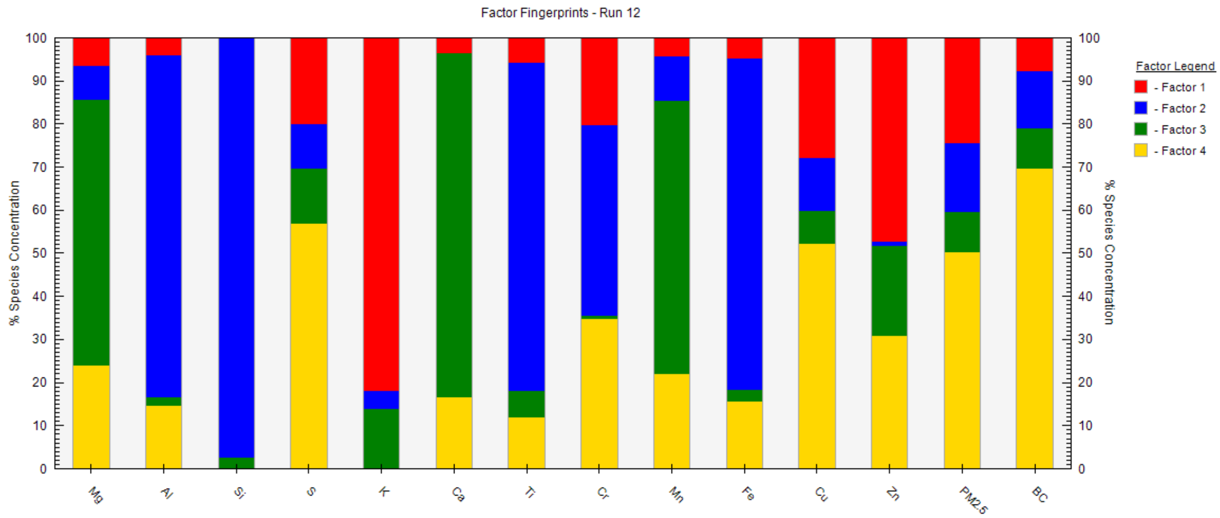

Figure 5.2. Factor fingerprints for personal exposure samples in Rwanda (n = 59). Factor 1 (red) is the probable biomass source. Factor 2 (blue) was identified as the probable crustal signal. Factor 3 (green) was identified as the probable agricultural source. Factor 4 (yellow) was identified as the possible gasoline or traffic emissions source.

CHAPTER 5

SUMMARY, CONCLUSIONS, AND FUTURE RESEARCH

SUMMARY

Approximately 3.8 billion people worldwide rely on solid fuels and use open cookstoves in the home for cooking and heating, resulting in exposures to hazardous air pollutants including $PM_{2.5}$, BC and CO, among others.[3–5] The majority of the people exposed reside in low- and middle-income countries (LMICs), and women and children are generally at the greatest risk for exposure due to the disproportionate amount of time they spend at home over the fire.[1] Exposure to household air pollution (HAP) is attributed to a large burden of disease globally, accounting for 2.31 million deaths in 2019 and attributing to 91 million years of healthy life lost.[120]

The HAPIN randomized controlled trial is a cookstove intervention trial that took place in four diverse LMICs: Guatemala, India, Peru, and Rwanda. This study sought to characterize HAP exposures in women who cook with traditional cookstoves compared to women who received an LPG stove and fuel intervention. In addition to the major aims of the HAPIN trial, many important research questions remain, some of which were addressed in this dissertation.

The first manuscript presents findings from a study where we measured personal exposures to and kitchen concentrations of NO_2 in participants enrolled in the main HAPIN trial. We randomly selected n=151 homes from Guatemala, n=101 homes from Peru, and n=36 homes from Rwanda. We found that study-wide, there was a significant reduction (Wilcoxon Rank Sum, $p<0.05$) in NO_2 personal exposures in intervention participants (median 12.6 ppb, IQR 9.6 - 17.9) compared to control (median 15.3 ppb, 10.7 – 25.7). Similarly, there was a significant reduction ($p<0.001$) in kitchen concentrations in intervention homes (median 17.9 ppb, IQR 11.7 – 31.0) compared to control homes (median 29.2 ppb, IQR 16.7 – 49.6). These findings suggest that LPG stoves can help reduce exposures to NO_2, though not all populations may see the same effects.

The second manuscript presents findings from a pilot study where we measured exposures in adolescent girls living with HAPIN participants in Jalapa, Guatemala. This study addressed another population of interest, as adolescent girls often help with household tasks including cooking and burning of household waste. Given that plastic waste is ubiquitous, we also characterized exposures to plastics. We measured 24-h personal exposures to $PM_{2.5}$ and BC via air monitoring, and urinary biomarkers of exposure to PAHs, phthalates, and BPA in girls from the biomass and LPG study arms. We found significant reductions in exposures to $PM_{2.5}$, BC, and PAHs in the LPG arm compared to biomass. Additionally, we compared PAH concentrations to age- and sex-matched participants of the National Health and Nutrition Examination Survey (NHANES) and found that our participants had significantly higher PAH concentrations, regardless of study arm.

Both BPA and phthalates are found in personal care products such as soap, shampoo hair spray, and nail polishes.[65] BPA is also found in a number of consumer materials including thermal receipt paper, linings of canned foods, electronics, pipes, coatings, and flame retardants.[67] Compared to age- and sex-matched NHANES participants (n = 113), we observed significantly higher ($p <0.001$) metabolite concentrations in our participants for five of the nine metabolites, regardless of study arm. BPA concentrations, on the other hand, were lower in our participants compared to age- and sex-matched NHANES participants. This study, though informative, was limited by sample size but has paved the way for a larger study currently underway at the same study site.

The third manuscript highlights a capacity building effort between three gravimetric laboratories from the HAPIN trial. During the pilot phase of the HAPIN trial and through the majority of the main phase of the trial, the gravimetric laboratory at UGA processed air monitoring filters for three of the four study sites: Guatemala, Peru, and Rwanda. The India site (SRIHER) has had their own gravimetric laboratory and processed their own filters, and the Guatemala site (UVG) started building their own gravimetric laboratory in 2019.

The purpose of this study was two-fold: 1) to show that regardless of where filters are weighed, we can achieve repeatable data for $PM_{2.5}$ and BC, and 2) to evaluate filter data in the UVG laboratory against data processed at UGA, which has a well-established gravimetric laboratory. This study not only showed repeatable data between UVG-UGA and SRIHER-UGA through our Bland-Altman analyses, but it also highlighted the successful capacity building effort at UVG, which resulted in the launch of their laboratory for HAPIN and future studies.

The fourth and final project reported preliminary results from a pilot source apportionment pilot study nested within HAPIN. We analyzed n=64 $PM_{2.5}$ samples from Guatemala and n=59 samples from Rwanda using x-ray fluorescence (XRF) to determine elemental composition and then used PMF to apportion sources. We resolved four potential sources in both study sites including biomass burning, a crustal source, an agricultural source, and gasoline. This pilot work informed a more robust study where we will analyze approximately 700 more filters from the Guatemala site. In addition to the 22 chemical elements analyzed in the pilot source apportionment project presented in this dissertation, we added antimony (Sb), which is a marker of garbage burning. This chemical tracer will be interesting for this study given that household waste burning has been reported as a common waste disposal method in Jalapa.

Overall, these four projects characterized HAP exposures in women across four LMICs enrolled in the HAPIN trial.

CONCLUSIONS

The projects within this dissertation build upon the main HAPIN trial by investigating additional exposures related to HAP. NO_2, while known to be a product of combustion, is not commonly studied in HAP settings, especially compared to $PM_{2.5}$, BC, and CO. Although clear reductions in concentrations were observed in Guatemala, there were no significant reductions in Peru. We were limited with sample size in Rwanda, but we only observed significant reductions in intervention kitchens and not in personal exposures. Additionally, we found that the newly-revised WHO annual guideline for NO_2 (approximately 5 ppb) and even some of the interim targets were exceedingly difficult to achieve in these settings. Ventilation patterns, stove stacking, and altitude may be influential factors for NO_2 exposures, but more work needs to be done to investigate these further.

At our study site in Jalapa, Guatemala, we found that adolescent girls, who often help with cooking and household waste burning, receive similar and oftentimes higher exposures than the mothers enrolled in the HAPIN trial living in the same households. We observed clear reductions in exposures to $PM_{2.5}$, BC, and PAHs in the intervention participants compared to control. We also observed that some sources of phthalates may be of concern in these populations, especially where plastic waste accumulates until it is disposed of via unsanitary waste disposal practices. Although BPA was detectable in our participants, levels were significantly lower compared to NHANES participants, regardless of study arm. A larger study is currently underway in Jalapa, which will hopefully further elucidate these variations in exposures.

In addition to field measurements, this dissertation also focused on laboratory methods and obtaining more information from the filter samples collected in the HAPIN trial. The successful capacity building effort between UGA and UVG has allowed for the UVG laboratory to process the filters for the Guatemala site in the first gravimetric laboratory of its kind in the country, to our knowledge. We also observed repeatable data between UGA and the SRIHER laboratory in India, which signifies that it does not matter where the filters are processed, as long as trained laboratory staff are following standardized protocols.

To date, the UGA laboratory has processed nearly 20,000 filters for the HAPIN trial. A lot of information can be obtained from them beyond $PM_{2.5}$ and BC concentrations through *post hoc* analyses like XRF and source apportionment. We were able to determine potential sources of $PM_{2.5}$ including biomass burning, an agricultural source, a gasoline source, and a crustal source. This project has much promise for future studies to determine what the health implications are for exposures to various sources of $PM_{2.5}$.

FUTURE RESEARCH

The HAPIN study is the largest study of its kind and offers much potential for continued research beyond the main study aims. In addition to the exciting work that is continuing at the Guatemala site to characterize HAP exposures in adolescent girls, the source apportionment study has also expanded and has the potential to continue expanding, first in Guatemala, and eventually in the other HAPIN sites as well. The source apportionment work will allow us to explore sources of $PM_{2.5}$ across study sites and to see if and how they vary in composition, which may have implications for differential toxicities and health effects upon exposure.

There is also potential to continue measuring NO_2 at these study sites. The start date of this project was significantly delayed due to onset of the COVID-19 pandemic and also limited our ability to ship real-time monitors between countries. In the future, it would be beneficial to measure real-time concentrations of NO_2, in addition to passive sampling, which is considered the gold standard, to be able to observe concentration peaks and changes during cooking periods. We were also limited to taking measurements at single timepoints within the households instead of repeat measurements, which would have allowed us to explore day-to-day and seasonal variation within homes.

The capacity building effort among the gravimetric laboratories was also limited by the pandemic in that technicians were not able to travel to the other countries' laboratories and work together in person. This limited our ability to learn about the intricacies and possible variations between laboratory protocols, which would have been helpful to more fully understanding the results. Nevertheless, our three laboratories overcame the challenges imposed by a global pandemic.

I was fortunate to have been able to start the capacity building and validation study at the laboratory in Guatemala (UVG) during my five-month assignment beginning in October 2019. I had tentative plans to visit the India laboratory (SRIHER) in April 2020 soon after my return from Guatemala, but my plans were promptly cancelled when the gravity of the COVID situation became apparent in March. My sister (who was also my roommate at the time) went with me to our laboratory during the shutdown and recorded a video of me going through the UGA laboratory protocol that I later uploaded to YouTube and shared with the other laboratories. One of the titles I considered for this video was “Capacity Building in the Era of COVID-19.”

Though the COVID situation is still very uncertain and continuously changing, we were able to host one of the laboratory technicians from UVG for a full week this past May (2022) to review training protocols, have her observe our technicians weighing and scanning filters, and to have her practice weighing and scanning filters at the UGA lab. We hope to continue this capacity building effort between the laboratories and to send one of our technicians to the India laboratory to repeat these same exercises. Continued capacity building is important in various forms including manuscript writing, especially among students from the various HAPIN sites, laboratory training, and field training and experiences. All of these elements have been instrumental for my professional and personal development, and I hope to be able to pay this forward and continue working with other sites and students in this manner.

This body of work contains four unique projects that are all intricately connected through characterizing HAP exposures in LMICs. The diversity in these projects underscores the interdisciplinary nature of this field and the interconnectedness of environmental, global, and public health. Such diversity in backgrounds and skillsets has been essential for the many successes of the HAPIN trial, and it has been my privilege to work alongside some of the top air pollution experts in the field, all with the common goal of improving environmental and human health. I am thrilled to have been a part of the HAPIN project, and I cannot wait to find out how I can continue making contributions to the field of environmental health science and toxicology, especially in the realm of household air pollution.

REFERENCES

1. World Health Organization. WHO | Household air pollution. *WHO* (2016).

2. Health Effects Institute. *State of Global Air 2018. Special Report*. (2018).

3. Clasen, T. *et al.* Design and Rationale of the HAPIN Study: A Multicountry Randomized Controlled Trial to Assess the Effect of Liquefied Petroleum Gas Stove and Continuous Fuel Distribution. *Environ. Health Perspect.* **128**, 047008 (2020).

4. Johnson, M. A. Air Pollutant Exposure and Stove Use Assessment Methods for the Household Air Pollution Intervention Network (HAPIN) Trial. *Environ. Health Perspect.* 9 (2020).

5. *Who guidelines for indoor air quality: selected pollutants*. (WHO, 2010).

6. Smith, K. R. *et al.* Effect of reduction in household air pollution on childhood pneumonia in Guatemala (RESPIRE): a randomised controlled trial. *Lancet* **378**, 1717–26 (2011).

7. Smith, K. R. *et al.* Personal child and mother carbon monoxide exposures and kitchen levels: methods and results from a randomized trial of woodfired chimney cookstoves in Guatemala (RESPIRE). *J Expo Sci Env. Epidemiol* **20**, 406–16 (2010).

8. Romieu, I. *et al.* Improved Biomass Stove Intervention in Rural Mexico: Impact on the Respiratory Health of Women. *Am. J. Respir. Crit. Care Med.* **180**, 649–656 (2009).

9. Mortimer, K. & Balmes, J. R. Cookstove Trials and Tribulations: What Is Needed to Decrease the Burden of Household Air Pollution? *Ann. Am. Thorac. Soc.* **15**, 539–541 (2018).

10. Mortimer, K. *et al.* A cleaner burning biomass-fuelled cookstove intervention to prevent pneumonia in children under 5 years old in rural Malawi (the Cooking and Pneumonia Study): a cluster randomised controlled trial. *The Lancet* **389**, 167–175 (2017).

11. Chillrud, S. N. *et al.* The effect of clean cooking interventions on mother and child personal exposure to air pollution: results from the Ghana Randomized Air Pollution and Health Study (GRAPHS). *J. Expo. Sci. Environ. Epidemiol.* **31**, 683–699 (2021).

12. Checkley, W. *et al.* Effects of a Household Air Pollution Intervention with Liquefied Petroleum Gas on Cardiopulmonary Outcomes in Peru. A Randomized Controlled Trial. *Am. J. Respir. Crit. Care Med.* **203**, 1386–1397 (2021).

13. Johnson, M. *et al.* Exposure contrasts of pregnant women during the Household Air Pollution Intervention Network randomized controlled trial. *medRxiv* 2021.11.04.21265938 (2021) doi:10.1101/2021.11.04.21265938.

14. World Health Organization. WHO indoor air quality guidelines: household fuel combustion. (2014).

15. Younger, A., Alkon, A., Harknett, K., Jean Louis, R. & Thompson, L. M. Adverse birth outcomes associated with household air pollution from unclean cooking fuels in low- and middle-income countries: A systematic review. *Environ. Res.* **204**, 112274 (2022).

16. Colbeck, I., Nasir, Z. A., Ali, Z. & Ahmad, S. Nitrogen dioxide and household fuel use in the Pakistan. *Sci. Total Environ.* **409**, 357–363 (2010).

17. Kephart, J. L. *et al.* Nitrogen dioxide exposures from LPG stoves in a cleaner-cooking intervention trial. *Environ. Int.* **146**, 106196 (2021).

18. Sun, L. & Wallace, L. A. Residential Cooking and Use of Kitchen Ventilation: The Impact on Exposure. *J Air Waste Manage Assoc* **71**, 830 (2021).

19. Kinney, P. L. *et al.* Prenatal and Postnatal Household Air Pollution Exposures and Pneumonia Risk. *Chest* **160**, 1634–1644 (2021).

20. Simkovich, S. M. *et al.* The health and social implications of household air pollution and respiratory diseases. *NPJ Prim Care Respir Med* **29**, 12 (2019).

21. Dutta, A. *et al.* Impact of prenatal and postnatal household air pollution exposure on lung function of 2-year old Nigerian children by oscillometry. *Sci. Total Environ.* 143419 (2020) doi:10.1016/j.scitotenv.2020.143419.

22. Katherine E. Woolley *et al.* Comparison of Respiratory Health Impacts Associated with Wood and Charcoal Biomass Fuels: A Population-Based Analysis of 475,000 Children from 30 Low- and Middle-Income Countries. *Int. J. Environ. Res. Public. Health* **18**, 9305–9305 (2021).

23. Katz, J. *et al.* Impact of Improved Biomass and Liquid Petroleum Gas Stoves on Birth Outcomes in Rural Nepal: Results of 2 Randomized Trials. *Glob. Health Sci. Pract.* **8**, 372–382 (2020).

24. Smith, K. R. Changing Paradigms in Clean Cooking. *Ecohealth* **12**, 196–199 (2015).

25. Burnett, R. T. *et al.* An integrated risk function for estimating the global burden of disease attributable to ambient fine particulate matter exposure. *Env. Health Perspect* **122**, 397–403 (2014).

26. Carter Ellison *et al.* Assessing Exposure to Household Air Pollution: A Systematic Review and Pooled Analysis of Carbon Monoxide as a Surrogate Measure of Particulate Matter. *Environ. Health Perspect.* **125**, 076002.

27. Clark, M. L. *et al.* Health and household air pollution from solid fuel use: the need for improved exposure assessment. *Env. Health Perspect* **121**, 1120–8 (2013).

28. Kirrane, E. F. *et al.* A systematic review of cardiovascular responses associated with ambient black carbon and fine particulate matter. *Environ. Int.* **127**, 305–316 (2019).

29. Bandowe, B. A. M. *et al.* The chemical composition and toxicological effects of fine particulate matter (PM2.5) emitted from different cooking styles. *Environ. Pollut.* **288**, 117754 (2021).

30. Harmsen, M. J. H. M. *et al.* Co-benefits of black carbon mitigation for climate and air quality. *Clim. Change* **163**, 1519–1538 (2020).

31. Wang, Y. *et al.* Personal and household PM2.5 and black carbon exposure measures and respiratory symptoms in 8 low- and middle-income countries. *Environ. Res.* **212**, 113430 (2022).

32. Van Vliet, E. D. S. *et al.* Personal exposures to fine particulate matter and black carbon in households cooking with biomass fuels in rural Ghana. *Environ. Res.* **127**, 40–48 (2013).

33. Kim, Y. H. *et al.* Chemistry, lung toxicity and mutagenicity of burn pit smoke-related particulate matter. *Part. Fibre Toxicol.* **18**, 45 (2021).

34. Bové, H. *et al.* Ambient black carbon particles reach the fetal side of human placenta. *Nat. Commun.* **10**, 1–7 (2019).

35. Liu, N. M. *et al.* Evidence for the presence of air pollution nanoparticles in placental tissue cells. *Sci. Total Environ.* **751**, 142235 (2021).

36. World Health Organization. WHO Household energy database. *WHO* (2014).

37. Johnson, M. Exposures to PM2.5 Associated with LPG Stove and Fuel Interventions: Pilot Results from the HAPIN Trial. (2018).

38. World Health Organization. *WHO global air quality guidelines: particulate matter (PM2.5 and PM10), ozone, nitrogen dioxide, sulfur dioxide and carbon monoxide*. (World Health Organization, 2021).

39. Pope, D. *et al.* Exposure to household air pollution from wood combustion and association with respiratory symptoms and lung function in nonsmoking women: results from the RESPIRE trial, Guatemala. *Environ. Health Perspect.* **123**, 285–292 (2015).

40. Smith-Sivertsen, T. *et al.* Effect of Reducing Indoor Air Pollution on Women's Respiratory Symptoms and Lung Function: The RESPIRE Randomized Trial, Guatemala. *Am. J. Epidemiol.* **170**, 211–220 (2009).

41. Kalpana Balakrishnan *et al.* Household Air Pollution Exposures of Pregnant Women Receiving Advanced Combustion Cookstoves in India: Implications for Intervention. *Ann Glob Health* **81**, 375–385 (2015).

42. Balakrishnan, K. *et al.* Exposures to fine particulate matter (PM2.5) and birthweight in a rural-urban, mother-child cohort in Tamil Nadu, India. *Environ. Res.* **161**, 524–531 (2018).

43. Kephart, J. L. *et al.* Nitrogen dioxide exposures from biomass cookstoves in the Peruvian Andes. *Indoor Air* **30**, 735–744 (2020).

44. Pacheco, H. *et al.* NO2 levels after the COVID-19 lockdown in Ecuador: A trade-off between environment and human health. *Urban Clim.* **34**, 100674 (2020).

45. Lamichhane, D. K., Leem, J. H. & Kim, H. C. Associations between Ambient Particulate Matter and Nitrogen Dioxide and Chronic Obstructive Pulmonary Diseases in Adults and Effect Modification by Demographic and Lifestyle Factors. *Int. J. Environ. Res. Public. Health* **15**, 363 (2018).

46. Lyons, R., Doherty, R., Reay, D. & Shackley, S. Legal but lethal: Lessons from NO2 related mortality in a city compliant with EU limit value. *Atmospheric Pollut. Res.* **11**, 43–50 (2020).

47. Mele, M., Magazzino, C., Schneider, N. & Strezov, V. NO2 levels as a contributing factor to COVID-19 deaths: The first empirical estimate of threshold values. *Environ. Res.* **194**, 110663 (2021).

48. US EPA NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT, R. T. P. N. Integrated Science Assessment (ISA) for Oxides of Nitrogen – Health Criteria (Final Report, Jan 2016). https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=310879.

49. Lebel, E. D., Finnegan, C. J., Ouyang, Z. & Jackson, R. B. Methane and NOx Emissions from Natural Gas Stoves, Cooktops, and Ovens in Residential Homes. *Environ. Sci. Technol.* **56**, 2529–2539 (2022).

50. Belanger, K. *et al.* Household Levels of Nitrogen Dioxide and Pediatric Asthma Severity. [Miscellaneous Article]. *Epidemiology* **24**, 320–330 (2013).

51. Hansel, N. N. *et al.* A Longitudinal Study of Indoor Nitrogen Dioxide Levels and Respiratory Symptoms in Inner-City Children with Asthma. *Environ. Health Perspect.* **116**, 1428–1432 (2008).

52. Morales, E. *et al.* Association of Early-life Exposure to Household Gas Appliances and Indoor Nitrogen Dioxide With Cognition and Attention Behavior in Preschoolers. *Am J Epidemiol* **169**, 1327–1336 (2009).

53. Helen, G. St. *et al.* Exposure of Pregnant Women to Cookstove-Related Household Air Pollution in Urban and Periurban Trujillo, Peru. *Arch. Environ. Occup. Health* **70**, 10–18 (2015).

54. Mullen, N. A. *et al.* Results of the California Healthy Homes Indoor Air Quality Study of 2011-2013: impact of natural gas appliances on air pollutant concentrations. *Indoor Air* **26**, 231–245 (2016).

55. Paulin, L. M. *et al.* 24-h Nitrogen dioxide concentration is associated with cooking behaviors and an increase in rescue medication use in children with asthma. *Environ. Res.* **159**, 118–123 (2017).

56. Edjabou, M. E., Møller, J. & Christensen, T. H. Solid waste characterization in Kétao, a rural town in Togo, West Africa. *Waste Manag. Res.* **30**, 745–749 (2012).

57. Hiramatsu, A., Hara, Y., Sekiyama, M., Honda, R. & Chiemchaisri, C. Municipal solid waste flow and waste generation characteristics in an urban—rural fringe area in Thailand. *Waste Manag. Res.* **27**, 951–960 (2009).

58. Wiedinmyer, C., Yokelson, R. J. & Gullett, B. K. Global Emissions of Trace Gases, Particulate Matter, and Hazardous Air Pollutants from Open Burning of Domestic Waste. *Environ. Sci. Technol.* **48**, 9523–9530 (2014).

59. Zeng, C., Niu, D., Li, H., Zhou, T. & Zhao, Y. Public perceptions and economic values of source-separated collection of rural solid waste: A pilot study in China. *Resour. Conserv. Recycl.* **107**, 166–173 (2016).

60. Ferronato, N. & Torretta, V. Waste Mismanagement in Developing Countries: A Review of Global Issues. *Int. J. Environ. Res. Public. Health* **16**, 1060 (2019).

61. Environment, U. N. Global Waste Management Outlook. *UNEP - UN Environment Programme* http://www.unep.org/resources/report/global-waste-management-outlook (2017).

62. Cimmino, I. *et al.* Potential Mechanisms of Bisphenol A (BPA) Contributing to Human Disease. *Int. J. Mol. Sci.* **21**, 5761 (2020).
63. Abraham, A. & Chakraborty, P. A review on sources and health impacts of bisphenol A. *Rev. Environ. Health* **35**, 201–210 (2020).
64. Vasiljevic, T. & Harner, T. Bisphenol A and its analogues in outdoor and indoor air: Properties, sources and global levels. *Sci. Total Environ.* **789**, 148013 (2021).
65. Wang, Y. & Qian, H. Phthalates and Their Impacts on Human Health. *Healthcare* **9**, 603 (2021).
66. Filardi, T., Panimolle, F., Lenzi, A. & Morano, S. Bisphenol A and Phthalates in Diet: An Emerging Link with Pregnancy Complications. *Nutrients* **12**, 525 (2020).
67. ZIELIŃSKA, M., WOJNOWSKA-BARYŁA, I. & CYDZIK-KWIATKOWSKA, A. Sources and Properties of BPA. in *Bisphenol A Removal from Water and Wastewater* (eds. ZIELIŃSKA, M., WOJNOWSKA-BARYŁA, I. & CYDZIK-KWIATKOWSKA, A.) 3–28 (Springer International Publishing, 2019). doi:10.1007/978-3-319-92361-1_2.
68. Warner, G. R. & Flaws, J. A. Bisphenol A and Phthalates: How Environmental Chemicals Are Reshaping Toxicology. *Toxicol. Sci.* **166**, 246–249 (2018).
69. Wang, Y., Zhu, H. & Kannan, K. A Review of Biomonitoring of Phthalate Exposures. *Toxics* **7**, (2019).
70. Carli, F., Ciociaro, D. & Gastaldelli, A. Assessment of Exposure to Di-(2-ethylhexyl) Phthalate (DEHP) Metabolites and Bisphenol A (BPA) and Its Importance for the Prevention of Cardiometabolic Diseases. *Metabolites* **12**, 167 (2022).

71. Li, Z. *et al.* PM2.5 Associated PAHs and Inorganic Elements from Combustion of Biomass, Cable Wrapping, Domestic Waste, and Garbage for Power Generation. *Aerosol Air Qual. Res.* **19**, 2502–2517 (2019).

72. Hoffer, A. *et al.* Emission factors for PM_{10} and polycyclic aromatic hydrocarbons (PAHs) from illegal burning of different types of municipal waste in households. *Atmospheric Chem. Phys.* **20**, 16135–16144 (2020).

73. Wang, J. *et al.* Source profiles of PM2.5 emitted from four typical open burning sources and its cytotoxicity to vascular smooth muscle cells. *Sci. Total Environ.* **715**, 136949 (2020).

74. Dai, C. *et al.* Review on the contamination and remediation of polycyclic aromatic hydrocarbons (PAHs) in coastal soil and sediments. *Environ. Res.* **205**, 112423 (2022).

75. Zhang, N. *et al.* Characteristics, Source Contributions, and Source-Specific Health Risks of PM2.5-Bound Polycyclic Aromatic Hydrocarbons for Senior Citizens during the Heating Season in Tianjin, China. *Int. J. Environ. Res. Public. Health* **19**, (2022).

76. Aslam, R., Sharif, F., Baqar, M. & Shahzad, L. Source identification and risk assessment of polycyclic aromatic hydrocarbons (PAHs) in air and dust samples of Lahore City. *Sci. Rep.* **12**, 1–15 (2022).

77. Hamidi, E. N., Hajeb, P., Selamat, J., Lee, S. Y. & Abdull Razis, A. F. Bioaccessibility of Polycyclic Aromatic Hydrocarbons (PAHs) in Grilled Meat: The Effects of Meat Doneness and Fat Content. *Int. J. Environ. Res. Public. Health* **19**, (2022).

78. Kim, K.-H., Jahan, S. A., Kabir, E. & Brown, R. J. C. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environ. Int.* **60**, 71–80 (2013).

79. Zhang, H. *et al.* Characteristics and Influencing Factors of Polycyclic Aromatic Hydrocarbons Emitted from Open Burning and Stove Burning of Biomass: A Brief Review. *Int. J. Environ. Res. Public. Health* **19**, (2022).

80. Zhang, J. *et al.* Distribution characteristics of and personal exposure with polycyclic aromatic hydrocarbons and particulate matter in indoor and outdoor air of rural households in Northern China. *Environ. Pollut.* **255**, 113176 (2019).

81. Zhang, L. *et al.* Size distribution of particulate polycyclic aromatic hydrocarbons in fresh combustion smoke and ambient air: A review. *J. Environ. Sci.* **88**, 370–384 (2020).

82. Nguyen, K. *et al.* Improvement on recovery and reproducibility for quantifying urinary mono-hydroxylated polycyclic aromatic hydrocarbons (OH-PAHs). *J. Chromatogr. B* **1192**, 123113 (2022).

83. Koch, H. M., Preuss, R. & Angerer, J. Di(2-ethylhexyl)phthalate (DEHP): human metabolism and internal exposure – an update and latest results1. *Int. J. Androl.* **29**, 155–165 (2006).

84. Téllez-Rojo, M. M. *et al.* Prenatal urinary phthalate metabolites levels and neurodevelopment in children at two and three years of age. *Sci. Total Environ.* **461–462**, 386–390 (2013).

85. Liu, C. *et al.* Urinary biomarkers of phthalates exposure and risks of thyroid cancer and benign nodule. *J. Hazard. Mater.* **383**, 121189 (2020).

86. Wolff Mary S. *et al.* Pilot Study of Urinary Biomarkers of Phytoestrogens, Phthalates, and Phenols in Girls. *Environ. Health Perspect.* **115**, 116–121 (2007).

87. Biomonitoring: Bisphenol A (BPA). 19.

88. Lee, J. *et al.* Bisphenol A distribution in serum, urine, placenta, breast milk, and umbilical cord serum in a birth panel of mother–neonate pairs. *Sci. Total Environ.* **626**, 1494–1501 (2018).

89. Khamphaya, T., Pouyfung, P., Kuraeiad, S., Vattanasit, U. & Yimthiang, S. Current Aspect of Bisphenol A Toxicology and Its Health Effects. *Trends Sci.* **18**, 408–408 (2021).

90. Wang, Y. *et al.* Quantification of urinary mono-hydroxylated metabolites of polycyclic aromatic hydrocarbons by on-line solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* **409**, 931–937 (2017).

91. Yang, Z. *et al.* Human health risks estimations from polycyclic aromatic hydrocarbons in serum and their hydroxylated metabolites in paired urine samples. *Environ. Pollut.* **290**, 117975 (2021).

92. Li, Z. *et al.* Evaluation of exposure reduction to indoor air pollution in stove intervention projects in Peru by urinary biomonitoring of polycyclic aromatic hydrocarbon metabolites. *Environ. Int.* **37**, 1157–1163 (2011).

93. Hosten, A. O. BUN and Creatinine. in *Clinical Methods: The History, Physical, and Laboratory Examinations* (eds. Walker, H. K., Hall, W. D. & Hurst, J. W.) (Butterworths, 1990).

94. Barr, D. B. *et al.* Urinary Creatinine Concentrations in the U.S. Population: Implications for Urinary Biologic Monitoring Measurements. *Environ. Health Perspect.* **113**, 192–200 (2005).

95. Adedeji, A. O. *et al.* Investigating the Value of Urine Volume, Creatinine, and Cystatin C for Urinary Biomarkers Normalization for Drug Development Studies. *Int. J. Toxicol.* **38**, 12–22 (2019).

96. Kuiper, J. R., O'Brien, K. M., Ferguson, K. K. & Buckley, J. P. Urinary specific gravity measures in the U.S. population: Implications for the adjustment of non-persistent chemical urinary biomarker data. *Environ. Int.* **156**, 106656 (2021).

97. MacPherson, S., Arbuckle, T. E. & Fisher, M. Adjusting urinary chemical biomarkers for hydration status during pregnancy. *J. Expo. Sci. Environ. Epidemiol.* **28**, 481–493 (2018).

98. McClellan, R. O. Setting ambient air quality standards for particulate matter. *Toxicology* **181–182**, 329–347 (2002).

99. US EPA, R. 01. What are the Air Quality Standards for PM? | Air Quality Planning Unit | Ground-level Ozone | New England | US EPA. https://www3.epa.gov/region1/airquality/pm-aq-standards.html.

100. Feng, S., Gao, D., Liao, F., Zhou, F. & Wang, X. The health effects of ambient PM2.5 and potential mechanisms. *Ecotoxicol. Environ. Saf.* **128**, 67–74 (2016).

101. Pandey, P. *et al.* Temporal distribution of fine particulates (PM2.5, PM10), potentially toxic metals, PAHs and Metal-bound carcinogenic risk in the population of Lucknow City, India. *J. Environ. Sci. Health Part A* **48**, 730–745 (2013).

102. Kabera, T., Bartington, S., Uwanyirigira, C., Abimana, P. & Pope, F. Indoor PM $_{2.5}$ characteristics and CO concentration in households using biomass fuel in Kigali, Rwanda. *Int. J. Environ. Stud.* **77**, 998–1011 (2020).

103. Walker, E. S. *et al.* Exposure to household air pollution from biomass cookstoves and self-reported symptoms among women in rural Honduras. *Int. J. Environ. Health Res.* **30**, 160–173 (2020).

104. Viana, M. *et al.* Source apportionment of particulate matter in Europe: A review of methods and results. *J. Aerosol Sci.* **39**, 827–849 (2008).

105. Watson, J. G. *et al.* The USEPA/DRI chemical mass balance receptor model, CMB 7.0. *Environ. Softw.* **5**, 38–49 (1990).

106. Jaeckels, J. M., Bae, M.-S. & Schauer, J. J. Positive Matrix Factorization (PMF) Analysis of Molecular Marker Measurements to Quantify the Sources of Organic Aerosols. *Environ. Sci. Technol.* **41**, 5763–5769 (2007).

107. Reff, A., Eberly, S. I. & Bhave, P. V. Receptor Modeling of Ambient Particulate Matter Data Using Positive Matrix Factorization: Review of Existing Methods. *J. Air Waste Manag. Assoc.* **57**, 146–154 (2007).

108. NIST TN 1297: Appendix A. Law of Propagation of Uncertainty. *NIST* (2015).

109. Zhou, Z. *et al.* Chemical Characterization and Source Apportionment of Household Fine Particulate Matter in Rural, Peri-urban, and Urban West Africa. *Environ. Sci. Technol.* **48**, 1343–1351 (2014).

110. Lai, A. M. *et al.* Chemical composition and source apportionment of ambient, household, and personal exposures to PM2.5 in communities using biomass stoves in rural China. *Sci. Total Environ.* **646**, 309–319 (2019).

111. Raqib, R. *et al.* Association of household air pollution with cellular and humoral immune responses among women in rural Bangladesh. *Environ. Pollut.* **299**, 118892 (2022).

112. Strassmann, A. *et al.* NO2andPM2.5Exposures and Lung Function in Swiss Adults: Estimated Effects of Short-Term Exposures and Long-Term Exposures with and without Adjustment for Short-Term Deviations. *Environ. Health Perspect.* **129**, 17009 (2021).

113. Document Display | NEPIS | US EPA. https://nepis.epa.gov/Exe/ZyNET.exe/2000TAGZ.txt?ZyActionD=ZyDocument&Client=EPA&Index=1976%20Thru%201980&Docs=&Query=&Time=&EndTime=&SearchMethod

=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&UseQField=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5CZYFILES%5CINDEX%20DATA%5C76THRU80%5CTXT%5C00000006%5C2000TAGZ.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=4#.

114. Kumar, R. *et al.* Impact of Domestic Air Pollution from Cooking Fuel on Respiratory Allergies in Children in India. *Asian Pac. J. Allergy Immunol.* **26**, 213–22 (2008).

115. Mitra, P., Chakraborty, D. & Mondal, N. K. Assessment of household air pollution exposure of tribal women. *Sci. Total Environ.* **817**, 152869 (2022).

116. Cibella, F. *et al.* Effect of indoor nitrogen dioxide on lung function in urban environment. *Environ. Res.* **138**, 8–16 (2015).

117. Dėdelė, A. & Miškinytė, A. Seasonal variation of indoor and outdoor air quality of nitrogen dioxide in homes with gas and electric stoves. *Environ. Sci. Pollut. Res.* **23**, 17784–17792 (2016).

118. Ruiz-Mercado, I. & Masera, O. Patterns of Stove Use in the Context of Fuel–Device Stacking: Rationale and Implications. *EcoHealth* **12**, 42–56 (2015).

119. Piedrahita, R. *et al.* Exposures to and origins of carbonaceous PM2.5 in a cookstove intervention in Northern Ghana. *Sci. Total Environ.* **576**, 178–192 (2017).

120. State of Global Air 2020. 28 (2020).

121. Arif, M. *et al.* Assessment of Indoor & Outdoor Black Carbon emissions in rural areas of Indo-Gangetic Plain: seasonal characteristics, source apportionment and radiative forcing. *Atmos. Environ.* **191**, 227–240 (2018).

122. Arku, R. E. *et al.* Long-term exposure to outdoor and household air pollution and blood pressure in the Prospective Urban and Rural Epidemiological (PURE) study. *Environ. Pollut.* **262**, 114197 (2020).

123. Woolley, K. *et al.* Biomass cooking carbon monoxide levels in commercial canteens in Kigali, Rwanda. *Arch. Environ. Occup. Health* **0**, 1–11 (2020).

124. Grindler, N. M. *et al.* Exposure to Phthalate, an Endocrine Disrupting Chemical, Alters the First Trimester Placental Methylome and Transcriptome in Women. *Sci. Rep.* **8**, 6086 (2018).

125. Benjamin, S. *et al.* Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. *J. Hazard. Mater.* **340**, 360–383 (2017).

126. Ma, Y. *et al.* The adverse health effects of bisphenol A and related toxicity mechanisms. *Environ. Res.* **176**, 108575 (2019).

127. Akgül, S. *et al.* Bisphenol A and phthalate levels in adolescents with polycystic ovary syndrome. *Gynecol. Endocrinol.* **35**, 1084–1087 (2019).

128. Pratiti, R., Vadala, D., Kalynych, Z. & Sud, P. Health effects of household air pollution related to biomass cook stoves in resource limited countries and its mitigation by improved cookstoves. *Environ. Res.* **186**, 109574 (2020).

129. Rodríguez-Aguilar, M. *et al.* Respiratory health assessment and exposure to polycyclic aromatic hydrocarbons in Mexican indigenous population. *Environ. Sci. Pollut. Res.* **26**, 25825–25833 (2019).

130. da Silva Junior, F. C. *et al.* A look beyond the priority: A systematic review of the genotoxic, mutagenic, and carcinogenic endpoints of non-priority PAHs. *Environ. Pollut.* **278**, 116838 (2021).

131. Lee, K. K. *et al.* Adverse health effects associated with household air pollution: a systematic review, meta-analysis, and burden estimation study. *Lancet Glob. Health* **8**, e1427–e1434 (2020).

132. Johnson, M. *et al. Exposure contrasts of pregnant women during the Household Air Pollution Intervention Network randomized controlled trial*. http://medrxiv.org/lookup/doi/10.1101/2021.11.04.21265938 (2021) doi:10.1101/2021.11.04.21265938.

133. Huang, X. *et al.* Internal exposure levels of polycyclic aromatic hydrocarbons in children and adolescents: a systematic review and meta-analysis. *Environ. Health Prev. Med.* **24**, 50 (2019).

134. Murawski, A. *et al.* Polycyclic aromatic hydrocarbons (PAH) in urine of children and adolescents in Germany – human biomonitoring results of the German Environmental Survey 2014–2017 (GerES V). *Int. J. Hyg. Environ. Health* **226**, 113491 (2020).

135. Yang, M. *et al.* Fine and ultrafine airborne PM influence inflammation response of young adults and toxicological responses in vitro. *Sci. Total Environ.* **836**, 155618 (2022).

136. Zhang, L. *et al.* Personal PM2.5-bound PAH exposure, oxidative stress and lung function: The associations and mediation effects in healthy young adults. *Environ. Pollut.* **293**, 118493 (2022).

137. Clasen, T. *et al.* Design and Rationale of the HAPIN Study: A Multicountry Randomized Controlled Trial to Assess the Effect of Liquefied Petroleum Gas Stove and Continuous Fuel Distribution. *Environ. Health Perspect.* **128**, 47008 (2020).

138. Garland, C. *et al.* Black carbon cookstove emissions: A field assessment of 19 stove/fuel combinations. *Atmos. Environ.* **169**, 140–149 (2017).

139. Weinstein, J. R., Diaz-Artiga, A., Benowitz, N. & Thompson, L. M. Reductions in urinary metabolites of exposure to household air pollution in pregnant, rural Guatemalan women provided liquefied petroleum gas stoves. *J. Expo. Sci. Environ. Epidemiol.* **30**, 362–373 (2020).

140. Thornburg, J. *et al.* Pregnant Women's Exposure to Household Air Pollution in Rural Bangladesh: A Feasibility Study for Poriborton: The CHANge Trial. *Int. J. Environ. Res. Public. Health* **19**, 482 (2022).

141. Baluka, S. A. & Rumbeiha, W. K. Bisphenol A and food safety: Lessons from developed to developing countries. *Food Chem. Toxicol.* **92**, 58–63 (2016).

142. Adaji, E. E., Ekezie, W., Clifford, M. & Phalkey, R. Understanding the effect of indoor air pollution on pneumonia in children under 5 in low- and middle-income countries: a systematic review of evidence. *Env. Sci Pollut Res Int* **26**, 3208–3225 (2019).

143. Anenberg, S. C. *et al.* Air pollution-related health and climate benefits of clean cookstove programs in Mozambique. *Environ. Res. Lett.* **12**, (2017).

144. Aung, T. W. *et al.* Health and Climate-Relevant Pollutant Concentrations from a Carbon-Finance Approved Cookstove Intervention in Rural India. *Env. Sci Technol* **50**, 7228–38 (2016).

145. Hering, S. & Cass, G. The Magnitude of Bias in the Measurement of PM25 Arising from Volatilization of Particulate Nitrate from Teflon Filters. *J. Air Waste Manag. Assoc.* **49**, 725–733 (1999).

146. Liu, C.-N. *et al.* Sampling and conditioning artifacts of PM2.5 in filter-based samplers. *Atmos. Environ.* **85**, 48–53 (2014).

147. Perrino, C., Canepari, S. & Catrambone, M. Comparing the Performance of Teflon and Quartz Membrane Filters Collecting Atmospheric PM: Influence of Atmospheric Water. *Aerosol Air Qual. Res.* **13**, 137–147 (2013).

148. Altman, D. G. & Bland, J. M. Measurement in Medicine: The Analysis of Method Comparison Studies. *J. R. Stat. Soc. Ser. Stat.* **32**, 307–317 (1983).

149. TECHNICAL MEMORANDUM. 10.

150. Kim, K.-H., Jahan, S. A. & Kabir, E. A review of diseases associated with household air pollution due to the use of biomass fuels. *J. Hazard. Mater.* **192**, 425–431 (2011).

151. Almeida, S. m. *et al.* Ambient particulate matter source apportionment using receptor modelling in European and Central Asia urban areas. *Environ. Pollut.* **266**, (2020).

152. Mohammed, M. O. A. *et al.* Potential Toxicological and Cardiopulmonary Effects of PM2.5 Exposure and Related Mortality: Findings of Recent Studies Published during 2003-2013. *Biomed. Environ. Sci.* **29**, 66–79 (2016).

153. Shupler, M. *et al.* Global estimation of exposure to fine particulate matter (PM2.5) from household air pollution. *Environ. Int.* **120**, 354–363 (2018).

154. Huang, W., Baumgartner, J., Zhang, Y., Wang, Y. & Schauer, J. J. Source apportionment of air pollution exposures of rural Chinese women cooking with biomass fuels. *Atmos. Environ.* **104**, 79–87 (2015).

155. Johnson, T. M. *et al.* *Tools for Improving Air Quality Management : A Review of Top-down Source Apportionment Techniques and Their Application in Developing Countries*. https://openknowledge.worldbank.org/handle/10986/17488 (2011).

156. Ofosu, F. G., Hopke, P. K., Aboh, I. J. K. & Bamford, S. A. Biomass burning contribution to ambient air particulate levels at Navrongo in the Savannah zone of Ghana. *J. Air Waste Manag. Assoc.* **63**, 1036–1045 (2013).

157. Hopke, P. K. *et al.* PM source apportionment and health effects: 1. Intercomparison of source apportionment results. *J. Expo. Sci. Environ. Epidemiol.* **16**, 275–286 (2006).

158. Christian, T. J. *et al.* Trace gas and particle emissions from domestic and industrial biofuel use and garbage burning in central Mexico. *Atmospheric Chem. Phys.* **10**, 565–584 (2010).

159. Jayarathne, T. *et al.* Nepal Ambient Monitoring and Source Testing Experiment (NAMaSTE): emissions of particulate matter from wood- and dung-fueled cooking fires, garbage and crop residue burning, brick kilns, and other sources. *Atmospheric Chem. Phys.* **18**, 2259–2286 (2018).