ABSTRACT

Title of Document:	LUNG CANCER IN NEPAL: THE ROLE OF TRADITIONAL TOBACCO PRODUCTS AND COMBUSTION RELATED HOUSEHOLD AIR POLLUTION Greg A. Raspanti PhD 2016
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The burden of chronic diseases such as cancer is increasing in low and middle income countries around the globe. Nepal, one of the world's poorest countries, is no exception to this trend, with lung cancer as the leading causes of cancer deaths. Despite this, limited data is available on the environmental and behavioral risk factors that contribute to the lung cancer etiology in Nepal. The objectives of this dissertation are to: 1) investigate the ethnic differences in consumption of local tobacco products and their role in lung cancer risk in Nepal; 2) evaluate urinary metabolite of 1,3-butadiene as a biomarker of exposure to combustion related household air pollution (CRHAP); 3) investigate the association between CRHAP exposure and lung cancer risk using urinary

metabolite of 1,3-butadiene as a biomarker of exposure; 4) investigate the association between CRHAP exposure and lung cancer risk using questionnaire based measure of exposure.

Lung cancer cases (n=606) and frequency matched controls (N=606) were recruited from B.P. Koirala Memorial Cancer Hospital. We obtained biological samples and information on lifestyles including cooking habits and type of fuels used. We used liquid chromatograph tandem mass spectrometer (LC-MS/MS) to quantify urinary metabolites of 1,3-butadiene in urine samples. We employed a combination of logistic and linear regression models to detect any exposure-disease associations while controlling for known confounding variables.

Overall, we found that ethnic groups in Nepal use different tobacco products that have different differing cancer potency -we observed the highest odds ratios for the traditional tobacco products. The biomarker analysis showed strong evidence that monohydroxybutyl mercapturic acid is associated with biomass fuel use among participants. However, we did not find significant association between urinary MHMBA and lung cancer risk. When we used questionnaire based measure of exposure to household air pollution, we observed significant, dose-response associations between CRHAP exposure and lung cancer risk, particularly among never-smokers.

Our results show that important role of local tobacco products in lung cancer risk in Nepal. Furthermore, we demonstrate that CRHAP exposure is a risk factor for lung cancer risk, independent of tobacco smoking.

LUNG CANCER IN NEPAL: THE ROLE OF TRADITIONAL TOBACCO PRODUCTS AND HOUSEHOLD AIR POLLUTION

By

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Dedication

Dedicated to my lovely and patient wife Elizabeth and son Christopher.

Acknowledgements

First and foremost, I have to thank my wife Elizabeth for all of her love and support over the years. This would certainly not be possible without her hard work and patience to allow me to pursue my education. Furthermore, a major thank you to all of our family members for their support along the way. Secondly, a special thank you to my academic adviser Dr. Amir Sapkota. You have truly served as a strong role model both professionally and personally and I thank you for all of your guidance, support, and mentorship over the last six years. I also would like to acknowledge all of my committee members who have helped make this project a success. Lastly, everyone else who have contributed to my time and experience here at UMD namely the Dean's Office, Global Health Scholars program, and all professors and fellow students in the Maryland Institute of Applied Environmental Health.

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Chapter 1: Introduction

Globally, cancer incidence is increasing with approximately 70% of cancer related deaths occurred in low and middle income countries (LMICs) (IOM, 2007). In LMICs, lung cancer accounts for an estimated 1.37 million deaths annually and is the leading cause of cancer related mortality for men and third for women (WHO, 2012). Lung cancer is one of the most common cancer in both men and women in Nepal, one of the poorest countries in the world, accounting for 20% of all cancer cases (Binu et al., 2007; Pradhananga et al., 2009; "World Bank Nepal Data," 2012). The influence of tobacco on lung cancer is well known, but the differential potency of various tobacco products, such as hand rolled traditional bidi or choor/kankat that are commonly used in Nepal, remain unclear (Notani et al., 1977; Pednekar et al., 2011; Prasad et al., 2010, 2009).

Besides tobacco smoke, environmental exposures are major sources of morbidity and mortality across the globe. A recent study on the global burden of disease has identified combustion related household air pollution (CRHAP) as a critical and worsening environmental health concern (Lim et al., 2012), contributing to over 3.5 million deaths worldwide. Burning biomass fuels such as wood, charcoal, dung, and agricultural waste in the home and the resulting

CRHAP exposures have been associated with both chronic and acute health outcomes including various cancers, low birth weight, increased respiratory infection, cataracts, and cardiovascular complications (Bruce et al., 2015; Martin et al., 2013; Naeher et al., 2007, 2005; Sapkota et al., 2013). The components of CRHAP include many chemicals that are known or suspected to be carcinogenic. CRHAP emissions contain particulate matter, sulfur oxides, nitrogen oxides, carbon monoxide, polycyclic aromatic hydrocarbons, formaldehyde, and dioxins (Ding et al., 2012; EPA, 2007; Ward et al., 2008). The carcinogenicity of CRHAP resulting from biomass fuels was extensively evaluated by expert panel convened by the International Agency for Research on Cancer (IARC). Based on the available epidemiological studies at the time, the panel concluded that CRHAP resulting from combustion of coal was a known human carcinogen (Group 1). The group concluded that CRHAP resulting from combustion of biomass was a probable human carcinogen (Group 2A). The panel cited lack of epidemiological studies related to CRHAP from biomass as the main reason for the Group 2A classification (IARC, 2010a).

In light of myriad health outcomes related to CRHAPs resulting from combustion of biomass, researchers have been investigating various ways to quantify individual level measure exposure to CRHAP that can be used in epidemiological studies. Biological markers (biomarkers) are attractive option as they allow researchers to quantify total exposures across various routes and

pathways. Biomarker also provide a measure of absorbed dose, rather than potential dose, and account for inter-individual variability related to occupation, home environment, and time activity pattern. For CRHAP exposure, many biomarkers have been proposed, but none have achieved the desired specificity and sensitivity (Rylance et al., 2013; Simpson and Naeher, 2010). Two urinary metabolites of 1,3-butadiene – monohydroxybutyl mercapturic acid (MHBMA) and dihydroxybutyl mercapturic acid (DHBMA) have been used as a biomarker of exposure to combustion related pollution in both occupational and environmental settings (Albertini et al., 2001; Richard J Albertini et al., 2003; Fustinoni et al., 2004; Gustafson et al., 2007; Ruchirawat et al., 2010; Sapkota et al., 2006). Others have suggested MHMBA and DHMBA as a potential biomarker of exposure to CRHAP exposure.

We propose to address these questions using hospital based case-control study conducted in Nepal. Our specific aims are:

Specific Aim 1: investigate the ethnic differences in consumption pattern of local tobacco products and their role in lung cancer risk in Nepal**.**

Hypothesis 1-1: Traditional tobacco products will increase lung cancer risk more dramatically compared with commercial tobacco products.

Specific Aim 2: Evaluate MHBMA and DHMBA as biomarker of exposure to CRHAP from biomass combustion.

Hypothesis 2-1: Participants who use biomass for cooking will have higher urinary concentration of MHMBA and DHMBA.

Specific Aim 3: Quantify the risk of lung cancer associated with CRHAP using biomarker based measure of exposure

Specific Aim 4: Quantify risk of lung cancer associated with CRHAP using questionnaire based measure of exposure.

Hypothesis 3-1: Long term exposure to CRHAP resulting from biomass combustion will be associated with increased lung cancer risk among Nepalese population

Results from this study will provide first ever quantitative estimate of the role of local tobacco product and CRHAP exposure on lung cancer etiology in Nepal. The data will help inform local public health policies designed to address the increasing lung cancer burden in Nepal.

Dissertation Outline:

This dissertation is organized into 7 distinct chapters. The overall dissertation aims, objectives, and hypotheses is presented in Chapter 1. Introductory material including an overview of chronic diseases and specifically lung cancer in low and middle income countries (LMICs) is contained in the Background section (Chapter 2). Also contained in Chapter 2 includes a review of the current literature surrounding household air pollution (CRHAP) from biomass cooking fuels, human exposure to CRHAP, and measuring personal exposure via biomarkers. Also included in this chapter is a review of the literature and background material about traditional tobacco products and associated health outcomes. Lastly, Chapter 2 includes background material surrounding the details of this present study and data collection protocols.

Chapter 3 is the first peer-reviewed manuscript that investigates lung cancer risk associated with traditional tobacco use as mediated by ethnic group (Raspanti et al., 2015). This manuscript addresses a large research gap focusing on differential tobacco use among ethnic groups in Nepal and how various tobacco use patterns differentially influence lung cancer within these groups. We analyzed tobacco use by focusing on traditional tobacco (bidi, choor/kankat, and hookah) as well as commercial tobacco products (filtered and non-filtered

cigarettes). The analysis was stratified by self-identified ethnic group to determine lung cancer effect modification across these sub populations.

Chapter 4 is a draft of the manuscript that focuses on measuring personal exposure to CRHAP from biomass cooking fuels. Here, we used the primary metabolites of 1,3 butadiene, which is a known human carcinogen, to estimate personal-level dose as related to cooking fuel. These mercapturic acids have been used to measured personal exposures from a variety of sources including traffic and occupational origins. Using these well validated biomarkers, we gained insight into personal exposures to this known carcinogen derived from cooking fuels and cooking behaviors in Nepal. The association between these metabolites and lung cancer risk provides a significant advancement in measuring and understanding CRHAP exposures faced by billions of people around the world.

Chapter 5 is a draft manuscript expanding on the biomarker analysis in the previous chapter. We utilized the urinary biomarker data derived from the previous chapter and analyzed the association with lung cancer risk. Utilizing data collected from the study participants, we were able to control for many known environmental and behavioral contributions to observed biomarker concentrations. Major challenges exist in using a transient biomarker to be reflective of a long term exposure. Our study population may provide a unique

opportunity to use the metabolites of 1,3 butadiene as associated with lung cancer risk. By understanding this association, we can contribute to unveiling the pathway starting from initial CRHAP exposure to carcinogenesis.

Chapter 6 is a peer-reviewed manuscript investigating lung cancer risk associated with CRHAP exposures from biomass cooking fuels (Raspanti et al., 2016). In this manuscript, we created lifetime profiles of cooking fuel usage across all reported residences. We calculated total years of exposure to biomass cooking fuels (coal, wood, biomass, and kerosene) and modern fuels (natural gas and electricity) and compared lung cancer risk associated with usage of these fuels among a highly exposed population. Our analysis was stratified by tobacco usage to reduce the influence of this well-established cocktail of carcinogens. Here, we were able to confidently attribute lung cancer risk to biomass cooking fuel exposure in a dose-response manner. These results contribute significantly to understanding the impact of biomass cooking fuels and negative health outcomes in LMICs.

Lastly, Chapter 7 provides an overall synthesis of the dissertation as organized by each previous chapter. This synthesis contains an improved view of lung cancer in Nepal through the results of this dissertation. Also, we discuss the limitations of the research and directions for future work.

Chapter 2: Background

Common misperceptions exists that low income areas only need to focus valuable and often limited resources on immediate, infectious disease response; however, chronic disease account for an estimated 50% of disease burden within low and middle income countries. Recent estimates by the WHO estimate that nearly 80% of chronic disease burden lie in low and middle income countries (WHO, 2010). In fact, this increasing burden of disease can potentially cost $84 billion USD in economic production in these highly impacted areas (Abegunde et al., 2007). While these perceptions are not entirely misplaced, chronic diseases, such as cancer, are often overlooked or not fully understood. Major catalysts in the battle to combat, control, and reduce chronic diseases in these regions is unequal distribution of resources and access to high quality health care in a timely manner (Ebrahim et al., 2013; Fitzmaurice et al., 2015; Miranda et al., 2008; Nugent, 2008; WHO, 2010). While the plight of low income areas are often viewed through the historical lens of development among current high income areas, it is incorrect to view this process linearly and homogenous. Similar intervention and development strategies that work in one area of the world will not necessarily be as successful elsewhere. Cultural, social, and other contextual differences exist and can often interfere with the most well-intentioned efforts

(Aikins et al., 2012; Di Cesare et al., 2013). While large scale education aimed at behavior change have been attempted, small scale efforts that increase local capacity and infrastructure may be the most appropriate response given the global climate (Pisani, 2011).

Lung Cancer Burden in LMICs

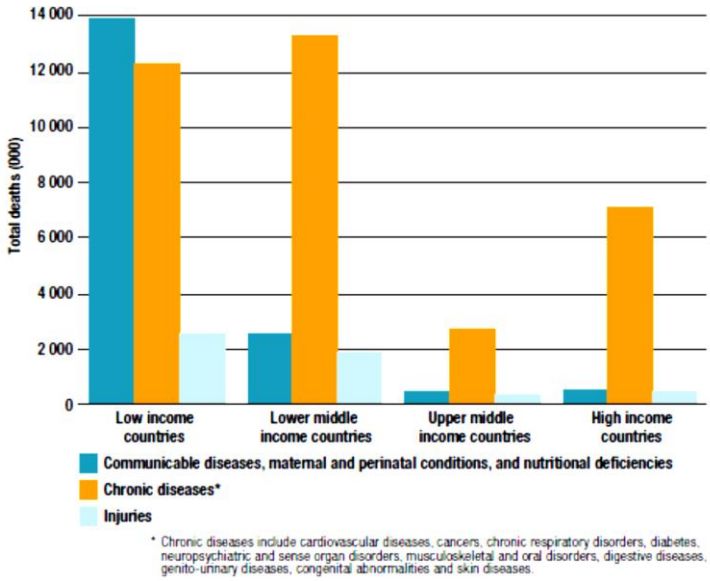
Mortality from cancer is on the rise, particularly among low and middle income countries around the world (IARC, 2012; WHO, 2012). Of the estimated 8.2 million deaths globally attributed to cancer, approximately 70% occurred in low and middle income countries (Frenk, 2009; WHO, 2012). It has been postulated that these numbers grossly under estimate the true burden of cancers in the developing world. Lack of access to preventive medical care as well as lack of access to care following cancer detection can severely increase mortality due to cancer. Furthermore, the increase in life expectancy, increased caloric intake, and other unhealthy habits have increased cancer rates in the developing world. Economic development has also been associated with increasing cancer incidence due to an increase in occupational and environmental exposures (Hashim and Boffetta, 2014; Lee and Hashibe, 2014). Other social indicators such as poverty, educational attainment, and family income have been shown to influence cancer incidence and mortality (de Vries et al., 2014; INCTR, 2014). Along with lack of surveillance, lack of monetary resources, lack of qualified physicians and nurses,

and other missing health infrastructure, cancer has and will continue to devastate the developing world (INCTR, 2014; WHO, 2010).

Often, low and middle income countries bear the largest burden of disease as shown in Figure 1. In most of these areas, lung cancer is the leading contributor to cancer deaths accounting for an estimated 1.59 million deaths (IARC, 2012). Majority of these cases are related to tobacco smoking, which accounts for roughly 22% of global cancer deaths and 71% of lung cancer deaths (Beaglehole et al., 2011; IOM, 2007; WHO, 2012). It has been estimated that up to one billion deaths can be adverted with targeted, global tobacco control efforts (Beaglehole et al., 2015). While tobacco use in high income areas is declining due to increased income and awareness, low income area are just entering the "tobacco epidemic" and smoking rates are expected to increase (Chiosi et al., 2015; Kuper et al., 2002; Lee and Hashibe, 2014; Martiniuk et al., 2010). Not only has tobacco smoking been commonly associated with lung cancer, but recent data confirm the influence of tobacco smoking on a variety of cancers (Beaglehole et al., 2015; WHO, 2015). Along with the well documented potency of tobacco, growing concern surrounding environmental contributions to lung cancer risk has been highlighted in recent publications (Bruce et al., 2015; Gordon et al., 2014; Lim et al., 2012; Lim and Seow, 2012; Sapkota et al., 2008). Through

improved cancer prevention strategies, IARC estimates that 2 million lives could be saved by 2020 and 6.5 million by 2040 (IARC, 2012).

Figure 1: Projected Deaths by Major Cause (WHO, 2005)



Lung Cancer Treatment in Nepal

Lung cancer is one of the most common cancer in both males and females in Nepal – one of the poorest countries in the world with 30 million people – accounting for 20% of all cancer cases (Binu et al., 2007; Pradhananga et al., 2009; "World Bank Nepal Data," 2012). Historically, cancer rates have been difficult to estimate due to poor cancer registry systems in Nepal. In 1991, Bir Hospital located in the capital city of Kathmandu was founded and offered rudimentary cancer services which included chemotherapy and surgery (Subedi and Sharma, 2012). Since then, more facilities have opened with a growing effort

on the part of the Nepalese government to prioritize health care to the highly rural Nepalese population. This effort has not been without setbacks. With poverty and limited education in much of the country, improving basic health care services and access has proven challenging, not to mention cancer prevention and control efforts. The vast majority of cancer services are concentrated in the Central, Kathmandu areas with limited reach to other parts of the country. Most recent estimates highlight B.P. Koirala Memorial Cancer Hospital (BPKMCH) as the top cancer treatment facility, serving the most cases in the county (Piya and Acharya, 2012; Subedi and Sharma, 2012). BPKMCH was founded in 1999 as a collaborative effort between the government of Nepal and the government of China to improve the oncology gap in Nepal (Piya and Acharya, 2012). This comprehensive cancer center boasts the top technological approaches to cancer care in the entire country. As observed in Figure 2, cancers of the trachea and lung are the leading cancers in 4 out of the 6 cancer centers in the country. In BPKMCH, cancers of the trachea and lung rank second behind cancer of the cervix and uterus.

Figure 2: Most Common Cancers by Hospital in Nepal (adapted from Subedi & Sharma, 2015)

Institutions	Region	I	II	III
Teaching Hospital, Manipal College of Medical Science, Pokhara, Kaski.	Western Region	Ca Head & Neck	Ca Lung	Ca Cervix Uteri
B.P.K. Memorial Cancer Hospital, Bharatpur, Chitwan.	Central Region	Ca Cervix Uteri	Ca Trachea & Lung	Ca Breast
Bir Hospital, NAMS, Kathmandu.	Central Region	Ca Trachea & Lung	Ca Stomach	Ca Breast
Bhaktapur Cancer Hospital, Bhaktapur.	Central Region	Ca Trachea & Lung	Ca Breast	Ca Cervix Uteri
Teaching hospital, Tribhuvan University, Kathmandu.	Central Region	Ca Trachea & Lung	Ca Stomach	Ca Breast
B.P.K. Memorial Institute of Medical sciences, Dharan, Sunsari.	Eastern Region	Ca Trachea & Lung	Ca Breast	Ca of Buccal cavity

Major challenges still exist in cancer treatment, control, and prevention in Nepal. One of the most important challenges is reaching the often remote rural populations. Nepal is known for its rough and unforgiving terrain and with an estimated 90% of the population living in rural areas, major efforts need to be made to provide improved basic health care to these regions. Secondly, the cost of care can further restrict those in rural poverty to break into the health care system. Lastly, extending health communication and education surrounding behavior change (ex. Tobacco cessation) to these populations remains a challenge (Farmer et al., 2010; Piya and Acharya, 2012). Given the challenges faced, large opportunities arise to make significant impacts on cancer in Nepal. Namely, strengthening the proactive approach of early detection and prevention has the

ability to greatly reduce many forms of cancer. A prime example would be an increased effort to reduce or eliminate tobacco use among Nepalese youth to attenuate ballooning lung cancer rates (Piya and Acharya, 2012; Subedi and Sharma, 2012). With great challenges comes great opportunity.

CRHAP Exposure from Biomass Fuels and Lung Cancer Risk

Overall, global biomass fuel use in the home for primary cooking purposes is estimated between 40-52% or approximately 2.8 billion people (Lim et al., 2012; Rehfuess et al., 2006). Estimates of global biomass fuel use ranges from 77% in African regions to less than 5% in developed nations (Rehfuess et al., 2006). Many components of CRHAP emissions are either known or suspected human carcinogens. The pyrolysis of organic material creates emissions that contain particulate matter, sulfur oxides, nitrogen oxides, carbon monoxide, polycyclic aromatic hydrocarbons, volatile organic compounds, formaldehyde, and dioxins (Abdullahi et al., 2013; Ding et al., 2012; EPA, 2007; Lim and Seow, 2012; Ward et al., 2008). In addition, the pyrolysis of coal emits lead, arsenic, and fluorine (Abdullahi et al., 2013; Desai et al., 2004). This influence of CRHAP emitted from burning biomass fuels on health is disproportionately observed in LMICs and extremely wide spread. There is a large need to better

understand and quantify the relationship between CRHAP exposures and health outcomes.

Recent publications have identified household air pollution, or CRHAP, to be a significant contributor to lung cancer and other pulmonary and cardiovascular illnesses (Barregard et al., 2008, 2006; Boman et al., 2003; Ganesh et al., 2011; Kampa and Castanas, 2008; Kurmi et al., 2012; Lam et al., 2012; Lee et al., 2013; Lim et al., 2012; Lim and Seow, 2012; Mortimer et al., 2012; Naeher et al., 2007, 2005; Rehfuess et al., 2009; Rylance et al., 2013). More specifically, about 3.5 million direct premature deaths from indoor exposures and 0.5 million deaths from outdoor air pollution caused by CRHAP, totaling nearly 4 million deaths annually is attributed to CRHAP (Lim et al., 2012). The effects are especially drastic in low and middle income regions where CRHAP is cited as the most important single environmental risk factor (Lim et al., 2012). Traditionally, the impact of using biomass fuels have disproportionately impacted women and children (Bates et al., 2013; Delfino et al., 2006; Desai et al., 2004; Epstein et al., 2013). Furthermore, the effects of using biomass fuels can extend to environmental degradation, global climate change, increased outdoor air pollution, and increased social inequalities (Gorin et al., 2006; IARC, 2010a).

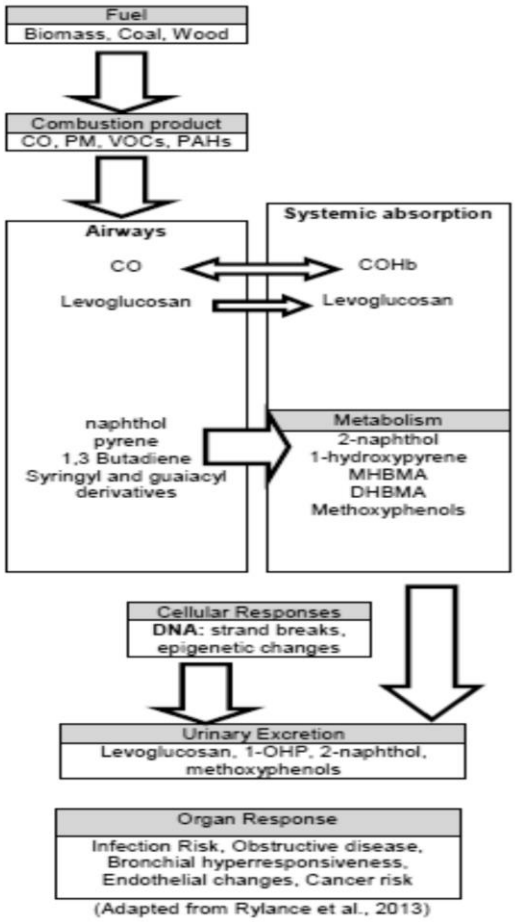
Previous research has clearly established the negative health impacts related to CRHAP from indoor biomass exposure including increased respiratory

infection, decreased lung function, aggravated asthma, irregular heartbeat, various cancers, heart attacks, and premature death in people with preexisting heart and lung diseases, inflammation, oxidative stress, neurologic development in children, and many others (Barregard et al., 2008, 2006; Delfino et al., 2006; Hashibe et al., 2010; Hejl et al., 2013; Lim and Seow, 2012; Sapkota et al., 2013, 2008; Seow et al., 2014; Stockfelt et al., 2012). Recent research has proposed that acute wood smoke exposure may have immunotoxic effects by impairing the pulmonary macrophages, effectively increasing the infection rates (Naeher et al., 2005; Rylance et al., 2015). Similarly, recent studies have shown increased urinary mutagenicity indicating DNA damage and mutation in those using biomass cooking fuels (Long et al., 2014). A number of various exposure assessment strategies have been used to estimate wood smoke exposure. Ambient air monitoring provides insight into the chemical composition of the air in a desired area which is very useful in large scale research projects. This method fails to provide accurate personal exposure measurements and ultimately no information regarding internal dose. Personal exposure measurements are taken in the individual's breathing zone which is generally defined as within 30 cm of the participant's nose and/or mouth (Nieuwenhuijsen, 2003). This method provides greater precision regarding potential personal exposure compared with ambient air monitoring; however, this does not provide information about internal dose. Personal monitoring is extremely difficult for large scale studies due to the cost

and time commitments. By far, the most effective and accurate method to measure wood smoke exposure is through measuring biomarkers.

Potential Biomarkers for CRHAP Exposure

Figure 3: Biomass Exposure Pathway



(Adapted from Rylance et al., 2013)

As defined by the EPA, biomarkers are measureable substances or characteristics in the human body that can be used to monitor the presence of a

chemical in the body, biological responses, or adverse health effects (EPA, 2012). Various biomarkers and the associated metabolic pathways have been investigated to measure wood smoke exposure in different human media. The most common method has been through urine samples. The research and need to identify an appropriate biomarker that can quickly and accurately quantify wood smoke exposure has been growing; however, no consensus has been reached in the scientific community on an appropriate urinary biomarker. Numerous studies have investigated urinary levoglucosan, polycyclic aromatic hydrocarbon metabolites, and volatile organic compound metabolites as potential indicators of wood smoke exposure, which traditionally has focused on the use of wood stoves in the home for cooking and heating purposes in acute exposure settings (Boman et al., 2003). These previous studies have yielded conflicting results into appropriate urinary biomarkers of wood smoke exposure. There has been no comprehensive evaluation of these biomarkers in high exposure settings which provides the greatest insight into urinary biomonitoring of wood smoke exposure. The need to develop a useful and effective wood smoke biomarker can advance current research knowledge and have the potential to reduce respiratory illnesses and even death. Ideally, a biomarker of wood smoke should include the following characteristics: 1) It should be uniquely derived from wood smoke; 2) it should be a relatively abundant constituent of wood smoke, such that ambient exposure levels generate sufficiently high biomarker levels to be measured reproducibly; 3)

the parent compound should be chemically stable in the environment, and the compound and it's metabolites should be chemically stable in biological samples (Naeher et al., 2005). In this project, we investigated wood smoke biomarkers with the goal of characterizing the effects of CRHAP and traditional tobacco products on lung cancer risk in Nepal.

Polycyclic Aromatic Hydrocarbon Metabolites

A large number of wood smoke related biomarkers have been investigated, but none have been identified as a universal target to measure wood smoke exposure conclusively. Depending on the health outcome of interest, many types of biomarkers in various media have been investigated. A popular biomarker to quantify wood smoke exposure has been urinary PAH metabolites (Kato et al., 2004; Nethery et al., 2012; Pruneda-Álvarez et al., 2012; Wang et al., 2008). Such metabolites are easily quantified in urine samples and can indicate an increased risk for cardiovascular illness and potential carcinogenic risk (Ruchirawat et al., 2010). Most notably, 1-hydroxypyrene and 2-naphthol are commonly used PAH metabolites tested in urine. Kato and colleagues concluded that urinary 2-naphthol was the most sensitive indicator of wood smoke exposure in a group of charcoal workers in Brazil (Kato et al., 2004). In a group of healthy women in Mexico, 1-hydroxypyrene levels were higher in women who use firewood as the primary cooking and heating fuel (Pruneda-Álvarez et al., 2012).

Furthermore, a study conducted in Peru focusing on pregnant women found that levels of OH-PAH metabolites, such as 2-hydroxy-fluorene and 3-hydroxy-fluorene, were significantly higher in women who exclusively used wood as a primary heating and cooking fuel when compared with groups who used natural gas or other combination of fuels (Adetona et al., 2013). When the women in Peru are compared to results from the 3rd National Health and Nutrition Examination Survey, women cooking with wood in Peru had 8 times higher 1-hydroxypyrene levels (Adetona et al., 2013). Also, using high efficiency wood stoves can greatly reduce the levels of 1-hyroxypyrene levels found in urine (Torres-Dosal et al., 2008).

Difficulties arise to conclusively identify wood smoke exposure due to the many sources of PAHs in the ambient air. Smoking, exposure to environmental tobacco smoke, car traffic, occupational exposure, and even diet can influence an individual's exposure to PAHs (Aquilina et al., 2010; Nethery et al., 2012; Siwińska et al., 1999). Although identifying PAH metabolites in human samples does indicate exposure to some sort of combusted organic material, it proves very difficult to conclusively identify wood smoke as the primary exposure. Similar to many other compounds, PAH exposure can occur via other routes besides inhalation and it has been suggested that inhalation may be the lowest contributor to overall PAH exposure (Aquilina et al., 2010).

Methoxyphenols

A second popular group of biomarker for wood smoke exposure is the methoxyphenol class of compounds. Methoxyphenols are byproducts of the burning of lignin which binds to cellulose in wood and contributes to the strength and hardness of wood (Clark, 2004). Emerging research shows promise in using urinary methoxyphenols to examine exposure to wood smoke. Not all methoxyphenol compounds are appropriate to use as biomarkers for wood smoke exposure (Clark et al., 2007; Dills et al., 2006; Neitzel et al., 2009). In a study conducted of healthy adults in Seattle who reported no exposure to wood smoke, guaiacol, 4-methylguaiacol, eugenol, and vanillin were found in all urine samples collected (Dills et al., 2001). Such results indicate that there exist some background level of certain methoxyphenols and exposure may be universal to some extent. The type of wood burned also contributes to the type of methoxyphenol biomarkers in urine. When hardwood is burned, the levels of urinary syringols are predominant, while the burning of softwood increase the level of urinary guaiacols (Dills et al., 2001). Geographic variations in type of wood use burned for fuel would complicate direct comparisons of exposure. Urinary methoxyphenols were found to be highly correlated with carbon monoxide and to a lesser extent levoglucosan exposure (Neitzel et al., 2009).

Similar to other biomarkers, methoxyphenols are highly influenced by dietary factors, reducing their specificity to wood smoke exposure.

Carboxyhemoglobin

Carboxyhemoglobin found in blood is a result of carbon monoxide binding to hemoglobin in red blood cells. Carbon monoxide is a common byproduct of incomplete combustion processes. The concentration of carbon monoxide following the burning of organic material is extensive and can potentially be used as a biomarker for wood exposure. The reduction of blood carboxyhemoglobin levels were observed after the instillation of high efficiency wood burning stoves when compared to using open fires (Torres-Dosal et al., 2008). Similarly, indoor levels of CO have been shown to be significantly reduced by installing a simple chimney stove (Smith et al., 2011, 2010; Smith-Sivertsen et al., 2009). However, carboxyhemoglobin levels are also very highly associated with smoking and second hand smoke exposure, complicating the relationship to wood smoke exposure.

Urinary Levoglucosan

Levoglucosan is a byproduct of burning cellulose which is common in wood (Migliaccio et al., 2009). Furthermore, levoglucosan has shown promise for its specificity to wood smoke. Levoglucosan is noted as being emitted in high concentrations from wood burning, it is associated almost exclusively with aerosols, and it is stable and non-reactive in the atmosphere for at least 10 days (Fraser and Lakshmanan, 2000; Yttri et al., 2005). Also, levoglucosan is not found in the smoke of lignite or semibitumious coal, but has been identified in small concentrations of cigarette smoke (Fabbri et al., 2008; Nolte et al., 2001; Simoneit et al., 1999). Urinary levoglucosan was found to be excreted rapidly from the body after exposure with a half-life of about 7 hours leaving a short window for sample collection (Moshammer et al., 2012). A recent study of the emissions from burning various types of wood has observed and quantified similar concentrations of levoglucosan in all types of wood examined. This indicates that levoglucosan can be used to examine wood smoke exposure in various geographic regions due to the universal presence in all types of wood smoke (Fine et al., 2002). This yields great promise in the use of levoglucosan as a biomarker of wood smoke exposure that can be used on a global scale compared with methoxyphenols.

One potential downside to urinary levoglucosan is the metabolic similarity with glucose. It has been postulated the glucose can sometimes be metabolized

into urinary levoglucosan effectively overestimating wood smoke exposure; however, Migliacco and colleagues concluded that glucose metabolism does not influence urinary levoglucosan concentrations (Migliaccio et al., 2009). Subsequently, the ingestion of caramel candy and other similar products are often treated as confounding variables (Bergauff et al., 2010; Moshammer et al., 2012). A major limiting factor is the uncertainty surrounding the metabolism of levoglucosan in humans. A recent study suggests that levoglucosan is excreted quickly and chemically unchanged (Moshammer et al., 2012). Furthermore, levoglucosan is a major organic component of particulate matter 2.5 and there seems to be background levels present in all urine samples reducing the ability to accurately quantify wood smoke exposure (Bergauff et al., 2010; Gorin et al., 2006). It has been estimated that levoglucosan is the main constituent in fine particulate emissions from wood burning, contributing about 18%-30% of the organic fine particulate emissions (Schauer et al., 2001). Alternatively, another study has determined that using levoglucosan alone is not sufficient to use as an indication of ambient wood smoke contributions to PM, rather levoglucosan needs to be used in tandem with other chemical components of wood smoke (Hedberg et al., 2006). Urinary levoglucosan has been proposed to be an indication of PAH exposure specifically in areas where wood is a primary fuel source as well (Wallner et al., 2013). Levoglucosan is also present in the pyrolysis of other biomass such as tobacco, grass, crops, or rice straw (Naeher et

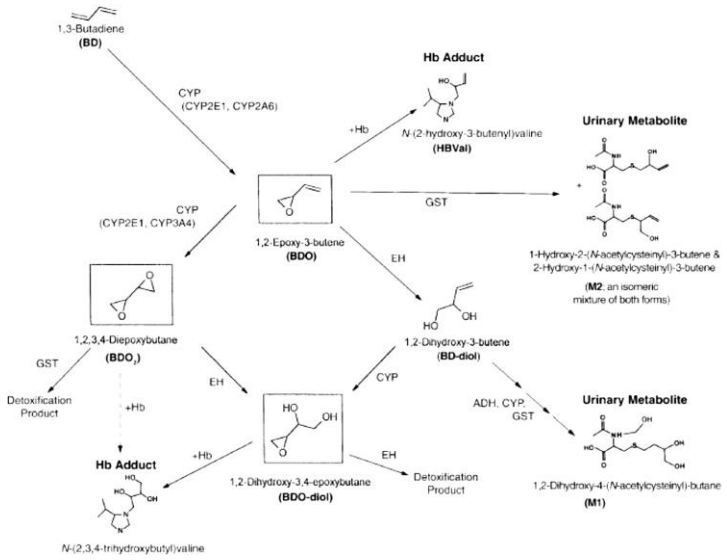
al., 2005). Urinary levoglucosan has shown great promise in areas where wood is the major fuel source however some concerns remain about urinary levoglucosan as an effective biomarker for wood smoke exposure due to high variability in human diets (Bergauff et al., 2010; Fabbri et al., 2008; Hinwood et al., 2008; Rylance et al., 2013). In low and middle income countries, the use of EPA certified wood stoves has shown significant reduction in indoor airborne levoglucosan levels when compared with open fires indicating the strong relationship between levoglucosan and wood smoke (EPA, 2007). Strong evidence exists for levoglucosan to be used as a urinary biomarker for wood smoke exposure; however, many limiting factors and conflicting studies have decreased the scientific consensus.

Metabolites of 1,3 Butadiene

Due to the carcinogenicity of 1,3 butadiene, the ability to accurately measure human exposure and associated health risk is necessary in many different settings. 1,3 butadiene is a colorless gas mainly used in rubber production and plastic manufacturing (ATSDR, 2012). An estimated 6 billion pounds of 1,3 butadiene is produced annually in the United States with another 600 million pounds imported (ATSDR, 2012). An estimated 60% of this manufactured and imported 1,3 butadiene is used in synthetic rubber manufacturing mainly used in

car and truck tires (ATSDR, 2012). It is released into the environment via industrial release, but also commonly released as a byproduct of gasoline combustion in vehicles. A noted natural source of 1,3 butadiene is forest fires, but most environmental contributions are of anthropogenic origins. 1,3 butadiene has an environmental half-life of about 6 hours and is highly volatile (ATSDR, 2012). The vast majority of human exposure is via inhalation and 1,3 butadiene is further absorbed into the bloodstream. On its own, 1,3 butadiene is not carcinogenic or biologically reactive, but is it bioactivated into carcinogenic products as shown in Figure 4 (R J Albertini et al., 2003).

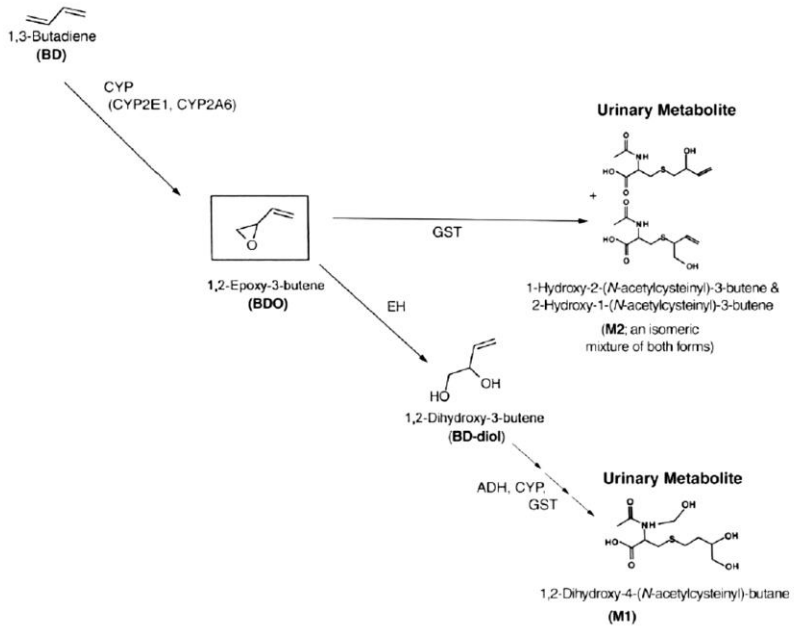
Figure 4: Toxicokinetics of 1,3 Butadiene (R J Albertini et al., 2003)



Catalyzed predominately by cytochrome P450 2E1, 1,3 butadiene is oxidized into 1,2-Epoxy-3-butene (BDO), further oxidation produces BDO2. A

second pathway detoxifies BDO via hydrolysis and glutathione *S*-transferase create 1,2-dihydroxy-3-butene (BD-diol) (Richard J Albertini et al., 2003; ATSDR, 2012). BD-diol and BDO2 can both be transformed in 1,2 Dihydroxy 3,4 epoxybutane or BDO-diol. BDO, BDO-diol, and BDO2 all have the ability to interact with proteins including DNA causing DNA adducts leading to mutations and ultimately carcinogenesis if left unresolved (Richard J Albertini et al., 2003; ATSDR, 2012). The estimated biological clearance of 1,3 butadiene is about 10 hours following inhalation (ATSDR, 2012). Monohydroxy-3-butenyl mercapturic acid (MHBMA) and 1,2-dihydroxybutyl mercapturic acid (DHBMA) are two major metabolites of 1,3 butadiene commonly measured in urine highlighted in Figure 5 (Albertini et al., 2001; Boogaard et al., 2001; Fustinoni et al., 2004; R F Henderson et al., 1996; Sapkota et al., 2006; Urban et al., 2003).

Figure 5: Highlighted Detoxification of 1,3 Butadiene into Major Urinary Metabolites [REFs]



According the EPA's Integrated Risk Information System, the chronic airborne reference concentration is set at 0.9 ppb (EPA, 2014). This is based mouse models of developmental and reproductive outcomes as there is no human based data on these health endpoints. Recently, the EPA has changed their classification of 1,3 butadiene from a "probable human carcinogen" to conclude that there exists "sufficient evidence" based on occupational epidemiologic studies that 1,3 butadiene is carcinogenic to humans with the proposed target organ to be the lymphohematopoietic system due to evidence of increased lymphoma and leukemia (Richard J Albertini et al., 2003; EPA, 2014). Other notable regulations include OSHA's 8-hour TWA for 1,3 butadiene exposure is 1

ppm and a short term exposure limit of 5 ppm for 15 min (OSHA, 2013). While industry is the main avenue for environmental release, growing evidence has identified residential wood combustion as another source of concern.

Recent publications have shown that indoor 1,3 butadiene levels are higher among those who burn wood for cooking and/or heating subsequently increasing personal exposure (Gustafson et al., 2007; Sällsten et al., 2006). Difficulties arise when targeting MHBMA/DHBMA as they are ubiquitous in the ambient air. Similar to other biomarkers, both metabolites are created from the combustion of organic material not specific to wood smoke. Other sources of MHBMA/DHBMA include industrial processes, vehicle emissions, tobacco smoke, and dietary influences (Albertini et al., 2001; ATSDR, 2012; Gustafson et al., 2007; Sapkota et al., 2006; Soeteman-Hernández et al., 2013). The ubiquitous presence of 1,3 butadiene raises concern about the usefulness of MHBMA/DHBMA as indicators of wood smoke. Much of the research has focused on 1,3 butadiene exposures in occupational settings, which are not generalizable to the general population (Albertini et al., 2001; Richard J Albertini et al., 2003; Boogaard et al., 2001; Sapkota et al., 2006). The main advantage of using MHBMA/DHBMA is the validated and established laboratory methods (Osterman-Golkar and Bond, 1996; Sapkota et al., 2006; Schettgen et al., 2009; Urban et al., 2003). Using metabolites of 1,3 butadiene may not be the most

appropriate in areas heavily influenced by vehicle traffic and industrial sources; however, such metabolites may be useful in low and middle income agricultural populations. Rural areas, especially in low and middle income countries, are less impacted by vehicle traffic and industrial sources compared with large urban centers. These geographic and socio-economic characteristics allow the research team to investigate MHBMA/DHMBA as indicators of wood smoke exposure.

Role of Local Tobacco Products on Lung Cancer Risk

Mortality from cancer is on the rise, particularly among low and middle income countries around the world (WHO, 2012). Lung cancer is the leading contributor to cancer deaths accounting for an estimated 1.59 million deaths (WHO, 2012). Majority of these cases are related to tobacco smoking, which accounts for roughly 22% of global cancer deaths and 71% of lung cancer deaths (IOM, 2007; WHO, 2012). However, there is considerable variability in the type of tobacco products used, particularly in the low income countries. Understanding the risk associated with these local tobacco products is important to inform more meaningful and culturally competent intervention strategies.

Increasing body of literature suggests that the potency of these local tobacco products is not similar (IARC, 2010b). For example, traditional tobacco products are used nearly 7-8 times more frequently than commercial tobacco

products (WHO, 2008). Bidi cigarettes are hand-rolled loose tobacco contained within a leaf, commonly found in India and Southeast Asia (WHO, 2008). The amount of tobacco used and inhalation rates and volumes vary dramatically even within individuals making it difficult to estimate personal usage. As with commercial cigarettes, bidi smoke contains a wide range of known and suspected carcinogens, including the highly addictive nicotine (Prasad et al., 2009; WHO, 2008). Bidi usage has been strongly connected with socioeconomic status. Usually, they are offered as a cheaper alternative to commercial tobacco products and used predominately by rural, low income populations. Often these populations have limited access to health care or tobacco cessation efforts (WHO, 2008). Furthermore, bidi usage has been associated with diabetes, cardiovascular health effects, and various cancers (Ganesh et al., 2011; Gupta et al., 2001; Jussawalla and Jain, 1979; Kolappan, 2002; NHRC, 2010; Notani et al., 1977; Pais et al., 2000; Prasad et al., 2009; WHO, 2008).

Several studies from India have shown that bidi smoking is associated with higher risk of lung cancer risk compared to commercial cigarettes (Dikshit and Kanhere, 2000; Ganesh et al., 2011; Gupta et al., 2001; Jussawalla and Jain, 1979; Notani and Sanghvi, 1974; Notani et al., 1977; Prasad et al., 2010; Sharma and Bansal, 2013; WHO, 2008). The relationship between tobacco smoking and lung cancer has long been established however the influence of traditional

tobacco products in low and middle income countries is less certain. Differences in health perceptions may increase in the use of these traditional tobacco products as they as incorrectly deemed safer than commercial products (WHO, 2008). Similarly, the frequency of use, variation in inhalation volume and force, and amount of tobacco per each "cigarette" will influence carcinogenesis (IARC, 2010b; O'Connor, 2012; Pednekar et al., 2011).

A brief examination of our pilot data revealed that there is considerable variation in smoking habits between gender and ethnic subgroups as well as geographical region. Males are known to smoke more frequently and for longer duration compared to women (Chawla et al., 2010; Noronha et al., 2012). Variability in female smoking prevalence is linked to how smoking is perceived across the subgroups. Among Brahmins and Chettris, smoking by females is considered socially unacceptable in contrast to Rai/Limbu/Magar/Tharu/Other where smoking is more acceptable. In addition to the differences in smoking prevalence across ethnic subgroups, there is considerable difference in the type of local tobacco products consumed by these subgroups.

Hospital-based Lung Cancer Study in Nepal

Nepal is a dynamic and diverse country where tobacco use and exposure to household air pollution from biomass cooking fuels is prevalent. Our study

design and study population is large and diverse enough to generalize our findings to Nepal as a whole. This provides a unique opportunity to investigate these major environmental concerns on a country-level with large scale implications. We have confidence that our approach can be duplicated in other low and middle income countries allowing for country-specific analysis of environmental contributions to lung cancer resulting in targeted and culturally competent public health interventions. Overall, we are confident that our investigation into environmental contributions to lung cancer risk in Nepal can serve as a basis for targeted public health interventions aimed at exposure reduction. Removing or reducing household air pollution and tobacco exposure can drastically increase the health of the Nepali population.

A hospital-based case-control study was conducted at the B.P. Koirala Memorial Cancer Hospital (BPKMCH), located in the city of Bharatpur, Chitwan District, Nepal, from November 2009 through December 2012. Located 150 kilometers southwest of Kathmandu, BPKMCH is the major cancer hospital in Nepal. The details regarding participant recruitment and biological sample collections have been described previously (Hashibe et al., 2010; Raspanti et al., 2015). In brief, 606 incident lung cancer cases and 606 age and gender matched controls were recruited from the hospital after receiving informed consent.

The inclusion criteria for a lung cancer case were as follows: 1) they are 18 years of age or older 2) they are a resident of Nepal for at least five years and 3) they were admitted to BPKMCH. The eligible cases were recruited as soon as possible following lung cancer diagnosis with a target interval of one day and a maximum interval of 4 weeks. A trained medical staff reviewed medical records to extract relevant diagnostic information, including the date and method of diagnosis, histological type, tumor location, stage, and grade. Final diagnosis of lung cancer was confirmed with histological, cytological, or X-ray based evidence. The control population was selected from various hospital visitors to frequency match the distribution of the case population by age, sex, ethnicity, and residence. The controls were visitors at BPKMCH excluding, family members of participating lung cancer cases. Prior to field implementation, standardized lifestyle and food frequency questionnaires were translated into Nepali language by native speakers and pilot tested in the field. Locally trained interviewers collected information on demographic characteristics, education, residential mobility throughout lifetime, type of cooking and heating fuel used at each residence, occupational history, and family history of cancer. The study was approved by the Institutional Review Board at the University of Utah, University of Maryland as well as the Government of Nepal (Nepal Health Research Council).

Biological samples were collected from all study participants following informed consent and administration of the questionnaire. The samples collected include urine, blood, hair, nail, and buccal swabs from all study participants. Samples were collected according to a standardized protocol by trained hospital staff members. The samples were stored in -80° C freezers on site until transport to the University of Maryland. The samples were shipped on dry ice and arrived at the University of Maryland Exposure Assessment laboratory in the School of Public Health. The biological samples were immediately transferred to -80° C freezers in the laboratory until analysis. Samples were thawed at room temperature prior to analysis.

The goal of this dissertation is to investigate environmental contributions to lung cancer in Nepal. The first manuscript focuses on tobacco as a contributor to lung cancer. This manuscript investigates the differential effects of traditional tobacco products and commercial tobacco products on lung cancer risk. Furthermore, we investigated the relationship between tobacco smoking patterns of ethnic subgroups and the contribution to overall lung cancer risk. The second manuscript aims to explore household air pollution originating from biomass cooking fuel use contributes to lung cancer risk. Here, we categorize fuel use into modern and biomass cooking fuel types and we analyze how duration and type of biomass cooking fuel use can influence lung cancer risk. Lastly, our final manuscript aims to explore urinary biomarkers associated with household air

pollution derived from biomass cooking fuels. Using novel laboratory methods and analysis, this study fills a large research gap in measuring personal household air pollution exposure. Together, these three manuscripts provide insight into major environmental causes of lung cancer risk in Nepal.

This study is highly significant to public health as it deals with an important environmental exposure (CRHAP) affecting over 3 billion people as well as health outcome (lung cancer) that continues to be a leading cause of cancer mortality. Our findings will further the understanding of the role of CRHAP exposure in the carcinogenesis of the lung. Results will provide an impetus for targeted interventions that can potentially reduce lung cancer mortality in Nepal. Furthermore, an effective biomarker to quantify wood smoke exposure has not been identified and recent publications have identified this research gap (Clark et al., 2013; Martin et al., 2013; Rylance et al., 2013). This study will have a high public health impact because it will result in: 1) the identification of a useful and effect biomarker used to quantify wood smoke exposure 2) the quantification of lung cancer risk resulting from using biomass fuel use. The larger implications related to household air pollution may yield the greatest public health impact as this project can propel wood smoke exposure research in other LMICs that rely on wood as a primary heating and cooking fuel.

Chapter 3: Ethnic Variability in Consumption of Traditional Cancer Products and Lung Cancer Risk in Nepal

Raspanti, G.A., Hashibe, M., Siwakoti, B., Wei, M., Thakur, B.K., Pun, C.B., Milrod, C., Adhikari, S., Lee, Y.-C.A., Sapkota, A., 2015. Ethnic Variation in Consumption of Traditional Tobacco Products and Lung Cancer Risk in Nepal. Asian Pac J Cancer Prev 16, 5721–5726.

Abstract

Lung cancer is the leading contributor to cancer deaths in the developing world. Within these countries, significant variability exists in the prevalence of lung cancer risk, yet limited information is available whether some of the observed variability is associated with differences in the consumption pattern of local tobacco products with differing potency. We recruited 606 lung cancer cases and 606 frequency matched controls from the B.P. Koirala Memorial Cancer Hospital in Nepal from 2009-2012. We estimated odds ratios (ORs) and 95% Confidence Intervals (CI) for lung cancer risk associated with different tobacco products, using unconditional logistic regression. Unfiltered cigarettes tended to be the most frequently used across ethnic subgroup with about 53.7% of Brahmin, 60,1% of Chettri, and 52.3% of Rai/Limbu/Magar/Other. In contrast, about 39.9% of Madishe/Tharu smokers reported using bidi compared with only

27.7% who smoked unfiltered cigarettes. Among those who only smoked one type of product, choor/kankat smokers had the highest lung cancer risk (OR 10.2; 95% CI 6.2-16.6), followed by bidi smokers (OR 5.6; 95% CI 3.6-8.7), unfiltered cigarettes (OR 4.9; 95% CI 3.4-7.2), and filtered cigarettes (OR 3.4; 95% CI 2.2-5.3). A clear dose-response relationship was observed between increased frequency of smoking and lung cancer risk across all ethnic subgroups. These results highlight the important role of traditional tobacco products on lung cancer risk in the low income countries.

Introduction

Mortality from cancer is on the rise, particularly among low and middle income countries around the world (WHO, 2012). Of the estimated 8.2 million deaths globally attributed to cancer, approximately 70% occurred in low and middle income countries (IOM, 2007; WHO, 2012). In most of these areas, lung cancer is the leading contributor to cancer deaths accounting for an estimated 1.59 million deaths (IARC, 2012). Majority of these cases are related to tobacco smoking, which accounts for roughly 22% of global cancer deaths and 71% of lung cancer deaths (IARC, 2012; IOM, 2007; WHO, 2012). However, there is considerable variability in the type of tobacco products used, particularly in the

low income countries. With a projected increase in lung cancer in the region, understanding the lung cancer risk associated with these local tobacco products is important to inform more meaningful and culturally competent intervention strategies (Bhagabaty et al., 2015; D'Souza et al., 2013; Thapa and Sayami, 2014).

Lung cancer is the most common cancer in both males and females in Nepal – one of the poorest countries in the world with 30 million people – accounting for 20% of all cancer cases (Binu et al., 2007; Pradhananga et al., 2009; "World Bank Nepal Data," 2012). Nepalese men are known to smoke more frequently and for longer duration compared to women (Chawla et al., 2010; Noronha et al., 2012). Approximately, 52% of Nepalese men smoke some form of tobacco products, while 13% of women reported smoking tobacco (USAID, 2012). The most common used tobacco product in Nepal is filtered cigarettes accounting for approximately 30% of male smokers (USAID, 2012). But there is considerable variation in female smoking prevalence across race and ethnicity as well as geographic areas. This variability in female smoking prevalence is linked to how smoking is perceived across the subgroups. Among Brahmins, smoking by females is considered socially unacceptable in contrast to Rai/Limbu/Magar/Other where smoking by females is more acceptable. In addition to the differences in smoking prevalence across ethnic subgroups, there is considerable difference in the type of local tobacco products consumed by these

subgroups. Limited work has been conducted in Nepal, while an increasing body of literature suggests that the potency of these local tobacco products is not similar in neighboring India (Jayalekshmy et al., 2008; Noronha et al., 2012; Pednekar et al., 2011; Prasad et al., 2010, 2009). For example, several studies in India have shown that bidi smoking is associated with higher risk of lung cancer risk compared to commercial cigarettes (Dikshit and Kanhere, 2000; Ganesh et al., 2011; Gupta et al., 2001; Jussawalla and Jain, 1979; Notani and Sanghvi, 1974; Notani et al., 1977; Prasad et al., 2010). Smokers in Nepal use additional loose tobacco that they hand roll into a cigarette, commonly locally referred to as choor/kankat and cancer potency of suck products remains unknown.

In this paper, we use data from hospital-based case control study from Nepal to i) quantify lung cancer risk associated with local tobacco products that are commonly used in Nepal (bidi and choor/kankat) versus commercial tobacco smoking (filtered and unfiltered cigarettes) and ii) investigate if the observed racial/ethnic differences in lung cancer risk in Nepal is associated with the differences in type of local tobacco product used.

Materials and Methods

A hospital-based case-control study was conducted at the B.P. Koirala Memorial Cancer Hospital (BPKMCH), located in the city of Bharatpur, Chitwan

District, Nepal, from November 2009 through December 2012. Located 150 kilometers southwest of Kathmandu, BPKMCH is the major cancer hospital in Nepal. The details regarding participant recruitment and biological sample collections have been described previously (Hashibe et al., 2010). In brief, 606 incident lung cancer cases and 606 controls were recruited from the hospital after receiving informed consent.

The inclusion criteria for a lung cancer case were: 1) 18 years of age or older 2) resident of Nepal for at least five years and 3) admitted to BPKMCH. The eligible cases were recruited as soon as possible following lung cancer diagnosis with a target interval of one day and a maximum interval of 4 weeks. A trained medical staff reviewed medical records to extract relevant diagnostic information, including the date and method of diagnosis, histological type, tumor location, stage, and grade. Final diagnosis of lung cancer was confirmed with histological, cytological, or X-ray based evidence. The control population was selected from various hospital visitors to frequency match the distribution of the case population by age (+/-) 5 years, sex, ethnicity, and residence. The controls were visitors at BPKMCH excluding, family members of participating lung cancer cases. Prior to field implementation, standardized lifestyle and food frequency questionnaires were translated into Nepali language by native speakers and pilot tested in the field. Locally trained interviewers collected information on demographic characteristics, education, residential mobility throughout lifetime,

type of cooking and heating fuel used at each residence, occupational history, and family history of cancer. The study was approved by the Institutional Review Board at the University of Utah, University of Maryland as well as the Government of Nepal (Nepal Health Research Council).

Tobacco use data was derived from questionnaires completed immediately following participant enrollment. Participants were asked if they have smoked more than 100 cigarette/bidi/kankat/choor over their lifetime. Non-smokers are classified as those who answered "no" to the aforementioned question and non-smokers are used as the reference group during analysis. If answered "yes", the participant then reported the age of starting and stopping (if appropriate) and quantity used per day for each type of tobacco product individually. We computed a lifetime profile of smoking habits based on duration and frequency of each type of tobacco products used. Furthermore, these profiles were categorized to reflect single vs. multiple product users. The type of tobacco products included: filtered cigarettes, unfiltered cigarettes, bidi, and choor/kankat. The last category (choor/kankat) is loose local tobacco products that individuals wrap themselves. Dichotomous variables were created to reflect the use of each type of tobacco products. A variable reflecting duration and frequency of smoking was created for each product type [PACKYEARS = reported duration of smoking in years x frequency of smoking / 20]. These product specific PY variables were summed to generate total pack years.

In addition, we also computed exposure to household air pollution derived from biomass cooking fuels (CRHAP) based on residential history and type of fuel used for cooking at each residence. We calculated a composite index of SES using scores for level of education, household income, and crowdedness (number of individuals living per room) as previously described (Ghosh and Ghosh, 2009; Sapkota et al., 2008)

We used unconditional logistic regression to estimate the odds ratios (ORs) and 95% confidence intervals (95% CIs) for lung cancer risk associated with different traditional and commercial tobacco products and duration of tobacco use. The models were adjusted for sex (male/female), ethnicity, zone of residence, age, CRHAP index, and SES index. These potential confounding variables were chosen based on prior literature documenting their influence on lung cancer risk and smoking habits. We considered adjusting for family history of lung cancer to account for the influence of genetic susceptibility. However, only two individuals in the study reported a family member with lung cancer and in both instances the family members were smokers.

Results

The demographic characteristics of the study participants are provided in Table 2. In this study, males accounted for 56% of the total study population

compared to 44% for females. In general, cases tended to be slightly older and less educated compared to controls. The vast majority of both cases (88%) and controls (91%) were Hindus. The largest ethnic groups for cases were Chettri, Brahmin, Magar, and Madishe (20.5%, 19.3%, 17.3%, and 10.1% respectively). In comparison, the largest control groups were Brahmin, Chettri, Madishe, and Magar accounting for 25.2%, 17.2%, 12.9%, and 7.4% of the control population respectively. The largest difference between cases and controls observed in this study was in the Magar caste, accounting for 17.3% of cases but only 7.4% of the controls. About 23.7% of cases and 29.7% of controls are classified as "other". Ethnic groups not included in the questionnaire include Janajati and Dalit groups as well as other smaller sub-populations not captured by the questionnaire options.

Overall, the average years of tobacco use was 40.3 years. Compared to controls, lung cancer cases significantly smoked tobacco longer (mean in years: 55.2 vs. 25.5; *p-value < 0.001)* and more frequently (PY: 30.4 vs. 17.6; *p-value < 0.001)*. We observed variation in smoking prevalence and type of tobacco products used within ethnic subgroups (Table 3). Ethnic subgroups were combined based upon cultural and geographical similarities. Across all subgroups, unfiltered cigarettes was the most frequently used tobacco product, with prevalence ranging from 60.1% of Chettri to 52.3% of Rai/Limbu/Magar/Other. A noted exception to this was the Madishe/Tharu group for whom the

predominant tobacco product was bidi (39.9%) followed by unfiltered cigarettes (27.7%).

Not surprisingly, ever smokers had an increased risk of lung cancer when compared to those who reported never smoking (OR 4.95; 95% CI 3.50-7.01). We observed increased lung cancer risk among females compared with males (OR 1.76; 95% CI 1.34-2.36) and older participants (OR 1.05; 95% CI 1.03-1.06). Furthermore, we observed a decreasing lung cancer risk as SES increased (OR 0.76; 95% CI 0.66-0.87). Overall, all types of tobacco product usage were independently associated with increased lung cancer risk compared with nonsmokers (Table 4). Specifically we observed highest product specific risk for choor/kankat (OR 11.2; 95% CI 6.6-19.3) followed by bidi (OR 6.1; 95% CI 4.2-9.1), unfiltered cigarettes (OR 5.6; 95% CI 3.9-8.1), and filtered cigarettes (OR 4.2; 95% CI 2.8-6.2) when compared to nonsmokers. Across ethnic subgroups, all tobacco products were associated with an increased lung cancer risk compared with non-smokers; however, the degree of the association varied by ethnic subgroup. Among Brahmins the highest product specific OR was observed for choor/kankat, followed by bidi and unfiltered cigarettes [OR (95% CI) 11.7 (5.0-27.4), 10.9 (5.1-23.1), 9.2 (4.5-18.7) respectively]. Chettri, Madishe/Tharu, and Rai/Limbu/Magar/Other groups showed similar patterns with the highest product specific ORs observed for choor/kankat [OR (95% CI) 9.1 (3.5-23.4), 14.1 (5.6-35.9), and 6.9 (3.6-13.1) respectively], followed by bidi [OR (95% CI) 6.9 (3.1-

15.8), 7.2 (3.4-35.9), and 5.2 (3.0-8.8) respectively] and unfiltered cigarettes [OR (95% CI) 5.6 (2.5-12.5), 6.1 (2.9-12.8), and 4.5 (2.7-7.4) respectively].

We conducted sub analysis where multiple product users were assigned to a single product that was used for the longest duration. This did not change our overall findings significantly. Similarly to the previous analysis, the highest product specific ORs were observed for choor/kankat (OR 10.2; 95% CI 6.2-16.6) followed by bidi (OR 5.6; 95% CI 3.6-8.7) and unfiltered cigarettes (OR 4.9; 95% CI 3.4-7.2). We observed the similar product specific effects when stratified across all ethnic subgroups.

We detected associations between the number of tobacco products used and lung cancer risk (Table 5). Lung cancer risk appeared to increase linearly with number of tobacco products consumed, with a noted exception for Madishe/Tharu groups (Table 5). Overall, the highest observed OR was for those who reported using 3 or more types of tobacco products (OR 7.0; 95% CI 4.3-11.4) compared with nonsmokers. Similar trends were observed for every ethnic group with highest lung cancer risk among those reporting using 3 or more types of tobacco.

To investigate if the frequency and duration of smoking has a different effect across the ethnic subgroups, we stratified the analysis for tobacco PY and lung cancer risk by ethnicity (Table 6). Within each group, we detected exposure-

response relationships between the tobacco PY and the risk of lung cancer ($P_{TREND} < 0.001$). We observed potential variability in the strength of association across ethnic subgroups. For example, the effect estimate for the highest exposure group (30+ PY) varied from 9.1 (CI 4.7-17.9) for Rai/Limbu/Other group to 23.7 (CI 9.5-59.2) for Brahmin group.

Discussion

We analyzed data from a hospital based case-control study of lung cancer in Nepal to investigate possible differences in lung cancer risk across different ethnic subgroups with differences in smoking prevalence as well as the specific types of tobacco products consumed.

We observed significant differences among the type of tobacco used by ethnic subgroups. Large percentages of Brahmin, Chettri, and Rai/Limbu/Magar/Other smokers used unfiltered cigarettes compared to Madishe/Tharu smokers who tended to use bidi. Analysis focused on product specific effects showed those using choor/kankat had the highest risk followed by bidi and unfiltered cigarettes. The differences observed between those smoking multiple types of tobacco products may be driven by underlying social and cultural perceptions held by the specific group.

A clear exposure-response relationship was observed, both for number of tobacco smoked and tobacco-PY and lung cancer risk. This association persisted across ethnic subgroups. One surprising finding is the strength of association for duration of tobacco used and lung cancer risk across the ethnic subgroups. For the longest duration of exposure, the strongest effect was observed among Brahmins, although their prevalence of consumption for the two highest risk products (choor/kankat and bidi) was lower than the other groups. This is consistent with the product specific risk (Table 4) as well as the risk associated with number of tobacco products smoked.

There are several strengths of this study. This is the first study to provide local tobacco specific risk estimates on lung cancer risk among a very diverse and underserved population in Nepal. The relatively large sample size allowed us to look at product specific risk. There are some limitations associated with our study as well. Mainly, complete independence was not achieved within the product specific tobacco categories. Our aim was to identify the product specific lung cancer risk. Secondly, the information regarding the tobacco types were assessed using a questionnaire, so potential recall bias cannot be ruled out. Even though it may be difficult to directly compare all types of tobacco smoking, we can reasonably conclude that smoking traditional tobacco products increases the risk of lung cancer more dramatically when compared with the nonsmoking population.

The implications of such results reinforce the dangerous health concerns surrounding smoking in low income countries. More precisely, those who smoke traditional or local types of tobacco products are at a higher risk for lung cancer when compared to other types of tobacco smoking and nonsmoking groups. The explanation of these differences may lie in the industrial processing of commercial tobacco compared to locally grown tobacco or that people may smoke traditional tobacco products more frequently due to misplaced perceptions of safety. Filtering of commercial cigarettes may play a role as well, but does not fully explain the commercial unfiltered cigarettes. While all tobacco products are harmful, our results show that the relative potency of the tobacco products is different. While risk perception is relatively high surrounding the role of tobacco and lung cancer, risk perception about other health effects, namely heart disease and other cancers, related to tobacco use is comparatively low which allows room for improved health messaging surrounding tobacco use (Gupta and Johnson, 2014; Gupta and Kumar, 2014; Peltzer and Pengpid, 2014). This comprehensive evaluation of various tobacco products and smoking habits within ethnic groups in Nepal is the first of its kind and further research is needed to investigate the cultural and social norms driving differential tobacco use.

Conclusions

In summary, some variation was observed between types of tobacco used across ethnic groups. Similarly, a marked variability was observed in the potency of local tobacco products, particularly choor/kankat. Our findings suggest these cheaper products that smokers buy in bulk and roll themselves are more harmful. This poses a particular challenge since the extensive warning signs that have been used in the packaging materials to warn against the harm of tobacco products are not applicable for such loose products. A separate intervention strategy is warranted to warn users of such cheap local products, who are more likely to be of low SES even by the LMIC standards.

Author Contributions

All authors contributed significantly to the conceptualization, execution, and/or reporting of this research project. Bhola Siwakoti, Binary Kumar Thakur, and Chin Bahadur Pun recruited study participants and collected original data. Greg Raspanti and Amir Sapkota analyzed the data, conceived the research questions, and drafted the manuscript. Charles Milrod conducted literature review and contributed to manuscript drafts. Yuan-Chin Amy, Mia Hashibe, Subodh

Adhikari, and Mei Wei contributed to data analysis and final manuscript preparation.

Conflict of Interest

The authors declare no conflict of interest.

Manuscript 1 Tables

Table 2: Demographic Characteristics

		Lung Cancer Cases		Controls		
		N	%	N	%	X^2 *(p-value)*
Age						98.5 (<0.001)
	<40	27	4.5	24	3.9	
	40-49	45	7.4	121	19.9	
	50-59	152	25.1	231	38.1	
	60-69	251	41.4	179	29.5	
	70 +	131	21.6	51	8.4	
Gender						6.0 (0.01)
	Male	338	55.8	380	62.7	
	Female	268	44.2	226	37.3	
SES Index						49.3 (<0.001)
	Q1 (Low)	180	29.7	109	18	
	Q2 (Mid-Low)	170	28.1	150	24.8	
	Q3 (Mid-High)	153	25.6	148	24.4	
	Q4 (High)	103	17	199	32.8	
Ethnicity						47.5 (<0.001)
	Brahmin	117	19.3	153	25.3	
	Chettri	124	20.5	104	17.2	
	Rai	20	3.3	14	2.3	
	Madishe	61	10.1	78	12.9	
	Limbu	17	2.8	4	0.7	
	Magar	105	17.3	45	7.4	
	Tharu	21	3.5	28	4.6	
	Other	141	23.3	180	29.7	
Fuel Use						3.2 (0.52)
	Modern Fuel	42	6.9	48	7.9	
	Wood Only	555	91.6	541	89.3	
	Coal Only	4	0.7	6	1	
	Biomass Only	4	0.7	9	1.5	
	Kerosene Only	1	0.2	2	0.3	

Zone of Residence						56.6 (<0.001)
	Bagmati	9	1.5	9	1.5	
	Bheri	36	6.1	24	4	
	Dhaulagiri	25	4.2	17	2.9	
	Gandaki	91	15.3	55	9.2	
	Janakpur	33	5.6	40	6.7	
	Karnali	11	1.9	4	0.7	
	Koshi	59	9.9	50	8.4	
	Lumbini	116	19.5	114	19.1	
	Mahakali	13	2.2	14	2.4	
	Mechi	24	4	24	4	
	Narayani	72	12.1	162	27.2	
	Rapti	45	7.6	38	6.4	
	Sagarmatha	41	6.9	36	6	

Table 3: Prevalence of Smoking by Ethnicity

	Non-Smokers		Choor/Kankat		Bidi		Filtered Cigarettes		Unfiltered Cigarettes	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Brahmin	12 (3.7%)	65 (24.1%)	28 (10.4%)	13 (4.8%)	34 (12.6%)	21 (7.8%)	36 (13.3%)	40 (14.8%)	83 (30.7%)	63 (23.3%)
Chettri	9 (3.5%)	35 (15.4%)	38 (16.7%)	15 (6.6%)	46 (20.2%)	18 (7.9%)	39 (17.1%)	29 (12.7%)	86 (37.7%)	51 (22.3%)
Madishe/Tharu	10 (5.4%)	52 (27.6%)	11 (5.8%)	6 (3.2%)	49 (26.1%)	26 (13.8%)	19 (10.1%)	27 (14.4%)	28 (14.9%)	25 (13.3%)
Rai/Limbu/Magar/Other	28 (5.1%)	92 (17.1%)	62 (11.8%)	25 (4.8%)	95 (18.0%)	57 (10.8%)	67 (12.7%)	52 (9.9%)	189 (35.9%)	89 (16.9%)

Table 4: Product Specific Odds Ratios by Ethnicity

	Overall			Brahmin			Chettri		
	CS/CNt	OR*	95 % CI	CS/CNt	OR*	95 % CI	CS/CNt	OR*	95 % CI
Non smokers	59/244	1	-	12/65	1	-	9/35	1	-
Choor/Kankat	113/39	10.2	6.2-16.6	17/9	13.5	5.8-31.2	29/11	9.8	2.9-25.5
Bidi	111/74	5.6	3.6-8.7	11/14	8.7	3.9-19.8	18/11	5.9	2.4-14.2
Filtered Cigarettes	74/89	3.4	2.2-5.3	20/26	3.7	1.7-8.1	17/11	2.5	1.1-5.7
Unfiltered Cigarettes	245/157	4.9	3.4-7.2	55/39	6.7	3.2-14.1	51/35	4.3	1.9-9.9

	Madishe/Tharu			Rai/Limbu/Magar/Other		
	CS/CNt	OR*	95 % CI	CS/CNt	OR*	95 % CI
Non smokers	10/52	1	-	28/92	1	-
Choor/Kankat	12/4	16.4	6.5-41.4	55/15	8.1	4.3-15.4
Bidi	39/19	8.6	3.7-20.1	43/30	4.6	2.6-8.3
Filtered Cigarettes	9/15	3.8	1.7-8.6	28/37	2.2	1.2-3.9
Unfiltered Cigarettes	11/15	5.4	2.5-11.7	128/67	3.8	2.2-6.4

*adjusted for age, sex, SES, CRHAP exposure, zone of residence

Table 5: Number of Tobacco Products Used and Lung Cancer Risk by Ethnicity

# of Products Used	Overall			Brahmin			Chettri		
	CS/CNt	OR*	95 % CI	CS/CNt	OR*	95 % CI	CS/CNt	OR*	95 % CI
0	59/244	1	-	12/65	1	-	9/35	1	-
1	302/231	4.2	2.9-6.1	55/54	6	2.9-12.6	63/38	3.9	1.7-9
2	150/84	6.3	4.1-9.6	36/21	9.9	4.5-22.1	20/19	6.9	2.9-16.6
3+	96/47	7	4.3-11.4	16/12	13.5	5.5-33.6	33/11	10.5	3.8-28.5

# of Products Used	Madishe/Tharu			Rai/Limbu/Magar/Other		
	CS/CNt	OR*	95 % CI	CS/CNt	OR*	95 % CI
0	10/52	1	-	28/92	1	-
1	39/36	4.8	2.0-11.5	145/103	3.4	2.0-5.8
2	22/12	9.2	3.2-26.3	72/32	5.5	2.9-10.5
3+	8/15	5.9	1.6-21.9	39/17	5.1	2.3-11.0

*adjusted for age, sex, SES, CRHAP exposure, zone of residence

Table 6: Tobacco Pack Years and Lung Cancer Risk by Ethnicity

	Overall			Brahmin			Chettri		
	CS/CNt	OR*	95 % CI	CS/CNt	OR*	95 % CI	CS/CNt	OR*	95 % CI
0 (Non Smokers)	59/244	1	-	12/65.0	1	-	9/35.0	1	
1-15.0	175/210	3.165	2.2-4.6	27/45	5.1	2.4-10.8	39/33.0	3.4	1.4-7.8
15.01 - 30	150/79	8.8	5.5-14.4	23/18	10.5	4.5-24.1	29/19.0	7.5	3.0-19.2
30 +	223/73	16.2	9.7-27.2	57/24	23.7	9.5-59.2	48/16.0	17.4	6.4-47.3
			p trend <0.001			*p trend* <0.001			*p trend* <0.001

	Madishe/Tharu			Rai/Limbu/Magar/Other		
	CS/CNt	OR*	95 % CI	CS/CNt	OR*	95 % CI
0 (Non Smokers)	10/52.0	1	-	28/92.0	1	-
1-15.0	27/37	3.4	1.4-8.4	82/95	2.5	1.4-4.3
15.01 - 30	17/11.0	8.9	2.9-26.9	81/31	6.2	3.2-11.9
30 +	25/7.0	19.9	6.0-66.0	93/26	9.1	4.7-17.9
			p trend <0.001			*p trend* <0.001

*adjusted for age, sex, SES, CRHAP exposure, zone of residence

Chapter 4: Urinary Metabolites of 1,3-Butadiene as a Biomarkers of CRHAP Exposure from Combustion of Biomass Fuels – Findings from Nepal

Abstract

INTRODUCTION: Nearly half the global population relies on biomass cooking fuels. Exposure to combustion related household air pollution (CRHAP) resulting from combustion of such biomass fuels has been attributed to nearly 4 million deaths each year globally. Ongoing epidemiological studies have suffered from lack of robust exposure metric that captures the miscellany of CRHAP exposure including type of fuels used, presence/absence of separate kitchen, ventilation, and time activity pattern. Expert panels convened by the National Institute of Health offered several solutions, including use of urinary biomarker to capture overall exposure. Many urinary biomarkers have been investigated to estimate personal exposure levels, but a scientific consensus has not been reached. In our study, we measured urinary metabolites of 1,3 butadiene in a heavily CRHAP exposed Nepali adult population.

METHODS: We used control population from a previously completed lung cancer case-control study. Urine samples from 587 controls were analyzed for urinary metabolite of 1,3-butadiene (monohydroxybutyl mercapturic acid: MHBMA and dihydroxybutyl mercapturic acid: DHBMA) using liquid chromatograph tandem mass spectrometer (LC-

MS/MS). We used multivariate linear regression models to analyze the association between biomass fuel use and urinary MHBMA and DHBMA levels while controlling for known confounders including age, sex, SES, and smoking status.

RESULTS: The mean MHBMA and DHBMA concentrations were 375.7 $\pm$ 313.8 and 444.7 $\pm$ 428.9 ng/mL respectively. Mean concentrations of both metabolites were higher among biomass fuel users compared to modern fuel users. Furthermore, we observed a significant association between urinary MHBMA and biomass fuel use status (*p-value* = 0.006) while controlling for known confounders. We did not observe such association for DHBMA

CONCLUSION: Overall, our results indicate that urinary MHBMA is associated with biomass fuel usage, and can serve as a potential biomarker of recent exposure to biomass fuel use.

Introduction

Combustion related household air pollution (CRHAP) resulting from combustion of biomass fuel is of significant concern to public health (Lim et al., 2012; WHO, 2014). Previous studies have shown the negative health impacts of CRHAP exposure including increased respiratory infection, decreased lung function, asthma exacerbation, cancers, heart attacks, premature death in people with preexisting heart and lung diseases, inflammation, oxidative

stress, impaired neurologic development in children, and others (Barregard et al., 2008, 2006; Delfino et al., 2006; Hashibe et al., 2011; Irigaray et al., 2007; Josyula et al., 2015; Lim et al., 2012; Sapkota et al., 2013, 2008; Stockfelt et al., 2012). Recent studies have proposed that acute CRHAP exposure may have immunotoxic effects by impairing the pulmonary macrophages, effectively increasing infection rates and subsequent diseases (Naeher et al., 2005; Rylance et al., 2015).

Epidemiological studies of focusing on CRHAP have suffered from a lack of suitable exposure metric as the traditional bulky air monitors are not an option in remote areas of the world where CRHAP exposure is the most prevalent. Such areas lack often lack roads and electricity necessary to operate such monitors. With this challenge in mind, the National Institute of Health convened a panel of expert to identify suitable exposure metric and set of pressing research questions related to CRHAP exposure from the combustion of biomass fuels (Gordon et al., 2014; Martin et al., 2013). The expert panel recommended use of novel biomarker as one of the potential solution that can capture inter-individual variability in exposure resulting from types of fuels used, ventilation, presence of separate kitchen, time activity pattern, and cooking duration. As defined by the EPA, biomarkers are measureable substances or characteristics in the human body that can be used to monitor the presence of a chemical in the body, biological

responses, or adverse health effects (EPA, 2012). Various biomarkers and the associated metabolic pathways have been investigated to measure CRHAP exposure in different human media. The most common method has been through urine samples due to the non-invasive nature of collection. The research and need to identify an appropriate biomarker that can quickly and accurately quantify CRHAP exposure has been growing; however, no consensus has been reached in the scientific community on an appropriate urinary biomarker. Numerous studies have investigated urinary levoglucosan, methoxyphenols, polycyclic aromatic hydrocarbon metabolites, and volatile organic compound metabolites as potential indicators of CRHAP exposure, which traditionally has focused on the use of wood stoves in the home for cooking and heating purposes in acute exposure settings (Bergauff et al., 2010; Boman et al., 2003; Clark et al., 2007; Dills et al., 2006, 2001; Hinwood et al., 2008; Mastral and Callén, 2000; Migliaccio et al., 2009; Sarigiannis et al., 2015). These previous studies have yielded conflicting results into appropriate and effective urinary biomarkers of CRHAP exposure. There has been no comprehensive evaluation of these biomarkers in high exposure residential settings which provides the greatest insight into urinary biomonitoring of CRHAP originating from biomass cooking fuels exposure. Ideally, a biomarker of CRHAP should include the following characteristics: 1) It should be uniquely derived from biomass cooking fuels; 2) it should be a relatively abundant constituent of CRHAP, such that

ambient exposure levels generate sufficiently high biomarker levels to be measured reproducibly; 3) the parent compound should be chemically stable in the environment, and the compound and it's metabolites should be chemically stable in biological samples (Naeher et al., 2005). Of the biomarkers investigated, metabolites of 1,3 butadiene are of particular interest.

International Agency for Research on Cancer (IARC) has identified 1,3 butadiene, a component of CRHAP, as a known human carcinogen, therefore quantifying individual level exposure to 1,3 butadiene among a highly exposed population may provide insights into mechanisms involving carcinogenicity of CRHAP exposures. Monohydroxy-3-butenyl mercapturic acid (MHBMA) and 1,2-dihydroxybutyl mercapturic acid (DHBMA) are two major metabolites of 1,3 butadiene commonly measured in urine (Albertini et al., 2001; Boogaard et al., 2001; Fustinoni et al., 2004; Rogene F Henderson et al., 1996; Sapkota et al., 2006; Urban et al., 2003). Difficulties arise when targeting MHBMA/DHBMA as 1,3 butadiene is part of automobile exhaust and is therefore ubiquitous in the ambient air, particularly in the urban environment. Similar to other biomarkers, both metabolites are created from the combustion of organic material not specific to CRHAP. Other sources of MHBMA/DHBMA include industrial processes, tobacco smoke, and dietary influences (ATSDR, 2012; EPA, 2014). The ubiquitous presence of 1,3 butadiene raises concern

about the usefulness of MHBMA/DHBMA as indicators of CRHAP. Much of the research has focused on 1,3 butadiene exposures in occupational settings, which are not generalizable to the general population (Albertini et al., 2001; Richard J Albertini et al., 2003; Boogaard et al., 2001; Fustinoni et al., 2004). The main advantage of using MHBMA/DHBMA is the validated and established laboratory methods especially for urine sample analysis (Osterman-Golkar and Bond, 1996; Sapkota et al., 2006; Schettgen et al., 2009; Urban et al., 2003). Using metabolites of 1,3 butadiene may not be appropriate in areas heavily influences by vehicle traffic and industrial sources; however, such metabolites may be useful in low and middle income agricultural populations. Rural areas, especially in LMICs, are less impacted by vehicle traffic and industrial sources compared with large urban centers and these areas often rely heavily upon biomass cooking fuels.

In this study, we investigated the urinary concentration of 1,3 butadiene metabolites among healthy Nepalese adults and the association to biomass cooking fuels. Our findings will further the understanding of personal exposure to CRHAP originating from biomass cooking fuels across Nepal. Results will provide an impetus for targeted interventions that can directly reduce harmful exposures and CRHAP related diseases in Nepal. Furthermore, an effective biomarker to quantify CRHAP exposure has not been identified and recent publications have highlighted this

research gap (Rylance et al., 2013; Simpson and Naeher, 2010). The larger implications related to CRHAP may yield the greatest public health impact as this project can propel exposure research in other LMICs that rely heavily on biomass fuels as a primary heating and cooking fuel.

Materials and Methods

Study Population

A hospital-based case-control study was conducted at the B.P. Koirala Memorial Cancer Hospital (BPKMCH), located in the city of Bharatpur, Chitwan District, Nepal, from November 2009 through December 2012. Located 150 kilometers southwest of Kathmandu, BPKMCH is the major cancer hospital in Nepal. The details regarding participant recruitment and biological sample collections have been described previously (Hashibe et al., 2011; Raspanti et al., 2015). In brief, 606 incident lung cancer cases and 606 age (+/- 5 years) and gender matched controls were recruited from the hospital after receiving informed consent. To eliminate any effect of lung cancer, we limited our analysis to the 606 cancer-free participants.

The control population was selected from various hospital visitors to frequency match the distribution of the lung cancer case population by age (+/-) 5 years, sex, ethnicity, and residence. The controls were visitors at BPKMCH excluding, family members of participating lung cancer cases. Prior to field implementation, standardized lifestyle and food frequency questionnaires were translated into Nepali language by native speakers and pilot tested in the field. Locally trained interviewers collected information on demographic characteristics, education, residential mobility throughout lifetime, type of cooking and heating fuel used at each residence, occupational history, and family history of cancer. The study was approved by the Institutional Review Board at the University of Utah, University of Maryland as well as the Government of Nepal (Nepal Health Research Council).

Urine Collection

Each participant was given a unique identification number to correspond to biological samples and questionnaire data collected. Biological samples were collected by trained hospital staff at BPKMCH. Following recruitment and questionnaire administration, spot urine samples were collected from each participant. The participants were given verbal instructions and supplies needed to collect their own urine. The samples were stored in -80° C

freezers until shipment to the University of Maryland. The samples were shipped on dry ice and immediately stored in freezers upon receipt.

Chemicals

Isometric mixture of (R,S)-N-Acetyl-S-[1-(hydroxymethyl)-2-propen-1-yl)-L-cysteine and (R,S)-N-Acetyl-S-[2-hydroxy-3-buten-1-yl)-L-cysteine (MHBMA), N-Acetyl-S-(3,4-dihyroxybutyl)-L-cysteine (DHBMA), and the deuterated analog (R,S)-N-Acetyl-S-[1-(hyrdoxymethyl)-2-propenyl)-L-cysteine-D6 (IS) were purchased from Toronto Research Chemicals, Ontario, Canada (Catalog #A179005, #A173710, and #A179007 respectively).

Sample Preparation

Samples were stored in -80° C freezers until analysis. The samples were thawed at room temperature prior to extraction. Urine samples were extracted using a "dilute and shoot" approach. Simply, 500 µL of acetonitrile was combined with 500 µL of urine. Each sample was spiked with 10 µL of 20 µg/mL MHBMA-*d6* internal standard

creating a final concentration of 400 ng/mL. The samples were vortex-mixed and centrifuged for 10 minutes at 17,100 g's. We removed 980 µL of supernatant leaving the precipitate undisturbed and injected 10 µL into the HPLC system.

Sample Analysis

Samples were analyzed with high performance liquid chromatography tandem mass spectrometry (LC-MS/MS) using Shimadzu HPLC system coupled with an API3000 tandem MS/MS. The total flow rate was 250 μL per minute with a constant binary mobile phase consisting of 80% acetonitrile and 20% HPLC-grade water with 0.1% ammonium hydroxide for a total run time of 2.5 minutes with a 40° C oven temperature.

MHBMA, DHBMA, and MHBMA-*d6* were detected in ESI negative mode. MHBMA and DHBMA were detected using the MRM transition of *m/z 232.2.3→101.2* and *m/z 250.1→120.7* respectively. Both compounds were quantified using MHBMA-*d6* internal standard MRM transition *m/z 238.1→107.0*. A solvent based 12 point calibration curve was used for quantification (1000, 750, 500, 200, 100, 50, 10, 1, 0.5, 0.1, 0.01, and 0 ng/mL). The calculated concentrations increased linearly with an R^2 value greater than 0.995 for both metabolites.

Sample Recovery/Limit of Detection

Recovery tests were conducted using 500 μL human urine samples spiked with MHBMA, DHBMA, and internal standard and extracted as above. A 5 x 1 mL aliquots were spiked with 20 μL of 10 μg/mL working stock and 10 μL of 10 μg/mL internal standard (400 ng/mL of MHBMA and DHBMA; 200 ng/mL IS) and extracted as previously described. We divided the calculated metabolite concentration by the known spiked concentration to create a percentage of recovery.

To determine limit of detection (LOD), we spiked 7 non-study participant human urine samples with 5 μL of 1 μg/mL working stock and 10 μL of 10 μg/mL internal standard (100 ng/mL of MHBMA and DHBMA; 200 μg/mL IS). These samples were run as unknowns and quantified using calibration curve. We calculated standard deviation of the 7 samples and multiplied the standard deviation of the estimated concentrations by the Student's *t*-test critical value at 99% with 6 degrees of freedom to calculate the limit of detection (LOD). Concentrations below the LOD were assigned values designated as LOD / square root of 2 as described previously (Finkelstein and Verma, 2001; Hornung and Reed, 1990).

Quality Control/Quality Assurance

QA/QC samples were included in each batch to monitor analytical accuracy of the instrument. We included a double blank, spiked mobile phase, and a spiked urine sample. Coefficient of variation (CV) was calculated for each metabolite, which is a commonly used evaluation of inter-batch accuracy.

Statistical Analysis

All statistical analysis was performed using SAS 9.4 (Cary, NC). We checked MHBMA and DHBMA for normal distribution using Kolmogorov-Smirnov goodness-of-fit test. To test for difference in mean metabolite concentrations by fuel type and smoking status, we used Student's *t-test* as appropriate. We used chi-square tests to investigate correlation between potential categorical confounding variables to include in regression models in order to reduce multicollinearity and ensure a parsimonious model. To investigate the impact of residential biomass cooking fuel use on observed urinary MHBMA and DHBMA concentrations, we created multiple linear regression models following log transformation of the metabolites to ensure normal distribution. In these models we controlled for known

common confounding variables including age, gender, ethnicity, SES (Low, Mid-Low, Mid-High, and High), tobacco use (current, former, and non-smokers), and secondhand smoke exposure (SHS; yes/no). We analyzed potential confounders for collinearity to reduce effects of highly correlated confounding variables included simultaneously in the model. As our targeted metabolites are transient in nature, we focused on the most recent reported residence for fuel type.

To minimize the impact of tobacco smoking, we stratified our analysis by self-reported tobacco use and reported results separately. We investigated all reported methods of cooking (boiling, shallow frying, deep frying, and steaming) by adding them individually into the model to test significance, overall model fit, and influence on regression coefficients. In addition, we investigated cooking behavior effect modification of the exposure-metabolite association by including interaction terms into the regression models.

Results

A total of 606 participants were included in this study. Of the 606 participants, 19 participants lacked urine samples. Demographic characteristics of the study population is provided in Table 7. In short, nearly 77% of

participants reported using biomass fuels in their current residence. The majority of our study population consisted of participants between the ages of 50-59 (38.12%) and males made up 62.71% of the study population. The vast majority of the participants reported living in a rural area (86.14%) and in the mid-low SES group (36.6%). Not surprisingly, the majority of participants reported being a current or former tobacco smoker (59.74%).

We recovered approximately 123% of MHBMA and 163% of DHBMA using spiked urine samples which were included in all sample batches. We adjusted the calculated concentrations for recovery rates and the distribution statistics can be found in Table 8. We observed a mean MHBMA concentration of 375.7 $\pm$ 313.8.6 ng/mL and a mean DHBMA concentration of 444.7 $\pm$ 428.9 ng/mL. We observed a maximum MHBMA concentration of 1528 ng/mL and a maximum DHBMA concentration of 2773 ng/mL. As expected, we observed higher mean and median concentrations of both biomarkers among biomass fuel users compared to modern fuel users as well as higher concentrations among smokers compared to non-smokers. Specifically, we observed a statistically significantly higher mean MHBMA concentration among biomass fuel users compared to modern fuel users (*p-value = 0.05*). Furthermore, wood users had a significantly higher mean MHBMA concentration compared to modern fuel users (*p-value = 0.04*). Similar significant results were not found for DHBMA concentrations.

We created multivariate linear regression models to investigate the relationship between fuel use and metabolite concentrations as reported in Table 9. After controlling for known confounders, we observed a significant increase in MHBMA concentrations with biomass fuel use [exp(β)=1.16; *p-value=0.006*] as well as wood use [exp(β)=1.19; *p-value=0.002*]. More specifically, biomass fuel use at home increased urinary MHBMA concentrations by 16% and wood use at home increased MHBMA concentration by 19%. For DHBMA concentrations, we did not observe any significant associations between fuel use and observed metabolite concentrations. Furthermore, we did not observe any significant associations between either metabolite concentrations and tobacco use, SES status or gender.

To reduce the impact of tobacco smoking further, we stratified our analysis by whether the participant reported using smoking tobacco (Table 10). We created models for each fuel category individually. Across never smokers and ever smokers, we observed increased concentrations of MHBMA with increased wood use compared to modern fuel users (16% and 23% respectively). Similarly, overall biomass fuel users had a 15% increase in MHBMA concentrations compared to modern fuel users among ever smokers with borderline significance. When stratified by cooking behavior, we observed significantly higher MHBMA concentrations among overall biomass fuel and wood

only users compared to modern fuel users among shallow frying groups. As expected, higher metabolite levels were observed among the participants who smoke than the non-smokers, but our results suggest that using biomass cooking fuels can increase personal exposure to 1,3 butadiene, which is a known human carcinogen, independently from tobacco use (Edward et al., 2014; IARC, 2010a, 2006).

Discussion

Recent studies have identified improved exposure assessment tools to measure personal exposures to CRHAP resulting from biomass cooking fuels as an important environmental health priority (Hosgood et al., 2014; Lim et al., 2012; Rylance et al., 2013). With nearly half the global population using biomass cooking fuels, the need to understand personal exposure and resulting health effects is critical (WHO, 2014). CRHAP from biomass cooking fuels is a complex mixture of particles and pollutants, including known human carcinogens such as 1,3 butadiene (EPA, 2007; IARC, 2006; Noonan et al., 2012; Ward et al., 2008; Zhang and Smith, 1996). Measuring the two primary urinary metabolites of 1,3 butadiene, MHBMA and DHBMA in urine, has been used in other settings to estimate personal exposure to this known carcinogen originating from traffic exhaust and other non-cook fuel related sources

(Albertini et al., 2001; Boogaard et al., 2001; Fustinoni et al., 2004; Sapkota et al., 2006; Smith et al., 2001). To the best of our knowledge, this study is the first of its kind to target urinary MHBMA and DHBMA as biomarkers of exposure to CRHAP from biomass cooking fuels in a highly exposed population in Nepal.

While our results are unique, the concentrations of urinary MHBMA and DHBMA are comparable to studies in other populations and settings. A noted study among a Czech rubber manufacturing plant reported mean post-exposure urinary MHBMA and DHBMA concentrations among the highest exposed group of 120.7 ng/mL and 4647 ng/mL respectively, while our reported mean concentrations of both MHBMA and DHBMA are 375.7 ng/mL and 444.7 ng/mL in the total study population (Albertini et al., 2001; Richard J Albertini et al., 2003). Interestingly, our reported mean MHBMA is nearly 3 times the concentration reported among the highest exposed Czech workers and our DHBMA is nearly 10 times lower than the highest exposed Czech workers. Prior to our analysis, we did expect our observed concentrations to be higher than previous studies due to the intense and widespread exposures in our study population. Similarly, our study population consisted of many smokers and those exposed to SHS. Difficulties arise when comparing exposures across different populations and environments, but we are confident that our results reflect accurate personal exposures to CRHAP from biomass cooking fuels.

Most importantly, our results highlight MHBMA as a strong candidate to be used as a biomarker of CRHAP exposure from biomass cooking fuels. Our results show that MHBMA concentrations are significantly higher among overall biomass fuel users, as well as wood users, after controlling for many known demographic and socioeconomic factors. Furthermore, the significant increases among never smokers show the true contributions of CRHAP to urinary biomarker concentration independent from tobacco use. Previously, IARC has classified wood/biomass use as a potential human carcinogen; however, our results show that biomass fuel exposure does in fact significantly increase personal exposure to known human carcinogens, but the final link to carcinogenesis remains unclear.

Only 8% of our participants reported deep frying as a cooking method for fish, meats, and/or vegetables compared with 98% who reported shallow frying. We did not observe any noted associations between urinary metabolite levels and boiling or steaming cooking methods which adds further confidence to our results. All fuel and cooking method interaction terms were not significant and removed from the final analysis. Participants were not asked the frequency or preference of cooking method, rather if they used the method for meats, fish, or vegetables.

In this study, we aimed to quantify urinary biomarkers MHBMA and DHBMA to investigate their association to biomass cooking fuel use. Overall, we analyzed urine samples from 587 participants and created multivariate linear

regression models controlling for known confounders such as age, ethnicity, gender, tobacco use, SHS exposure, SES, and cooking methods. We investigated numerous potential confounding variables by adding them individually to the model and comparing model fit statistics. In all, we decided to remove variables reflecting ventilation, urban/rural status, presence of a separate kitchen, and self-reported level of smokiness during cooking as these variables were highly correlated to cooking fuel type and/or SES. Our decision to remove these variables from the final analysis is reflective of our desire to retain parsimony and reduce any influence of multicollinearity within our analysis.

The diverse and large study population is a major strength of this study. Secondly, we used well validated HPLC-MS/MS analytical methods for MHBMA and DHBMA metabolite detection and quantification. Also, we were able to control for many known confounding effects that could influence our observed metabolite concentration. Namely, we had robust measures of tobacco use, frequency, and type of products smoked to allow us to control for these effects confidently. Similarly, we were able to include individual cooking methods and interactive effects of cooking methods and fuel type. While this study had numerous strengths, we also encountered limitations. While biomarkers are identified as "gold standards" in exposure assessments, it is extremely difficult to identify source specific biomarkers of exposure. By nature, biomarkers include exposure across all pathways and sources, making it

very difficult to isolate exposures from biomass cooking fuels only. We controlled and stratified our analysis to remove the effects of many known confounding variables to the best of our ability. We did not normalize for urinary creatinine levels. In the future, we will perform additional analysis for urinary cotinine and levoglucosan to better characterize CRHAP exposure in this population.

In conclusion, we observed significant associations between urinary MHBMA and individual level CRHAP exposure from biomass cooking fuels. These results can be directly applied to invention-based cook stoves studies to reduce the burden of disease associated with CRHAP and biomass cooking fuels. While our results are promising, further research is needed into MHBMA, DHBMA, and other potential urinary biomarkers of exposure in other communities and regions to better estimate personal level exposure to CRHAP.

Manuscript 2 Tables:

Table 7: Demographic Characteristics			
		N	%
Fuel Type			
	Modern Fuel	141	23.27
	Biomass Fuel	465	76.73
	Wood Only	437	72.11
	Biomass Only	28	4.62
Age			
	<40	24	3.96
	40-49	121	19.97
	50-59	231	38.12
	60-69	179	29.54
	70 +	51	8.42
Gender			

	Male	380	62.71
	Female	226	37.29
Ethnicity			
	Brahmin	153	25.25
	Chettri	104	17.16
	Rai/Limbu/Magar/Other	243	40.10
	Madishe/Tharu	106	17.49
SES Index			
	Low	150	24.75
	Mid-Low	222	36.63
	Mid-High	109	17.99
	High	125	20.63
Tobacco Use			
	Non-Smoker	244	40.26
	Ever Smoker	362	59.74
Urban/Rural Status			
	Urban	84	13.86
	Rural	522	86.14
Secondhand Smoke Exposure			
	No	456	75.25
	Yes	150	24.75

Table 8: Distribution Statistics

		MHBMA (ng/mL)						DHBMA (ng/mL)				
		N	Mean	SD	Median	Range	p-value[a]	Mean	SD	Median	Range	p-value[a]
ALL		606	375.7	313.8	261	<LOD-1528		444.7	428.9	282.8	<LOD-2773	
Biomass Fuel Use	Modern Fuel	141	349.9	315.8	248.8	<LOD-1528	REF	437.7	458.2	280.4	<LOD-2773	REF
	Biomass Fuel	465	383.6	313.1	271.5	<LOD-1496	0.04	446.8	420.1	283.1	<LOD-1748	0.4
	Biomass Only	28	274.6	234.4	207.3	<LOD-984	0.8	345.9	343.7	225.8	<LOD-1258	0.2
	Wood Only	437	390.8	316.6	282.1	<LOD-1496	0.05	453.5	242.2	285.9	<LOD-1748	0.6
Smoking Status	Non Smokers	244	376.8	323	255.3	<LOD-1439	REF	447.2	436	268.7	<LOD-2282	REF
	Ever Smokers	362	375	307.8	265	<LOD-1528	0.5	443	424.5	293.6	<LOD-2773	0.8

a=Log transformed metabolite mean concentrations were compared using Student's *t-test* with modern fuel use and non-smokers as reference groups.

Table 9: Selected Linear Regression Results - Only Current Residence

	Label	N	MHBMA[a]				DHBMA[a]			
			β	exp(β)[b]	% Change	*p-value*	β	exp(β)[b]	% Change	*p-value*
Current Fuel Use	Modern	141	REF	REF	REF	REF	REF	REF	REF	REF
	Wood	437	0.17	1.19	19	0.002	0.08	1.09	9	0.18
	Biomass	28	0.01	1.01	1	0.96	-0.11	0.90	-10	0.4
	Wood+Biomass	465	0.15	1.16	16	0.006	0.06	1.06	6	0.3
Tobacco Use	Never	244	REF	REF	REF	REF	REF	REF	REF	REF
	Ever	362	0.03	1.03	3	0.5	0.01	1.01	1.00	0.77
SES	Low	146	REF	REF	REF	REF	REF	REF	REF	REF
	Mid-Low	226	-0.11	0.90	-10	0.12	-0.09	0.91	-9	0.27
	Mid-High	109	-0.10	0.90	-10	0.25	-0.11	0.90	-10	0.21
	High	127	-0.07	0.93	-7	0.4	-0.06	0.94	-6	0.51
Gender	Female	226	REF	REF	REF	REF	REF	REF	REF	REF
	Male	382	-0.01	0.99	-1	0.9	0.02	1.02	2	0.74
SHS	No	458	REF	REF	REF	REF	REF	REF	REF	REF
	Yes	148	-0.13	0.88	-12	0.01	-0.15	0.86	-14	0.01
Deep Fry Meat/Fish/Vegetables	No	556	REF	REF	REF	REF	REF	REF	REF	REF
	Yes	50	-0.25	0.78	-22	0.002	-0.32	0.73	-27	0.0002
Shallow Fry Meat/Fish/Vegetables	No	15	REF	REF	REF	REF	REF	REF	REF	REF
	Yes	591	0.25	1.28	28	0.07	0.28	1.32	32	0.07

a=MHBMA/DHBMA concentrations are log transformed and results presented have been exponentiated;

b= age and ethnicity also included in model;

Table 10: Stratified Analysis [a]

	N	MHBMA				DHBMA			
		β	exp(β[a)]	% Change	*p-value*	β	exp(β[a)]	% Change	*p-value*
Overall									
Modern	141	REF	REF	REF	REF	REF	REF	REF	REF
Wood Only	437	0.17	1.19	19	0.002	0.08	1.09	9	0.18
Biomass Only	28	0.01	1.01	1.00	0.96	-0.11	0.90	-10	0.4
Wood+Biomass	465	0.15	1.16	16	0.006	0.06	1.06	6	0.3
Smoking Status									
Never Smoker									
Modern	72	REF	REF	REF	REF	REF	REF	REF	REF
Wood Only	163	0.15	1.16	16	0.04	0.09	1.10	10	0.2
Biomass Only	9	0.03	1.03	3	0.87	-0.21	0.81	-19	0.33
Wood+Biomass	172	0.16	1.17	17	0.07	0.08	1.09	9	0.36
Ever Smoker									
Modern	69	REF	REF	REF	REF	REF	REF	REF	REF
Wood Only	274	0.21	1.23	23	0.002	0.09	1.09	9	0.24
Biomass Only	19	0.03	1.03	3	0.83	0.01	1.01	1	0.97
Wood+Biomass	293	0.14	1.15	15	0.05	0.03	1.03	3	0.71
Cooking Method[b]									
Deep Fry									
Modern	13	REF	REF	REF	REF	REF	REF	REF	REF
Wood Only	33	-0.07	0.94	-6	0.6	-0.23	0.79	-21	0.07
Biomass Only	4	0.06	1.06	6	0.84	0.78	2.17	117	0.81
Wood+Biomass	37	0.03	1.03	3	0.87	0.00	1.00	0	0.99
Shallow Fry									
Modern	136	REF	REF	REF	REF	REF	REF	REF	REF
Wood Only	428	0.19	1.21	21	0.001	0.10	1.10	10	0.12
Biomass Only	27	0.02	1.02	2	0.88	-0.09	0.92	-8	0.51
Wood+Biomass	455	0.16	1.17	17	0.004	0.07	1.07	7	0.27

a=MHBMA and DHBMA coefficients have been exponentiated;

b=Participants reported regular cooking method for meat, fish, and/or vegetable;

Chapter 5: Urinary Biomarkers of CRHAP Exposure from Combustion of Biomass Fuels and Lung Cancer Risk

Introduction

In this chapter, we investigated the relationship between urinary metabolites of 1,3 butadiene and lung cancer etiology in a highly exposed population in Nepal. We utilized the data previous collected from the urine sample analysis and the questionnaire based data to create logistic regression models to estimate the association between observed urinary MHBMA and DHBMA concentrations as measures of personal-level exposure and lung cancer risk. As much of the methods and background material is presented in previous chapters, we will focus on the results of this analysis.

Results

A total of 606 confirmed lung cancer case and 606 frequency matched on age, gender, and geographical residence controls were included in this analysis. Overall, the majority of our lung cancer cases were male (55.78%), between the ages of 60-69 (41.42%), and in the lowest SES group (35.80%). Not surprisingly, nearly 90% of lung cancer cases reported tobacco smoking while only 59.7% of controls reported tobacco smoking (Table 11). We observed significant chi-square results for lung cancer risk and age, gender, ethnicity, SES, tobacco use,

cook stove ventilation, presence of a separate kitchen, and physician diagnoses tuberculosis (all *p-values* < 0.05). Interestingly, we observed a significant chi-square results for DHBMA quartiles (*p-value* = 0.02), but not for MHBMA quartiles (*p-value* =0.08).

The results of the logistic regression models can be found in Table 12. We did not observe any significant association between the log-transformed metabolite concentrations and lung cancer risk when controlling for known confounding variables. As expected, we observed significant associations between lung cancer risk and tobacco use (OR 5.64; 95% CI 3.80-7.59), age (OR 1.03; 95% CI 1.02-1.04), and females (OR 1.94; 95% CI 1.47-2.57).

To better control of the effects of tobacco, we stratified our analysis by smoking status in Table 13. Overall, we did not observe any considerable association between observed metabolite concentrations and lung cancer risk. Although, we did borderline significant association between increasing MHBMA concentration and lung cancer risk among the total population; however, this relationship remains unclear.

Discussion

Overall, our analysis did not provide evidence of an association between 1,3 butadiene measured through urinary biomarkers and lung cancer risk. We feel this is due to the very transient nature of biomarkers of exposure. In most cases, biomarkers of exposure are useful at measuring recent personal exposure to

environmental chemicals and the applicability of biomarkers of exposure at estimating long term, repeated exposures is limited. As useful as biomarkers of exposure are for measuring overall personal exposure to particular environmental chemicals, difficulties arise when focusing on a specific source of the pollution. In our analysis, we aimed to estimate personal exposure to CRHAP derived from biomass cooking fuels as measured by metabolites of 1,3 butadiene. While controlling for known contributions of personal 1,3 butadiene exposure from other non-biomass fuel sources, we were unable to uncover any conclusive statistical associations.

In conclusion, our analysis and results do not support our primary hypothesis that urinary 1,3 butadiene metabolite concentrations are significantly and directly associated with increased lung cancer risk among a highly exposure Nepali population. It is our interpretation that the highly transient nature of the 1,3 butadiene metabolites are useful for short term, recent exposure, but not useful for diseases with long latency periods such as lung cancer.

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Table 11: Demographic characteristics of the study population						
		Lung Cancer Cases		Controls		
		N	%	N	%	*X^2 (p-value)*
MHBMA Concentration in Quartiles						6.69 (0.08)
	Q1	176	29.04	165	27.23	
	Q2	135	22.28	156	25.74	
	Q3	161	26.57	130	21.45	
	Q4	134	22.11	155	25.58	
DHBMA Concentration in Quartiles						10.05 (0.02)
	Q1	158	26.07	185	30.53	
	Q2	134	22.11	156	25.74	
	Q3	166	27.39	124	20.46	
	Q4	148	24.42	141	23.27	
Age						98.49 (<0.0001)
	<40	27	4.46	24	3.96	
	40-49	45	7.43	121	19.97	
	50-59	152	25.08	231	38.12	
	60-69	251	41.42	179	29.54	
	70 +	131	21.62	51	8.42	
Gender						6.03 (0.01)
	Male	338	55.78	380	62.71	
	Female	268	44.22	226	37.29	
Ethnicity						47.48 (<0.0001)
	Brahmin	117	19.31	153	25.25	
	Chettri	124	20.46	104	17.16	
	Rai	20	3.3	14	2.31	
	Madishe	61	10.07	78	12.87	
	Limbu	17	2.81	4	0.66	
	Magar	105	17.33	45	7.43	
	Tharu	21	3.47	28	4.62	
	Other	141	23.27	180	29.7	
SES Index in Quartiles						20.24 (0.0002)
	Low	217	35.8	150	24.8	
	Mid-Low	170	28.1	222	36.6	
	Mid-High	94	15.5	109	18	

	High	125	20.6	125	20.6	
Tobacco Use						150.61 (<0.0001)
	Non-Smoker	59	9.7	244	40.3	
	Smoker	547	90.3	362	59.7	
Tobacco Packyears (PYs) in Quartiles	Q1 (0-0.125 PYs)	58	9.6	244	40.3	215.76 (<0.0001)
	Q2 (0.125-11.9 PYs)	175	28.9	210	34.7	
	Q3 (11.9-30 PYs)	150	24.8	79	13	
	Q4 (> 30 PYs)	223	36.8	73	12.1	
SHS	No	432	71.3	456	75.3	2.42 (0.12)
	Yes	174	28.7	150	24.7	
Ventilation	None	202	33.3	250	41.2	8.13 (0.004)
	Window/Chimney	404	66.7	356	58.8	
Level of Smokiness	Low	42	6.9	35	5.8	3.68 (0.29)
	Mid-Low	90	14.9	108	17.8	
	Mid-High	166	27.4	146	24.1	
	High	308	50.8	317	52.3	
Separate Kitchen	No	93	15.3	129	21.3	7.15 (0.008)
	Yes	513	84.7	477	78.7	
TB Status	Negative	518	85.5	562	92.7	16.46 (<0.0001)
	Positive	88	14.5	44	7.3	

Table 12: Odds Ratios (OR) and 95% Confidence Interval for Biomarker Concentration and selected confounders

		Cases (n)	Controls (n)	OR[b]	95% CI
MHBMA[a]		606	606	0.94	0.84-1.06
DHBMA[a]		606	606	1.05	0.94-1.16
Smoking Status	Never	59	244	1.00	-
	Ever	547	392	5.34	3.8-7.59
Age		606	606	1.03	1.02-1.04
Gender	Male	338	380	1.00	-
	Female	268	226	1.94	1.47-2.57
SES	Low	217	150	1.00	-
	Mid-Low	170	222	0.56	0.40-0.79
	Mid-High	94	109	0.52	0.34-0.77
	High	125	125	0.65	0.45-0.94
SHS Exposure	No	432	456	1.00	-
	Yes	174	150	0.33	0.99-1.79

a=Biomarker concentration (ng/mL) were log transformed

b=Model was adjusted for smoking status, age, gender, ethnicity, SES, and SHS exposure

Table 13: Odds Ratios and 95% Confidence Interval for Biomarker Concentration Quartile and lung cancer risk, stratified by smoking status

Quartile	Overall				Ever Smokers				Never Smokers			
	Case (n)	Control (n)	OR*	95 % CI	Case (n)	Control (n)	OR*	95 % CI	Case (n)	Control (n)	OR**	95 % CI
MHBMA												
Q1	176	165	1	-	158	95	1	-	18	70	1	-
Q2	135	156	0.69	0.48-1.0	123	96	0.64	0.43-1.0	12	60	0.85	0.36-1.98
Q3	161	130	1.06	0.75-1.51	147	84	0.97	0.65-1.46	14	46	1.19	0.52-2.71
Q4	134	155	0.72	0.51-1.03	119	87	0.71	0.47-1.07	15	68	0.69	0.30-1.54
			p-trend	0.04			*p-trend*	0.08			*p-trend*	0.62
DHBMA												
Q1	158	185	1	-	140	109	1	-	18	76	1	-
Q2	134	156	0.95	0.67-1.35	121	95	0.99	0.66-1.48	13	61	0.98	0.43-2.24
Q3	166	124	1.27	0.89-1.81	155	76	1.37	0.91-2.06	11	48	1.01	0.42-2.42
Q4	148	141	1.02	0.72-1.46	131	82	1.05	0.70-1.59	17	59	0.96	0.43-2.13
			p-trend	0.43			*p-trend*	0.4			*p-trend*	0.99

* Adjusted for age, sex, ethnicity, SES, SHS, total pack years

** Adjusted for age, sex, ethnicity, SES, SHS

Chapter 6: Household Air Pollution and Lung Cancer Risk among Never-Smokers in Nepal

Raspanti, G.A., Hashibe, M., Siwakoti, B., Wei, M., Kumar, B., Bahadur, C., Al-temimi, M., Lee, Y.A., Sapkota, A., 2016. Household air pollution and lung cancer risk among never-smokers in Nepal. Environ. Res. 147, 141–145. doi:10.1016/j.envres.2016.02.008

Abstract

More than half of the global population relies on biomass fuels (wood, charcoal, crop residue, dung) for cooking and/or heating purposes. Combustion related household air pollution (CRHAP) resulting from the use of these biomass fuels is of particular concern, given the overall prevalence as well as the intensity of exposure and the range of potential adverse health outcomes. Long term exposure to CRHAP is a major public health concern, particularly among women and children in low and middle income countries. In this study, we investigated the association between exposure to CRHAP and lung cancer risk among Nepalese population. Using a hospital-based case-control study (2009 - 2012), we recruited 606 lung cancer cases and 606 healthy controls frequency matched on age (+/- 5 years), gender, and geographical residence. We used unconditional logistic regression to compute odds ratios (ORs) and 95% Confidence Intervals (95% CI) for lung cancer risk associated with CRHAP exposures, adjusting for potential confounders (tobacco use, TB status, SES, age, gender, ethnicity, and exposure to

second hand smoke). In our overall analysis, we observed increased risk of lung cancer among those who were exposed to CRHAPs (OR: 1.78, 95% CI: 1.01-3.15). A more detailed analysis stratified by smoking status showed considerably higher risk of lung cancer associated with increasing duration of biomass specific CRHAP exposure, with evidence of an exposure-response relationship (P_{trend} = 0.05) that was more pronounced among never-smokers (P_{trend} = 0.01). Our results suggest that chronic exposure to CRHAP resulting from biomass combustion is associated with increased lung cancer risk, particularly among never-smokers in Nepal.

Introduction

Combustion related household air pollution (CRHAP) resulting from incomplete combustion of biomass fuels (wood, coal, agricultural waste, charcoal, and animal dung) for cooking and/or heating is a major global public health concern (Lim et al., 2012). Recent estimates suggest that 3.5 million deaths are attributable to CRHAP, while additional 500,000 deaths are attributed to outdoor air pollution originating from indoor source annually (Lim et al., 2012). The vast majority of these deaths occur in low and middle income countries (LMIC) where a significant proportion of the population relies on biomass fuels for cooking and/or heating (Gordon et al., 2014; IOM, 2007; Perez-Padilla et al., 2010; WHO,

2014). CRHAP is a complex mixture of pollutants including particulate matter, sulfur oxides, nitrogen oxides, carbon monoxide, polycyclic aromatic hydrocarbons, formaldehyde, and dioxins, to name few (Ding et al., 2012; EPA, 2007; Naeher et al., 2007; Pruneda-Álvarez et al., 2012; Ward et al., 2008). An increasing body of literature has illustrated the role of CRHAP in disease etiology of both acute and chronic health outcomes with women and children in LMIC bearing disproportionate disease burden (Adetona et al., 2013; Guarnieri et al., 2014; Lim et al., 2012; Naeher et al., 2007; Pokhrel et al., 2013; Pradhananga et al., 2009; Romieu et al., 2009; Smith et al., 2010, 2000; Smith-Sivertsen et al., 2009; WHO, 2014, 2013).

The carcinogenicity of CRHAP exposure was extensively evaluated by an expert panel convened by the International Agency for Research on Cancer in 2006 (IARC, 2010). The panel classified CRHAP from coal as a known human carcinogen (IARC Group 1), while CRHAP from biomass was classified as a possible human carcinogen (IARC Group 2A), citing lack of epidemiologic evidence (IARC, 2010). Since then few new studies have investigated the carcinogenicity of CRHAP particularly from biomass combustion (Sapkota et al., 2013, 2008) while others have conducted systematic review and meta-analysis of the literature (Bruce et al., 2015; Josyula et al., 2015; Martin et al., 2013). However, few studies have comprehensively investigated the risk of CRHAP related to biomass on lung cancer risk in high risk areas such as Nepal, where

75% of the population heavily relies on biomass for cooking (Ghimire et al., 2011).

Nepal is one of the poorest countries in the world with an estimated 24% of the nearly 30 million people living under $1 a day (WHO, 2009). Lung cancer is the most common cancer in men and third most common in women in Nepal (IARC, 2012). Compared with all cancers in Nepal, lung cancer accounts for 13% of new cancer incidence and 15% of cancer related mortality (IARC, 2012). Although, tobacco use is decreasing in Nepal, lung cancer incidence continues to rise and is projected to double by the year 2035 (Ferlay et al., 2010; Ghimire et al., 2011; IARC, 2012; WHO, 2009). In this study, we evaluate the role of CRHAP in lung cancer etiology in Nepal, one of the highest exposed areas globally.

Materials and Methods

A hospital-based case-control study was conducted at B.P. Koirala Memorial Cancer Hospital (BPKMCH), Chitwan District, Nepal, from November 2009 through December 2012. Located 150 kilometers southwest of Kathmandu, BPKMCH is the major cancer hospital in Nepal. The details regarding participant recruitment and biological sample collections have been described previously (Hashibe et al., 2011; Raspanti et al., 2015). In brief, 606 incident lung cancer cases and 606 age (+/- 5 years) and gender frequency matched controls were

recruited from the hospital. The inclusion criteria for a lung cancer case were: 1) 18 years of age or older 2) resident of Nepal for at least five years and 3) were admitted to BPKMCH. The eligible cases were recruited as soon as possible following lung cancer diagnosis with a target interval of one day and a maximum interval of 4 weeks. A trained medical staff member reviewed medical records to extract relevant diagnostic information, including the date and method of diagnosis, histological type, tumor location, stage, and grade. Final diagnosis of lung cancer was confirmed with histological, cytological, or X-ray based evidence.

The controls were visitors at BPKMCH excluding friends and family members of participating lung cancer cases, and were frequency matched by age, gender, and geographic residence. Prior to field implementation, standardized lifestyle and food frequency questionnaires were translated into Nepali language by native speakers and pilot tested in the field (Hashibe et al., 2011). Locally trained interviewers collected information on demographic characteristics, education, residential mobility throughout lifetime, type of cooking and heating fuel used at each residence, occupational history, and family history of cancer. The study was approved by the Institutional Review Board at the University of Utah, University of Maryland as well as the Government of Nepal (Nepal Health Research Council).

We computed a lifetime profile of exposure to CRHAP based on duration and type of biomass fuel used at each reported residence across the lifespan. We defined biomass fuel as wood, charcoal, agricultural waste and dung while modern fuel is defined as electricity and natural gas. For this analysis, individuals that predominantly used kerosene (>50% of their lifetime) were excluded (n=7) because while kerosene is a modern fuel, recent studies show that it has considerable adverse health impact (Bates et al., 2013; Epstein et al., 2013; Lam et al., 2012; Pokhrel et al., 2010). A variable reflecting duration of CRHAP exposure was created for each fuel type. These product specific variables were summed to generate total years of CRHAP exposures. We further categorized CRHAP exposure and fuel specific exposure into quartiles based on distribution of exposure among controls. Linear trends were examined by including the quartile variables as continuous within the models.

Tobacco user was defined as someone who smoked greater than 100 cigarettes or similar tobacco product in their lifetime. Furthermore, we calculated tobacco pack years based on the duration as well as frequency of each tobacco product smoked as described previously (Raspanti et al., 2015). Exposure to secondhand smoke (SHS) was captured by participant's indication of living or working with someone who actively smoked tobacco. We computed socioeconomic status (SES) index based on level of education, household monthly average income, and crowdedness in the home (number of individuals

per room) (Ghosh and Ghosh, 2009; Sapkota et al., 2008). This SES index reflects the contributions of multiple indicators and a high value on this index can be interpreted as high SES. We categorized the SES index into quartiles which reflect low, mid-low, mid-high, and high categories.

Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using multivariate logistic regression models to investigate the relationship between CRHAP exposure and lung cancer risk. We included age, gender, ethnicity, geographical region, tobacco use status, SHS exposure, and SES index (in quartiles) as confounders in the model as these are known contributors to lung cancer (Di Cesare et al., 2013; Hashibe et al., 2011; Raspanti et al., 2015). We included an interaction term between CRHAP exposure and gender to detect potential effect modification; however, this term was not significant and was removed from the final model. We also investigated the influence of residential characteristics of the home such as ventilation and smokiness during cooking by including them in the statistical model because the physical structure is known to contribute to the concentration and dispersion of CRHAP related pollutants (Batterman et al., 2006; Pokhrel et al., 2005; Seow et al., 2015; Zuraimi and Tham, 2008). Participants were asked what type of ventilation or smoke extraction was used during cooking in each residence. Response options included window, chimney, or none. Smokiness during cooking was determined by the participants classifying how much smoke was present in their homes during

cooking events. Participants were given the following options: cooking indoors with little smoke, some smokiness caused by cooking, much smokiness caused by cooking although no enough to irritate eyes, and much smokiness caused by cooking and enough to irritate eyes. Interaction terms were included in the model to examine any effect modification between ventilation and type of fuel used. Furthermore, we included physician diagnosed tuberculosis (TB) as a confounder in the model as prior studies have highlight the link between TB and lung cancer risk as well as biomass cooking fuels potential contribution to TB (Huang et al., 2015; Lin et al., 2014; Pokhrel et al., 2010; Skowroński et al., 2015).

Results

The demographic characteristics of the study population are presented in Table 14. In general, cases and controls were similar in terms of ethnicity and family income. Lung cancer cases tended to be slightly older, male, and less educated. The prevalence of smoking, as expected, was considerably higher among cases (Table 14).

As shown in Table 15, the association between CRHAP exposure and lung cancer risk was statistically significant based on the overall analysis (OR 1.78; 95% CI 1.03-3.15). Furthermore, we observed significant increased lung cancer risk among tobacco users (OR 5.57; 95% CI 3.93-7.87), older age (OR 1.03; 95%

CI: 1.01-1.04), and females (OR 2.07; 95% CI 1.58-2.72). Participants who had prior physician diagnosed TB had a considerably higher lung cancer risk compared to those who did not (OR 2.25; 95% CI 1.47-3.44). We also observed significant decreased lung cancer risk among those with higher SES status. We did not observe a significant association between lung cancer and presence of a separate kitchen, ventilation, or self-reported smokiness level during cooking and these variables were not included in the final model. Similarly, we did not observe a significant association between lung cancer risk and fuel, ventilation, and smokiness level interaction terms and were therefore removed from the final model. Lastly, participants who reported living or working with a smoker had a higher risk of lung cancer compared to those who did not (OR 1.37; 95% CI 1.02-1.84).

Results for duration of CRHAP exposure from biomass fuel use and lung cancer risk, stratified by smoking status is depicted in Table 16. Here, we focused solely on biomass as CRHAP exposure from combustion of coal has been classified as IARC Group 1 carcinogen (IARC, 2010). In the overall analysis, we observed evidence of an association between increased duration of CRHAP exposure and lung cancer risk (OR 1.71, 95% CI: 1.07-2.74 for 4^{th} vs 1^{st} quartile of exposure, $P_{trend} = 0.05$). Upon stratification, we observed an exposure-response relationship between increasing duration of exposure to CRHAP and lung cancer risk among never-smokers ($P_{trend} = 0.01$). Non-smokers that were at the highest

quartile of CRHAP exposure had considerably higher risk of lung cancer compared to those at the lowest quartile (OR 10.26; 95% CI 2.47-42.68). Among ever smokers, increased duration of CRHAP exposure was not associated with increased risk of lung cancer (P_{trend} = 0.6).

Discussion

CRHAP originating from the combustion of biomass fuels contains many harmful pollutants including known human carcinogens (IARC, 2010), and is a major contributor to the global burden of disease (Lim et al., 2012; WHO, 2014). Most recent IARC monograph has classified exposures to CRHAP from biomass combustion as probable human carcinogen (IARC, 2010), while subsequent comprehensive review and meta-analysis has linked exposures to CRHAP from biomass with lung cancer risk (Bruce et al., 2015). Our findings build upon this literature by documenting increased risk of lung cancer associated with exposure to CRHAPs from biomass among never-smoking Himalayan population.

Approximately 71% of Nepali households cook inside the main living space, while 20% cook in a separate building and 8% cook outside (Ghimire et al., 2011) emphasizing the need to better understand the relationship between CRHAP from biomass cooking fuels and health in this region. In our study, significant increase in lung cancer risk associated with CRHAP exposure was

similar to studies conducted in India and elsewhere (Gupta et al., 2001; Perez-Padilla et al., 2010; Sapkota et al., 2013). Our findings regarding increased lung cancer risk among females, low SES, tobacco users, and exposure to SHS are in line with our hypothesis and previous literature (Binu et al., 2007; Pradhananga et al., 2009).

Since tobacco consumption is a major risk factor for lung cancer, and residual confounding by smoking cannot be ruled out even when adjusting for it as a confounder, we stratified our analysis by ever and never smokers. This allowed us to investigate the lung cancer risk associated with CRHAP exposure, independent of tobacco smoking. We further restricted this analysis to participants with no co-exposure to coal. In doing so, we observed an exposure-response relationship for duration of CRHAP exposure and lung cancer risk among never smokers (P_{trend} = 0.01). This increase in odds of lung cancer risk is similar to what has been reported in a study conducted in neighboring India (Sapkota et al., 2008). Conversely, we did not observe any significant association between lung cancer risk and CRHAP exposure among ever smokers, even after adjusting for tobacco pack years. A potential explanation for this is the strength of association between tobacco smoking and lung cancer is considerably higher by comparison, so an incremental increase in lung cancer risk associated with CRHAP exposure would be hard to detect on top of the existing risk associated with tobacco.

Building characteristics, such as ventilation and smokiness during cooking activities are important factors to consider when estimating personal CRHAP exposure. Previous studies in China, Guatemala, Nepal, and Papua New Guinea have highlighted the potential benefits of properly ventilated cook stoves (Hu et al., 2014; McCracken et al., 2007; Smith et al., 2011, 2010; Smith-Sivertsen et al., 2009; Tielsch et al., 2014; Werry, 2005; Zhang and Smith, 2007). In our study, we did not observe a statistically significant association between either ventilation or smokiness during cooking to lung cancer risk. Lastly, we investigated urban versus rural residency as a potential confounding variable; however, this variable was highly correlated to our SES variable and we decided not to include the urban/rural classification to reduce multicollinearity.

There are several strengths of this study, including large diverse study population. Participants were recruited from every geographic regions of Nepal. The study population is among the highest exposed population in the world. This study is unique as it is one of the first to investigate the contribution of CRHAP exposure to lung cancer risk in Nepalese adults. A limitation of this study is the self-reported nature of key variables, which are prone to recall bias. However, the link between CRHAP exposure and lung cancer is relatively new, and as such our study participant's responses regarding their past biomass fuel use were not likely influenced by it. Large variability exists in personal CRHAP exposures even if individuals reported similar duration of exposure and type of fuel used. For

example, physical household characteristics such as presence/absence of ventilation, wall and roof structure, and size of the room may determine the true concentration and pollutant within homes. In addition, the time activity pattern, cooking frequency as well as time spent cooking each meal may determine individual's true exposures. These detailed variables were not part of our data collection, and could not be accounted for in our statistical analysis. Similarly, population density and outdoor-to-indoor infiltration also can contribute to CRHAP and related personal exposures. The vast majority of our study population reported using wood which limited our ability to compare different types of fuel.

Conclusion

In summary, this study aimed to estimate lung cancer risk associated with CRHAP exposures in Nepal. Our findings suggest that long term exposure to CRHAP from biomass is associated with increased lung cancer risks among never smokers. Public health strategies focused in mitigating cancer burden need to consider reducing CRHAP exposure, in addition to tobacco control.

Manuscript 4 Tables

Table 14: Demographic characteristics of the study population

		Lung Cancer Cases		Controls		
		N	%	N	%	X^2 *(p-value)*
CRHAP Exposure in Quartiles						37.62 (<0.0001)
	Q1 (0-46 years)	242	39.9	271	44.7	
	Q2 (46-56 years)	103	17	152	25.1	
	Q3 (56-65 years)	104	17.2	105	17.3	
	Q4 (> 65 years)	157	25.9	78	12.9	
Fuel Type						4.67 (0.32)
	Modern Fuel	30	5.0	40	23.3	
	Natural Gas	30	5.0	40	23.3	
	Biomass Fuel	576	95.0	566	93.4	
	Wood	563	92.7	548	90.4	
	Coal	5	0.8	5	0.8	
	Biomass	4	0.7	10	1.7	
	Kerosene	4	0.7	3	0.5	
Age						98.49 (<0.0001)
	<40	27	4.46	24	3.96	
	40-49	45	7.43	121	19.97	
	50-59	152	25.08	231	38.12	
	60-69	251	41.42	179	29.54	
	70 +	131	21.62	51	8.42	
Gender						6.03 (0.01)
	Male	338	55.78	380	62.71	
	Female	268	44.22	226	37.29	
Ethnicity						47.48 (<0.0001)
	Brahmin	117	19.31	153	25.25	
	Chettri	124	20.46	104	17.16	
	Rai	20	3.3	14	2.31	
	Madishe	61	10.07	78	12.87	
	Limbu	17	2.81	4	0.66	
	Magar	105	17.33	45	7.43	
	Tharu	21	3.47	28	4.62	
	Other	141	23.27	180	29.7	
SES Index in Quartiles						20.24 (0.0002)
	Low	217	35.8	150	24.8	
	Mid-Low	170	28.1	222	36.6	
	Mid-High	94	15.5	109	18	
	High	125	20.6	125	20.6	

Tobacco Use						150.61 (<0.0001)
	Non-Smoker	59	9.7	244	40.3	
	Smoker	547	90.3	362	59.7	
Tobacco Packyears (PYs) in Quartiles	Q1 (0-0.125 PYs)	58	9.6	244	40.3	215.76 (<0.0001)
	Q2 (0.125-11.9 PYs)	175	28.9	210	34.7	
	Q3 (11.9-30 PYs)	150	24.8	79	13	
	Q4 (> 30 PYs)	223	36.8	73	12.1	
SHS	No	432	71.3	456	75.3	2.42 (0.12)
	Yes	174	28.7	150	24.7	
Ventilation	None	202	33.3	250	41.2	8.13 (0.004)
	Window/Chimney	404	66.7	356	58.8	
Level of Smokiness	Low	42	6.9	35	5.8	3.68 (0.29)
	Mid-Low	90	14.9	108	17.8	
	Mid-High	166	27.4	146	24.1	
	High	308	50.8	317	52.3	
Separate Kitchen	No	93	15.3	129	21.3	7.15 (0.008)
	Yes	513	84.7	477	78.7	
TB Status	Negative	518	85.5	562	92.7	16.46 (<0.0001)
	Positive	88	14.5	44	7.3	

Table 15: Odds Ratios (OR) and 95% Confidence Interval for CRHAP exposure and selected confounders

		Cases (n)	Controls (n)	OR[b]	95% CI
CRHAP Exposure[a]	No	31	42	1.00	-
	Yes	575	564	1.78	1.01-3.15
Smoking Status	Never	59	244	1.00	-
	Ever	547	392	5.57	3.93-7.87
Age		606	606	1.03	1.01-1.04
Gender	Male	338	380	1.00	-
	Female	268	226	2.07	1.58-2.72
TB Status	Negative	518	562	1.00	-
	Positive	88	44	2.25	1.47-3.44
SES	Low	217	150	1.00	-
	Mid-Low	170	222	0.56	0.40-0.78
	Mid-High	94	109	0.49	0.32-0.72
	High	125	125	0.57	0.39-0.83
SHS Exposure	No	432	456	1.00	-
	Yes	174	150	1.34	1.01-1.80

a=CRHAP includes coal, wood, and biomass

b=Model was adjusted for smoking status, age, gender, TB status, SES, and SHS exposure

Table 16: Odds Ratios and 95% Confidence Interval for duration of CRHAP Exposure from biomass combustion and lung cancer risk, stratified by smoking status

Quartile	Overall				Ever Smokers				Never Smokers			
	Case (n)	Control (n)	OR*	95 % CI	Case (n)	Control (n)	OR*	95 % CI	Case (n)	Control (n)	OR**	95 % CI
Q1	241	268	1.00	-	201	122	1.00	-	40	146	1.00	-
Q2	101	149	0.89	0.59-1.35	94	97	0.83	0.50-1.37	7	52	1.21	0.41-3.54
Q3	103	104	1.10	0.70-1.74	99	76	0.93	0.55-1.58	4	28	2.74	0.65-11.53
Q4	153	77	1.71	1.07-2.74	145	62	1.23	0.73-2.09	8	15	10.26	2.47-42.68
			p-trend	0.05			*p-trend*	0.6			*p-trend*	0.01

* Adjusted for age, sex, ethnicity, SES, TB status, SHS, total pack years

** Adjusted for age, sex, ethnicity, SES, TB status, SHS

Q1=0-43 years; Q2=43.1-55; Q3=55.1-64; Q4 > 64

Chapter 7: Synthesis

To achieve our specific aims, we utilized previously collected data in a highly exposed population in Nepal. As described previously, we used a combination of questionnaire based data and biologic samples to test our hypotheses. Using various statistical approaches and well established laboratory methods, we feel confident that our results can contribute significantly to future research projects aimed at exposure reduction efforts focusing on CRHAP from biomass fuels and the differential influence of traditional tobacco products. More importantly, these results can be utilized by public health professionals aiming to reduce disease incidence and prevalence by targeted intervention-based approaches.

Ethnic Variability in Consumption of Traditional Tobacco Products and Lung Cancer Risk in Nepal

In this dissertation, we described the differential impact of traditional tobacco use on lung cancer risk among ethnic groups in Nepal. In our first manuscript, we described how differential use of tobacco across ethnic groups in Nepal can lead to varying risk of lung cancer. We created individual regression models for each ethnic group to analyze lung cancer risk specific to the group. We were able to show the highly significant increase of lung cancer risk attributed

to traditional tobacco products when compared to the non-smoking population. In doing this analysis, certain trends began to materialize that added further evidence of the deleterious effects of traditional tobacco products. The highest and most dramatic increases in lung cancer risk was observed among those who reported using traditional tobacco and those who reported using multiple tobacco products across all ethnic groups. While we expected the risk to be increased among these populations, we did not expect the increases to drastically different.

We are confident that our results can be generalized and extrapolated to other similar LMICs. While we understand cultural differences, our results can be used to inform on the ground tobacco cessation, reducing tobacco use initiation among teens, and overall educational outreach programs surrounding tobacco use. Our results highlight the need to include cultural competent inclusive messaging that includes or even prioritizes traditional tobacco products especially among the most vulnerable populations.

Historically, tobacco cessation and health messaging have targeted commercial tobacco cigarettes produced by large, multi-national companies. In the United States, successful legislative actions have limited or altogether eliminated tobacco advertising especially to young adults and teenagers who are more likely to start smoking. While these successes have been achieved, the implications have been isolated to high income countries. In LMICs, many challenges are faced by public health practitioners working to reduce tobacco use.

Some challenges include, but are not limited to varying cultural connections to tobacco, prioritization, wide-spread acceptance of tobacco use, limited resources, and cyclic poverty. As described in this dissertation, traditional tobacco use is more common among rural, poor populations where there is a misplaced perception of safety and it is within this framework which lies the opportunity to make significant impacts.

Urinary Metabolites of 1,3 Butadiene as Biomarkers of CRHAP Exposure from combustion of Biomass Fuels – Findings from Nepal

Our aim of using metabolites of 1,3 butadiene provided insight into measuring personal exposure to CRHAP originating from the combustion of biomass fuels. As mentioned earlier, a large research gap exists in the current scientific literature regarding biomarkers of exposure for CRHAP from biomass fuels. Attempts have been by many researchers targeting other metabolites and chemicals yielding mixed results. Our results are unique in that they provide evidence that MHBMA may be a useful non-invasive biomarker especially among the non-smoking population.

As with any biomarker based projects, many benefits and challenges exist. While biomarkers are a useful means of measuring overall personal exposure to environmental chemicals, difficulties arise when attempting to investigate or target a biomarker related to a specific source, such as biomass fuels. Our study

population provided a unique opportunity to target MHBMA and DHBMA as our population was mainly in rural, low income environments. We used a variety of statistical approaches to eliminate the other known contribution to personal MHBMA and DHBMA concentrations to garner a clearer picture to the contribution of CRHAP from the combustion of biomass fuels.

While these results are unique to our study population, we are confident that similar investigations can be completed in other countries and environments using urinary MHBMA as a measure of personal exposure to CRHAP from biomass fuels. Using biomarkers of exposure, researchers can be able to compare the efficiency and effectiveness of various cook stoves aimed at CRHAP reduction. The detection and validation of a biomarker of exposure to CRHAP from biomass fuels is a necessary first step in the ultimate reduction of diseases attributed to this wide-spread environmental exposure.

Urinary Biomarkers of CRHAP Exposure from combustion of Biomass Fuels and Lung Cancer Risk

In this chapter, we investigated the association between 1,3 butadiene metabolites and lung cancer risk. As expected, we did not see any association between the metabolites concentrations and risk of lung cancer. These results are not entirely unexpected as biomarkers of exposure tend to be very transient in nature. Therefore, they are useful in detecting and measuring recent personal

exposure to environmental pollutants, in this case 1,3 butadiene via CRHAP exposure from biomass fuels, but are not as reliable in predicting chronic diseases such as cancer. Cancer has a long latency period in which the contributions of many environmental chemicals play a cumulative role. It can be argued that measuring a recognized human carcinogen such as 1,3 butadiene in a CRHAP exposure assessment strategy can be useful in predicting cancer risk in certain situations. This can be especially true if the study population tends to be stationary and static in which participants report a single, long term residence with little to no change in cooking fuels, ventilation, or other known contributing factors to CRHAP. In this setting, a measurement of recent exposure can potentially be reflective of a person's long term, repeated exposure. Here, we may be able to surmise that the daily, repeated exposure to the measured carcinogen can contribute to carcinogenesis or lead in that direction when controlling for many confounding variables. In our study, about 50% of our study population reported living in multiple residences and the median years spent at the most recent reported residence is 45. After conducting this analysis, we feel that our study population did not fit the static, stationary characteristics to make a confident determination of exposure-disease relationship.

Household Air Pollution and Lung Cancer Risk among Never-Smokers in Nepal

In regards to CRHAP and biomass cooking fuels, recent estimates have shown the highly detrimental health impacts of using biomass cooking fuels in the home (Buchner and Rehfuess, 2015; Desai et al., 2004; IARC, 2010a; Kan et al., 2011; Lim et al., 2012; Lim and Seow, 2012; Naeher et al., 2005; Rehfuess et al., 2009; Sapkota et al., 2013, 2008; WHO, 2007). Not only does using biomass cooking fuel contribute negatively to health, it also has severe ecological impacts. Deforestation, loss of animal habitat, reducing agricultural productivity, and contributing to climate change are just some of the more wide-spread complications of biomass fuel use. In our projects, we aimed to investigate the role of using biomass cooking fuels and the risk of lung cancer. Similar to tobacco, some fuels such as coal, is a well-known and documented human carcinogen while other biomass fuels, such as wood, are not well understood. Here, our main interest was creating a lifetime profile of fuel use and investigate the relationship to lung cancer. Our results show that using biomass cooking fuels is a major risk factor for lung cancer especially among the non-smoking population. This increase in risk among the non-smoking population reveals the true contribution of CRHAP originating from the combustion of biomass fuels to lung cancer risk. Furthermore, these results can potentially be extrapolated to other LMICs with similar exposures. As mentioned previously, our results can be used to inform future research projects aimed at exposure and disease reduction. Also, the results can be used to propel the many efforts currently being made to

create more efficient cook stoves with improved ventilation that can greatly reduce CRHAP from biomass fuels and ultimately disease.

Lastly, we investigated how changing fuel types can influence lung cancer risk. In this analysis, we identified the participant's last and penultimate residences and the fuel used in each. We categorized the fuel change into categories reflecting whether the change was biomass-modern fuel, modern-biomass fuel, or no change at all as well as calculated the number of years since that change occurred. Our working hypothesis was that lung cancer risk will decrease directly with years since a biomass to modern fuel change. We expected to see lung cancer risk decrease once the biomass fuel exposure was removed and that this decrease will occur linearly over time. Following analysis, we did not see any significant evidence of a decrease in lung cancer risk among the 168 study participants who reported such fuel change. Among this group, the median years since biomass-modern fuel change was only 15 years which may not be enough time to observe a change in risk. Conversely, very few participants reported switching from a modern to a biomass fuel (~1%). These fuel changes and subsequent health outcomes is important in the sense that biomass fuel use is often used as an informal metric of a county's overall development. Higher percentage of biomass fuel use is often correlated to a lower level of economic development (Rehfuess et al., 2006).

Overall Synthesis and Future Research

Taken together, this dissertation highlights important environmental factors that significantly contribute to morbidity and mortality in low and middle income countries (LMICs). Recent studies and estimates have shown that many infectious and chronic diseases are projected to increase in LMICs even though well-known behavioral factors, namely tobacco smoking, are declining. Of major importance are lung cancer and other respiratory diseases such as tuberculosis (TB). The influence of traditional tobacco products and CRHAP from biomass cooking fuels on such respiratory diseases is not well understood. Similarly, recent publications have highlighted the need for improved exposure assessment tools to understand personal exposures to CRHAP from biomass cooking fuels (Clark et al., 2013; Martin et al., 2013; Rylance et al., 2013). The ability to accurately measure personal exposures to environmental pollutants is the first step in reducing hazardous exposures and associated negative health effects. The best tool to achieve this accuracy and reliability is via non-invasive biomarkers of exposure. Many attempts have been made to test various biomarkers in various human media and settings, but no consensus has been reached (Clark et al., 2013; Rylance et al., 2013; Simpson and Naeher, 2010). The concern surrounding previously underestimated or otherwise not well understood environmental threats, such as CRHAP from biomass fuels and traditional tobacco products, has

been growing. The aim of this dissertation was to investigate the influence traditional tobacco products and CRHAP from biomass fuels on lung cancer risk and to investigate biomarkers of exposure to CRHAP.

In a larger sense, recent events have highlighted a growing global commitment to the reduction of fossil fuel use in all sectors, including biomass fuels in the home, in an effort to reduce the impacts of a changing climate. Early contentions following these announcements have place the LMICs at a disadvantage. Historically, the now high income/developed world built itself upon the use of fossil fuels to spawn and incubate early technology and economic development. While we are now seeing the cumulative climactic impacts of the prolonged extortion of our environment, the high income areas are desiring cleaner solutions to our dependence on fuels. It is these climate goals placed upon resource starved LMICs that make such global efforts difficult and the old "top down" global development/health paradigm still firmly rooted in international relations. Better solutions are needed that start at the household level in the most vulnerable global populations. Starting with advancements in cook stove technology to increase efficiency and output with the ultimate goal of providing methods that are completely renewable. It cannot be stressed enough that the success of many project like cook stove interventions are heavily dependent on a "community based" rather than "community placed". In cook stove specific projects, many cultural and geographical aspects must be taken into

account. The type of cooking, type of fuel, cooking styles, cultural cuisines, and many others can determine the characteristics of the type of stove that will succeed in this area. There is not a "one size fits all" solution to CRHAP derived from biomass fuels for cooking and/or heating, but with targeted efforts by both communities and scientists, solutions can be reached. Such efforts need to include LMICs from the very beginning and at all critical decision points to ensure their voices are not stifled and true multilateral change achieved.

The cocktail of chemicals of which we are exposed to every day make isolating source specific contributions difficult. An improved project for isolating and measuring personal exposure to CRHAP from biomass fuels must include multiple ambient air measures and multiple biologic samples taken at various time points. Such time points need to include the following: baseline for comparison, during fire making/pre-cooking process, during cooking, immediately following cooking, during fire burn out, and at time points following to measure chemical clearance and other toxicokinetic properties. Chemical half-life is something that needs to be kept in the forefront of any similar project. The ability to accurately and reliably measure personal exposure in a non-invasive manner is imperative to testing the efficacy of cook stoves. Ultimately, cook stoves need to be evaluated not by ambient air measures, rather by personal exposure metrics such as biomarkers. This approach will yield the most accurate manner of efficacy

comparison with the goal of reducing exposures and subsequent diseases related to CRHAP from biomass cooking fuels.

In conclusion, chronic diseases such as lung cancer continue to grow across LMICs. It is in these areas where extreme poverty collides with a lack of resources to create an incubator for disease. Environmental health concerns surrounding using biomass fuels and continued tobacco use can be classified among the top health priorities. In this dissertation, we investigated the how the use of traditional tobacco impacts lung cancer risk ethnic groups differentially as well as the influence of biomass cooking fuels on lung cancer risk. Lastly, we attempted to measure personal level exposure to CRHAP derived from biomass cooking fuels using urinary biomarkers and analyze these biomarkers as related to lung cancer risk. Taken together, these manuscripts shed an important light into highly impactful environmental factors that contribute significantly to lung cancer risk in Nepal. As noted, these manuscripts lay the groundwork for more in-depth studies on traditional tobacco and biomass fuel use on a variety of respiratory diseases in different areas. It is difficult to extrapolate our findings to other countries or regions as many cultural, social, and environmental factors contribute to the exposure-disease relationship; however, we feel that our results are certainly reflective of those faced by millions of Nepali adults. We hope that this project is useful for the ultimate reduction of disease through targeted public

health interventions aimed at exposure reduction. It is in this context that lives can truly be saved.

Appendices

As part of my overall doctoral program, I was able to participate in a number of projects not directly related to my dissertation project. The outcome of one of these projects was the publication presented here. While not related to CRHAP from biomass fuels, we investigated an indoor aerosol environment in Washington DC. This manuscript was published in *Environmental Research* in 2015.

Volatile organic compounds and particulate matter in child care facilities in the District of Columbia: Results from a pilot study

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Abstract

Background: Many young children in the U.S. spend a significant portion of their day in child care facilities where they may be exposed to contaminants linked to adverse health effects. Exposure data on volatile organic compounds (VOCs) and particulate matter (PM) in these settings is scarce.

Objective: To guide the design of a larger exposure assessment study in urban child care facilities, we conducted a pilot study in which we characterized indoor concentrations of select VOCs and PM. Methods: We recruited 14 child care facilities in the District of Columbia (Washington, DC) and measured indoor concentrations of seven VOCs (n = 35 total samples; 2–5 samples per facility): benzene, carbon tetrachloride, chloroform, ethylbenzene, o-xylene, p-xylene, and toluene in all facilities; and collected real-time PM measurements in seven facilities. We calculated descriptive statistics for contaminant concentrations and computed intraclass correlation coefficients (ICC) to evaluate the variability of VOC levels indoors. We also administered a survey to collect general health

information on the children at- tending these facilities, and information on general housekeeping practices and proximity of facilities to potential sources of target contaminants.

Results: We detected six of the seven VOCs in the majority of child care facilities with detection frequencies ranging from 71% to 100%. Chloroform and toluene were detected in all samples. Median (range) concentrations for toluene, chloroform, benzene, o-xylene, ethylbenzene, and carbon tetra- chloride were: 5.6 mg/m3 (0.6–16.5 mg/m3), 2.8 mg/m3 (0.4–53.0 mg/m3), 1.4 mg/m3 (below the limit of detection or <LOD – 4.4 mg/m3), 1.1 mg/m3 (<LOD – 35.7 mg/m3), 1.0 mg/m3 (< LOD – 28.5 mg/m3), and

1.0 mg/m3 (< LOD – 1.6 mg/m3), respectively. The ICCs for the VOCs measured ranged from 0.32 to 0.75. Child care facility median concentrations for PM2.5 and PM10 were 20.1 mg/m3 and 26.3 mg/m3, respectively. Chlorine bleach, a source of chloroform, was used in almost all facilities, air fresheners and/or scented candles were used in half of the facilities, and at least one child in each facility had physician- diagnosed asthma (median asthma prevalence rate 10.2%).

Conclusion: We found quantifiable levels of VOCs and PM in the child care facilities sampled. Given that exposures to environmental contaminants during critical developmental stages may have long lasting impacts on children's health, larger studies are needed to characterize and identify sources of exposures to these and other indoor contaminants to develop exposure mitigation strategies.

Introduction

Most infants and young children in the United States spend a significant portion of their day in child care facilities. In the U.S., an estimated 6 million children under the age of five years are cared for at child care facilities (i.e., child care centers, nursery schools, preschools, and family home daycares) with some children attending for as much as 50 hours a week (Bureau U.C., 2011; Ax- elrad et al., 2013). Limited studies have characterized various in- door air contaminants previously linked to adverse health effects, including volatile organic compounds (VOCs) and particulate matter (PM) in childcare settings (Bradman et al., 2012; Canha et al., 2015; Fritz and Herbarth, 2001; Roda et al., 2011; St-Jean et al., 2012; Fromme et al., 2005; Kabir et al., 2012; Mainka and Zajusz-Zubek, 2015). Exposure to VOCs and PM in children has been linked to respiratory effects such as decreased lung function, inflammation, and airway obstruction; to increased allergen sensitization; and to the exacerbation of pre-existing respiratory conditions such as asthma (Goldizen et al., 2016; Harving et al., 1991; Koren et al., 1992; Mortimer et al., 2008; Norback et al., 1995; Salvi, 2007; Wieslander et al., 1997). Young children attending child care facilities may thus

experience chronic exposures to potentially deleterious contaminants during critical windows of development.

Exposures to environmental contaminants during early stages of development may have long lasting health impacts in infants and young children. Children are more vulnerable to the potential effects of indoor air contaminants because, compared to adults, they have higher respiratory rates and breathe a larger volume of air per unit body weight (ATDSR, 2015; USEPA, 2011). Additionally, due to their increased physical exertion, infants and young children tend to breathe more through their mouths allowing a greater volume of pollutants to be inhaled and reducing the effectiveness of one level of filtration (USEPA, 2011; Mott et al., 1997). They are also more vulnerable to environmental exposures because their major systems are still developing. In fact, their lungs are not fully developed until adolescence (De Luca et al., 2010; ER et al., 2000; Pinkerton, 2000). Children also play on the ground and their height places their breathing zones close to the floor which results in higher inhaled doses of select pollutants (e.g., toxic gases that are denser than air and layer close to the floor) than an adult would receive in the same room (ATDSR, 2015; USEPA, 2011).

VOCs in indoor environments may originate from several sources including building materials and furnishings (e.g., treated wood, (ATDSR, 2007; Brown, 1999; Yoon et al., 2011; Zuraimi et al., 2004) paint, (Yoon et al., 2011; ATDSR, 2010; ATDSR, 2015;

Hodgson et al., 2000) furniture, (USEPA, 2011; Brown, 1999; Yoon et al., 2011; Zuraimi et al., 2004) and carpet/flooring (Hodgson et al., 2000; Katsoyiannis et al., 2008); consumer products (e.g., electronics and toys) (ATDSR, 2010; Hodgson et al., 2000; Katsoyiannis et al., 2008; Adgate et al., 2004; Destaillats et al., 2008; Tirendi et al., 2009); cleaning supplies (e.g., disinfecting and sanitizing products) and deodorizers; (Nazaroff and Weschеler, 2004) and art supplies (Bradman et al., 2012; ATDSR, 2015; Mishra et al., 2015). Factors such as age (Jia et al., 2008) and structural quality of the facility (Wieslander et al., 1997; Cox et al., 2002; Wilke et al., 2004; Wan-Je and Sohn, 2009) as well as maintenance and cleaning practices (Nazaroff and Weschеler, 2004; Singer et al., 2006; Wolkoff et al., 1998) could also affect VOC levels in- doors. For example, poorly maintained facilities may be more prone to mold and pests potentially leading to frequent use of pesticides, which have been linked to higher VOC levels indoors (Gordon et al., 1999; Ott and Roberts, 1998; Neal and Spurlock, 2014; Chin et al., 2014). Proximity of the facilities to roadway traffic, gas stations, parking garages, or dry cleaners could also have an effect on the indoor air quality and impact indoor VOC and/or PM concentrations (Goyal, 2009; Guo et al., 2010; Janssen et al., 2001, 2003; van Vliet et al., 1997; Kheirbek et al., 2012; Kwon et al., 2006; Roda et al., 2013). PM may also be present on indoor floors/surfaces primarily from dust tracked indoors and outdoor air particles, which may be resuspended in air from human activity (USEPA, 2007).

While limited studies conducted in Canada, Europe, and Asia have reported the presence of some VOCs (Canha et al., 2015; Fritz and Herbarth, 2001; Roda et al., 2011; St-Jean et al., 2012; Yoon et al., 2011; Zuraimi and Tham, 2008) and/or PM (Canha et al., 2015; Fromme et al., 2005; Kabir et al., 2012; Mainka and Zajusz- Zubek, 2015; Beamer and Castaño, 2002) in child care settings, there is a dearth of data on VOCs and PM within U.S. childcare facilities (Bradman et al., 2012; Beamer and Castaño, 2002). Results from one U.S. study conducted in northern California by Bradman et al. (2012) indicate that several pollutants in child care facilities, including PM and VOCs such as chloroform, benzene, and ethylbenzene, reached exposure levels of concern (i.e., estimated exposures exceeded government health-based dose or exposure benchmarks). Many of the chemicals that reached levels of concern are also known carcinogens, are endocrine disruptors, may exacerbate asthma and other respiratory illnesses, and may alter neurocognitive functioning in children (Bradman et al., 2012). Another study conducted in California measured PM10 concentrations in one university child care center (Beamer and Castaño, 2002) and reported that children were exposed to higher amounts of PM10 than the adults in the same room. Although the Bradman et al. study (Bradman et al., 2012) provided baseline exposure levels for several contaminants in child care facilities in northern California, much remains to be known about the extent of exposures in these environments and how they may differ from facilities located in other geographic areas. To guide

the design of a larger exposure assessment study in child care facilities, we conducted a pilot study in which we characterized indoor concentrations of seven VOCs and PM in childcare facilities located predominantly in inner-city areas in Washington, DC.

Materials and methods

Child care facility recruitment

In collaboration with the Children's Environmental Health Network, approximately 314 child care center directors in the District of Columbia (Washington, DC) were initially contacted via email and letters between the Fall of 2012 and the Fall of 2013. Study staff followed up with Center Directors who responded to emails and letters to be briefed on the pilot study, determine their eligibility, and assess their willingness to participate. Recruitment of facilities for our study was a challenge due to limited resources (e.g., the lack of full time study staff available to focus on outreach and recruitment efforts) and general resistance from Center Directors to enroll in the study. Thus, our final sample size consisted of a convenience sample of 14 child care facilities. Eligible child care centers were licensed and had a Center Director willing to complete a baseline

questionnaire and provide researchers with access to the facility to conduct indoor environmental air sampling. For their participation, child care facilities were provided education materials on how to reduce indoor environmental exposures upon completion of the study. The University of Maryland Institutional Review Board reviewed and approved all study protocols and written informed consent was obtained from child care Center Directors prior to data and sample collection.

Data collection

Study staff administered a questionnaire to child care Center Directors to collect information on the number of children cared for at the facility and information on other factors that may have influenced indoor contaminant concentrations including proximity of the facility to major highways, dry cleaning facilities, industrial sites, and gas stations. For one facility, the Center Director was not available to complete the questionnaire so the Facilities Manager completed the questionnaire with prior authorization from the Center Director. We also queried Center Directors to collect information on the physical characteristics of the building such as heating sources, whether there was a garage attached to the facility, if any recent remodeling/painting had taken place, and information on general housekeeping practices and usage of air fresheners, scented candles, and pesticides. In addition, we collected information on the number of children with

physician-diagnosed asthma at each facility and whether there were any reports of children experiencing asthma attacks and/or wheezing episodes in the three months prior to sampling.

Air sample collection

To characterize VOCs within the facilities, we collected between two and five 10-h air samples inside the 14 child care facilities using SKC AirChek XR 5000 model 210 pumps (SKC Eighty Four, PA) fitted with SKC Anasorb Coconut Shell Charcoal sampling tubes (catalog # 226-09, SKC Inc, Eighty Four, PA). The total number of samples collected per facility was based on the facility size (e.g., in the case of large facilities with multiple floors, multiple monitors were used to collect a more comprehensive sample); we collected at least two samples per facility to ensure complete sample collection in the case of equipment failure and to assess indoor variability of VOC concentrations. The VOCs measured and their respective limits of detection (LOD) included: benzene (0.50 mg/m3), carbon tetrachloride (0.56 mg/m3), chloroform (0.29 mg/m3), ethylbenzene (0.45 mg/m3), o-xylene (0.72 mg/ m3), p-xylene (0.45 mg/m3), and toluene (0.35 mg/m3). Selection of these VOCs was based on their potential to impact health and presence of known indoor sources. Prior to sampling, pumps were calibrated to operate at 1 L/min. Pre and post sampling flow rates were measured in the field

in triplicates. Monitors were positioned on a tripod at an approximate height of 24–30” to represent the breathing zone of a child.

To assess exposure to PM we used a DUSTTRAK II Aerosol Monitor (TSI, Shoreview, MN) to measure real-time PM2.5 and PM10 (i.e., particles with an aerodynamic diameter 2.5 μm and 10 μm, respectively) in seven child care facilities. Concentrations of PM2.5 and PM10 were recorded every minute for 10 h for five facilities (n 600 measurements per child care facility), for four hours and 10 min for one facility (n 250 measurements), and for 45 min in one facility. Equipment malfunction during the study limited our ability to measure PM in all facilities and for the full 10 h. For our analyses, we excluded data from the childcare facility where PM was only measured for 45 min. All environmental samples (VOCs and PM) were collected from highly trafficked areas where children spent the majority of their time.

Laboratory analyses of VOCs

Prior to analysis, the charcoal tubes were spiked with 20 μl of an internal standard (AccuStandard Cat# CLP-PI-2.5X). The char- coal tubes were broken and the content transferred to 4 mL vial, to which a 2 mL of a 50:50 mixture of carbon disulfide and acetone was added. The samples were then sonicated for 30 min. Each vial was centrifuged for 5 min to separate the solid particles and only the

liquid supernatant was removed for analysis. The VOC samples were analyzed using Shimadzu QP2010 gas chromatograph mass spectrometer (GC/MS) (Shimadzu Biotech, Columbia, MD) in selective ion monitoring mode as previously described by Sapkota et al. (2006). Chromatographic separation was achieved using a Restek Rtx-624 column, 60 m x 0.25 mm ID with 1.4 mm film thickness (Restek Corp., catalog no. 10969). We deployed field blanks and laboratory blanks to check for potential contamination during the shipment process or inside the lab.

Quality assurance/quality control (QA/QC) of VOC sample analyses

For QA/QC purposes, we collected multiple field blanks (20% of total samples collected). These blank samples were analyzed using identical laboratory methods as those used for the study samples. All results were corrected for field blanks. We determined the percent recovery for all individual VOCs by spiking a known con- centration of VOCs to a clean sampling tube, and extracting them as described previously. The percent recovery rate was determined as the amount of VOCs recovered divided by the spiked amount, which ranged from 59 to 102%. We determined the LODs following the Code of Federal Regulations, part 136, Appendix B (https:// www.law.cornell.edu/cfr/text/40/part-136/appendix-B) (Protection of Environment, 2014). Briefly, we analyzed seven spiked samples and analyzed them following our analytical methods. The standard deviation of

these spiked samples was multiplied by the Student's t-value associated with the 99% confidence interval and 6 degrees of freedom. Sample concentrations below the LOD were imputed to LOD/2 (Finkelstein and Verma, 2001) and corrected for percent recovery.

2.5. Statistical analysis

We first summarized general facility characteristics and housekeeping practices. For VOCs, given multiple air samples were collected from each child care facility, we first calculated the arithmetic mean concentration for each VOC in each facility. We then calculated descriptive statistics using the respective VOC average concentration for each facility. If at least one sample for the child care facility had a concentration 4LOD then this analyte was considered detected. To evaluate the within- and between- facility variability and reproducibility of VOC concentrations, we also calculated intraclass correlation coefficients (ICC) using mixed effects models (Rabe-Hesketh and Skrondal, 2012). An ICC 40.75 indicates excellent reproducibility (i.e., concentrations are consistent from sample-to-sample and a single measurement sufficiently represents the average of the series of measurements over a specific time period), an ICC value between 0.4 and 0.75 indicates fair to good reproducibility, and an ICC of o0.4 indicates poor reproducibility (Rosner, 2006). Thus, low ICC values would indicate great within-

facility variability of VOC concentrations and that more samples per facility are needed to properly characterize indoor VOC exposure for the sampling period. Log 10-transformed VOC concentrations were used to calculate ICCs.

The real-time PM instrument used provided time-resolved PM measurements (i.e., particle count and concentration) at 1 min intervals. We calculated descriptive statistics for each center and used these center level measurements to calculate average PM concentrations across centers. We also assessed mean hourly PM concentrations at each facility to evaluate time trends in concentrations. We performed all statistical analysis in Stata 13.0 for Windows (StataCorp LP, College Station, TX, USA).

Results

General child care center characteristics

With the exception of two facilities, which served children up to age 12 years, the majority of child care centers (85.7%) served children between the ages of 6 weeks and 5 years. The number of children attending the child care centers ranged between 15 and 193 children (Mean 78.0 children). Among child care centers serving children up to age 5 years, the maximum number of children reported

attending was 160. General characteristics for participating child care facilities are presented in Table 1. Most of the child care centers were located in a commercial building (78.6%) and over half of the center respondents (64.3%) indicated that pesticides were applied on the premises for pest control. Half of the child care center respondents also reported that air fresheners and/or scented candles were used onsite and almost all center respondents (92.9%) stated that chlorine bleach (sodium hypo- chlorite, a source of chloroform) was used for sanitizing different surfaces in the facility. The most commonly reported surfaces sanitized with chlorine bleach included tables, bathrooms, and chairs, though respondents also reported the use of chlorine bleach to sanitize beds, changing tables, and children's toys. Over half (64.3%) of the child care centers were located more than 10 blocks from the nearest highway or dry cleaning facility and within five blocks from the nearest gas station. Approximately 30% of the facilities had 50% or more of usable carpeted indoor space (data not shown). While the majority of the facilities were located in an inner-city urban setting, only three facilities were near roads with heavy traffic and only one facility had an attached garage. Paint removal or remodeling activities within the prior two years of sampling was reported in 71.4% of the facilities; two Center Directors reported that painting was done while children and staff were present in the facility.

Center Directors from 13 of the 14 facilities sampled were able to provide information on health-related questions. We found that 30.8% and 46.1% of the

child care facility respondents reported having at least one child experience an asthma attack and a wheezing episode, respectively, within three months prior to sampling. Interestingly, all 13 child care facilities from which we collected health data had at least one child with physician-diagnosed asthma; 53.8% of the centers had at least 5 children with physician- diagnosed asthma. One center serving 89 children be- tween the ages of 6 weeks and 5 years reported having 25 (28.1%) children with physician-diagnosed asthma who were also taking asthma medication. The Center Director for this facility also re- ported that 5 children experienced asthma attacks and 10 children had wheezing episodes within three months prior to sampling. This facility was located less than one block away from the nearest highway and the Center Director also reported heavy traffic in close proximity to this facility. Overall reported physician-diagnosed asthma prevalence was 7.9% (median: 10.2%; range: 0.63– 28.1%); overall reported prevalence of children experiencing an asthma attack in the prior three months was 1.1%.

VOC and PM concentrations

We collected a total of 35 air samples from 14 child care facilities (two samples were collected from each of 11 child care facilities, four samples from each of two child care centers, and five samples from one center) serving 1092 children. Summary statistics for VOC concentrations are presented in Table 2. With the

exception of p-xylene, all VOCs measured had a detection frequency greater than 70%. Toluene and chloroform were detected in every child care facility. Median (range) concentrations for toluene, chloroform, benzene, o-xylene, ethylbenzene, and carbon tetrachloride were: 5.6 mg/m3 (0.6, 16.5 mg/m3); 2.8 mg/m3 (0.4, 53.0 mg/m3), 1.4 mg/m3 (<LOD, 4.4 mg/m3), 1.1 mg/m3 (<LOD, 35.7 mg/m3), 1.0 mg/m3 (< LOD, 28.5 mg/m3), and 1.0 mg/m3 (<LOD, 1.6 mg/m3), respectively.

Table 3 indicates the variability of concentrations between and within-facilities along with the respective ICCs for the VOCs measured and detected in more than one child care facility. The ICCs ranged from 0.32 to 0.75. Most of the variability in concentrations for benzene, chloroform, and toluene was largely due to between-facility differences suggesting that one sample may be sufficient to characterize exposure to these VOCs during a given day. For example, 25% of the variability in concentrations was due to differences within facilities for benzene while 26% of the variability was due to differences within facilities for chloroform and toluene. For carbon tetrachloride, ethyl benzene, and o-xylene we found that most of the variability in concentrations was due to within-facility differences (ICC=0.32–0.43) suggesting that multiple samples would be needed to properly characterize exposure to these VOCs within a given day.

Summary statistics for DustTrak PM measurements from six of the child care facilities are displayed in Table 4. Briefly, the one- minute PM2.5 and PM10

median (range) concentrations were 17.0 mg/m3 (7.0–128.0 mg/m3) and 19.0 mg/m3 (10.0–207.0 mg/m3), respectively; child care facility median (range) concentrations for PM2.5 and PM10 were 18.1 mg/m3 (14.0–34.1 mg/m3) and 23.9 mg/m3 (15.9–45.1 mg/m3), respectively. Peak median PM2.5 and PM10 concentrations were observed around 12:00 pm and, in general, concentrations seemed to decrease after noon and remain stable after 5:00 pm (Supplemental Fig. 1).

Discussion

In this pilot study, we characterized indoor concentrations of seven VOCs in 14 child care facilities and PM in six child care facilities in predominantly inner-city areas in Washington, DC. We also collected general information on participating facilities including chemical use indoors (e.g., cleaning products, pesticides, air fresheners), proximity to potential sources of VOCs and PM, as well as general information on the respiratory health of children enrolled at these facilities. All but one of the VOCs measured were detected in every center and PM was also widely detected in the facilities sampled.

While limited studies have documented the presence of indoor air pollutants in child care settings (i.e., child care/day care centers, nurseries, preschools), to our knowledge, this study is one of only two to characterize indoor concentrations of PM (PM2.5 and PM10) along with select VOCs in multiple U.S. child care

facilities. Brad- man et al., (2012) measured several VOCs in 34 California child care facilities including the seven VOCs measured in the present study. Compared to Bradman et al. (2012), median VOC concentrations in our study were 1.4–1.8 times higher. Maximum concentrations of frequently detected VOCs in our study were up to 14.3 times higher than those reported in California child care facilities. Differences in concentrations between these two studies may be due to differences in laboratory methods (e.g., limits of detection), quality of indoor ventilation in the facilities sampled, proximity of facilities to potential sources of target pollutants, and general facility housekeeping practices. For example, we detected chloroform (a VOC that may originate, among other sources, from consumer products containing chlorine bleach (Board CAR, 1990; Odabasi, 2008) in every air sample in our study while it was only detected in 38% of the facilities sampled by Bradman et al. (2012). This may reflect higher use of products containing chlorine bleach in the facilities we sampled compared to the California facilities. Although actual usage of chlorine bleach was not reported in the Bradman et al. study, the authors did report that chlorine bleach was stored in 65% of the facilities sampled. In our study, 92% of the facilities we sampled reported using chlorine bleach to sanitize and disinfect various indoor surfaces. While effective sanitation and disinfection is necessary to comply with childcare licensing regulations and to reduce the risk of infectious diseases in child care facilities, the use of some consumer products (e.g., those containing bleach or

sodium hypochlorite) may also lead to elevated levels of indoor pollutants known to be respiratory irritants. Additionally, recent increases in the concentration of bleach pro- ducts registered by the U.S. Environmental Protection Agency make diluting bleach correctly confusing and may expose facility staff to more irritating vapors when diluting the products before use. Alternatives for bleach-free disinfection and sanitation of surfaces in childcare settings that do not introduce other hazards should be explored (Agana, 2013; Program CCH, 2008; USEPA, 2013).

Carbon tetrachloride was detected in 86% of the facilities sampled; however, this VOC was not widely detected in child care centers in California (Bradman et al., 2012) where only 3% of the child care facilities sampled had detectable levels. Common sources of indoor exposure to carbon tetrachloride include building materials or products such as cleaning agents (Odabasi, 2008; ATDSR, 2005). To our knowledge, no other studies have assessed indoor concentrations of carbon tetrachloride in child care settings in the U.S. or elsewhere.

Compared with other studies conducted in child care facilities outside the U.S. (Fig. 1, Supplemental Table S1), particularly in Canada (St-Jean et al., 2012) and France (Canha et al., 2015; Roda et al., 2011), we generally observed comparable geometric mean (GM) concentrations for benzene, while higher GM benzene concentrations were reported in two studies conducted in Asia (Yoon et al., 2011; Zuraimi and Tham, 2008). We also observed lower arithmetic mean

benzene concentrations compared to those reported in German child care facilities by Fritz and Herbarth (2001). For ethylbenzene, we observed similar GM concentrations compared to those reported in child care facilities in Canada (Montreal) (St-Jean et al., 2012) and France (Paris) (Roda et al., 2011); while higher GM concentrations were observed in child care facilities in Clermont-Ferrand, France (Canha et al., 2015). In general, higher GM concentrations for toluene have been reported in child care facilities in other countries including Canada, Singapore, and France (Roda et al., 2011; St-Jean et al., 2012; Zuraimi and Tham, 2008). With the exception of child care facilities in Singapore (Zuraimi and Tham, 2008), we observed slightly higher GM concentrations for o-xylene in the facilities we sampled compared to child care facilities in Canada and France (Canha et al., 2015; Roda et al., 2011; St-Jean et al., 2012). Climate, season, location (urban vs. rural), indoor ventilation, sampling collection methods, laboratory analyses, building characteristics (e.g., proximity to potential pollutant sources), and general housekeeping practices may explain the noted differences among these studies; thus comparisons should be interpreted with caution. Limited work has been conducted to investigate children's exposure to PM specifically in child care settings in the U.S., with the majority of such studies conducted internationally. We observed similar mean PM2.5 concentrations compared to those reported by Bradman et al. (2012) in 40 child care facilities in California (21.777.8 μg/m3 vs. 19716 μg/m3, respectively) and Canha et al.

(2015) in 7 nursery schools in France (2179 μg/m3). The PM2.5 concentration for several classrooms and lunchrooms in three nurseries in Portugal, ranged from 18.7 μg/m3 to 48.94 μg/m3 (Branco et al., 2014) while concentrations ranged from 14.0 μg/m3
to 34.1 μg/m3 in the child care facilities we sampled. Another study conducted in Portugal investigated urban and rural nursery schools and found that PM2.5 concentrations ranged from 9.91 μg/ m3 to 30.14 μg/m3 across classrooms and lunchrooms (Nunes
et al., 2015).

With respect to mean PM10 concentrations, we observed lower concentrations (33.8 + 22.1 μg/m3) compared to those reported in prior studies conducted in the U.S. (California) by Beamer and Castaño (2002) in one university child care center (47.3 + 9.4 μg/ m3 and 68.9 + 24.7 μg/m3 in two different classrooms) and by Bradman et al. (2012) in 35 child care facilities (54.8 + 32.3 μg/m3). Higher mean PM10 concentrations have also been reported in other studies conducted in child care facilities in Korea, Poland, and Germany (Fromme et al., 2005; Kabir et al., 2012; Mainka and Zajusz-Zubek, 2015). Differences in PM concentrations between studies could be due to several factors including differences in ventilation, measurement methods (real-time vs. gravimetric), and proximity of facilities to potential PM sources.

Bradman et al. (2012) reported that PM2.5 and PM10 concentrations in 11 and 46%, respectively of the child care facilities they sampled exceeded 24-hour standards (i.e., concentrations not to be exceeded in a 24-h averaging period) based on state (PM10 = 50 μg/m³) and U.S. Environmental Protection Agency (USEPA) standards (PM2.5 = 35 μg/m³). In our small pilot study, one child care facility exceeded the 24-h PM10 state standard, while no facilities exceeded the PM2.5 USEPA standard. However, it should be noted that these standards are based on 24-h gravimetric sampling(California Air Resources Board, 2015) while we collected real-time PM data and were only able to sample half of the facilities recruited for 10 h or less, limiting direct comparisons. Also, our one time sample may not be representative of children's daily exposures.

The use of insecticides can also contribute to indoor VOC levels (Chin et al., 2014) and although insecticides were not measured in this pilot study, we found that over half of the facilities reported onsite applications. Many insecticides are neurotoxicants and have also been linked to adverse respiratory effects including various respiratory symptoms (e.g., wheezing or whistling in the chest, trouble going to sleep due to wheezing or whistling in the chest), and an increased risk for cough (Glaser, 2005; Raanan et al., 2015; Salameh et al., 2003). The frequent pesticide use reported in the sampled facilities highlights the need for education on and adoption of integrated pest management (IPM) to reduce pesticide exposures in these settings. IPM is a pest control approach that emphasizes

prevention techniques such as removing sources of food and water, and sealing possible entryways such as cracks and crevices, to minimize the use of chemicals. Least toxic pesticides are used sparingly as a last resort.

Half of the child care facilities sampled also reported using air fresheners and/or scented candles indoors. Air fresheners and candles can also affect indoor air quality by emitting VOCs and particulate matter (Singer et al., 2006; Lee et al., 2014). For ex- ample, Lee et al., (2014) observed significantly higher concentrations of benzene, toluene, and ethylbenzene in homes where air fresheners were applied compared to homes where they had not been applied.

Another important observation included the prevalence of physician-diagnosed asthma reported in the facilities we sampled. The prevalence of physician-diagnosed asthma (as reported by Childcare Center Director) ranged between 0.68 and 28.1% (Median 10.2%). Three out of the 13 childcare Center Directors that provided this information reported a higher prevalence of physician-diagnosed asthma compared to that reported by the Centers for Disease Control and Prevention for children 0-4 years of age in Washington, D.C. (11.4%) (Centers for Disease Control and Prevention, 2008) and in the general U.S. population (5.4%) (Bloom et al., 2012).

The main limitation of this study is the sample size, which limits our statistical analysis and the power to draw any inferences. Similar to other child care studies, sampling was limited to a convenience sample of child care facilities as

recruitment proved to be a challenge, potentially leading to selection bias. Our small sample size also limits generalizability of our results. Additionally, most of the facilities recruited were in buildings or churches, so we were not able to assess exposures in home-based child care centers where children may experience different exposures. Like other studies in child care settings, our study only provides a “snapshot” of indoor concentrations of the target pollutants measured on the days sampled. Thus, concentrations of target contaminants may not reflect contaminant levels observed on other days, during different seasons, and/or long-term averages. Limited resources to conduct this study also prevented us from measuring other VOCs and indoor air contaminants. Lastly, information collected in questionnaires was self-reported and potentially subject to recall bias. For example, we relied on in- formation provided by Center Directors on questions related to children's health (e.g., number of children with physician-diagnosed asthma) rather than confirming this with parents to obtain more accurate information.

Despite these limitations, our study has several strengths. First, this study is one of only two studies in the U.S. to characterize indoor air concentrations for the selected VOCs and PM in multiple child care facilities helping to fill a major exposure data gap (Bradman et al., 2012). Our study also focused on child care environments the majority of which were located in inner-city environments characterized by minority populations and under- served communities who may

already be experiencing disproportionate exposures to environmental contaminants. In addition, although we only sampled 14 child care facilities, these facilities served a total of 1,092 children; thus, our exposure data provides a "snapshot" on indoor child care exposures to a large population of predominantly inner-city children. Lastly, we obtained at least two samples per facility allowing us to evaluate variability of concentrations within and between facilities and to assess whether multiple samples would be necessary in future studies to properly characterize indoor exposures to select VOCs.

There are multiple lessons to be gained from this pilot study. As already noted, recruitment of childcare centers was a challenge. Since this pilot study was conducted without funding we did not have dedicated staff to help with recruitment efforts and relied heavily on letters and mass e-mails. A dedicated staff member to call center directors followed by in-person visits to the centers would likely improve enrollment considerably as the center di- rectors are pressed for time and responding to e-mail for study participation is not their priority. Future studies should also con- sider collecting concurrent indoor and outdoor samples to better characterize the indoor source contributions. Detailed inventories of potential exposure sources including information on frequency of use for consumer products would also be useful in informing potential areas of intervention. The usage pattern of such products is likely to vary across season and among facilities. Hence, future studies of longer duration are needed to

identify the temporal as well as spatial patterns of such uses to better inform targeted exposure mitigation interventions.

Indoor air quality has been the focus of several studies in an effort to characterize and reduce children's indoor environmental exposures; however, few studies have focused on child care environments. Our data suggest that children in predominantly inner-city environments in the Washington D.C. metro area are potentially exposed to elevated levels of VOCs and/or PM in child care facilities. Given the amount of time that children may spend in these facilities and their unique susceptibility to environmental exposures, educational outreach efforts that focus on sustainable strategies to improve indoor air quality in these settings is warranted. Future studies are needed to quantify children's individual exposure levels of indoor contaminants in such environments to assess the health impact of these exposures, and determine if such exposures can be mitigated through culturally competent mitigation programs specifically targeted towards child care professionals.

Competing financial interests statement

The authors declare that they have no financial conflicts of interest.

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None.

Human Subjects statement

The University of Maryland Institutional Review Board re- viewed and approved all study protocols and written informed consent was obtained from child care Center Directors prior to data and sample collection.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2015.12. 005.

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Table 1
General characteristics for participating child care center facilities ($N = 14$).

	N	(%)
Building facility type		
Commercial building	11	(78.6%)
Church	3	(21.4%)
Spray pesticides (center staff or contractor) inside and/or outdoors		
Yes	9	(64.3%)
No	2	(14.3%)
Do not Know	3	(21.4%)
Use air fresheners or candles (scented or unscented)		
Yes	7	(50.0%)
No	7	(50.0%)
Use chlorine bleach in the facility		
Yes	13	(92.9%)
No	1	(7.1%)
Facility proximity to nearest highway		
< 1 block	1	(7.1%)

1–5 blocks	3	(21.4%)
5–10 blocks	1	(7.1%)
> 10 blocks/not near a highway	9	(64.3%)
Facility proximity to nearest dry cleaning facility		
< 1 block	2	(14.3%)
1–5 blocks	3	(21.4%)
> 10 blocks/not near a dry cleaner	9	(64.3%)
Facility proximity to nearest gas station		
< 1 block	3	(21.4%)
1–5 blocks	6	(42.9%)
5–10 blocks	2	(14.3%)
> 10 blocks/not near a gas station	3	(21.4%)
Facility has an attached garage		
Yes	1	(7.1%)
No	13	(92.9%)
Any remodeling or paint removal in the facility done within the last 2 years preceding sampling		
Yes	10	(71.4%)
No	4	(28.6%)
Peeling or flaking paint inside or outside the facility		
Yes	7	(50.0%)
No	7	(50.0%)
Children present when any painting is done on the building		
Yes	2	(14.3%)
No	12	(85.7%)
Primary heating sources in the facility		
Electric	8	(57.1%)
Gas/oil	5	(35.7%)
Do not Know	1	(7.1%)

Table 2
Summary statistics for VOC concentrations in 14 child care facilities in Washington, DC[a].

VOC	LOD (μg/m^3)	DF%[b]	Mean	SD	GM	GSD	min	p25	p50	p75	max
Benzene	0.50	86	2.0	1.5	1.4	1.9	<LOD	0.9	1.4	3.6	4.4
Carbon tetrachloride	0.56	86	1.0	0.3	0.9	1.4	<LOD	0.8	1.0	1.2	1.6
Chloroform	0.29	100	7.3	13.8	2.7	3.5	0.4	1.1	2.8	4.2	53.0
Ethyl benzene	0.45	79	4.3	7.4	1.6	3.2	<LOD	0.6	1.0	4.7	28.5
o-xylene	0.72	71	5.0	9.3	1.8	3.4	<LOD	<LOD	1.1	5.2	35.7
p-xylene	0.45	7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.8
Toluene	0.35	100	7.2	5.7	4.8	2.0	0.6	2.2	5.6	14.2	16.5

LOD = Limit of detection.

[a] A total of 35 samples were collected from 14 child care facilities; we collected two samples from each of 11 facilities, four samples from each of two facilities, and five samples from one facility. Individual sample concentrations below the respective LOD were imputed to LOD/2 prior to calculating the average respective VOC concentration for each facility and subsequent descriptive statistics.

[b] DF% = detection frequency based on VOC detection at the facility level (i.e., if at least one sample had a VOC concentration > LOD then the chemical was considered detected for that child care facility).

Table 3
Variability of VOC concentrations in multiple samples from 14 child care facilities (n=35 total samples)[a].

	ICC[b]	$\sigma_{Between}$	σ_{Within}
Benzene	0.75	0.36	0.21
Carbon tetrachloride	0.43	0.14	0.16
Chloroform	0.74	0.55	0.33
Ethyl benzene	0.33	0.36	0.50
o-xylene	0.32	0.35	0.51
p-xylene[c]	NR	NR	NR
Toluene	0.74	0.41	0.25

[a] We collected two samples from each of 11 facilities, four samples from each of two facilities, and five samples from one facility for a total of 35 samples.

[b] Log 10-transformed concentrations for each VOC were used to calculate the ICC values reported. An ICC > 0.75 indicates excellent reproducibility, an ICC value between 0.4 and 0.75 indicates fair to good reproducibility, and an ICC of < 0.4 indicates poor reproducibility. Where $ICC = \sigma^2_{between}/(\sigma^2_{between} + \sigma^2_{within})$.

[c] p-xylene was only detected in one facility so no values are reported ("NR" = not reported).

Table 4
One-minute and child care facility averages for $PM_{2.5}$ and PM_{10} concentrations ($\mu g/m^3$)[a].

	One-minute averages ($\mu g/m^3$)[b]		Childcare averages for time sampled ($\mu g/m^3$)[c]	
Statistic	$PM_{2.5}$	PM_{10}	$PM_{2.5}$	PM_{10}
N	3250	3250	6	6
Mean	20.4	25.9	20.1	26.3
SD	9.1	17.5	7.2	10.5
GM	19.0	22.5	19.3	24.8
GSD	1.5	1.8	1.4	1.5
Minimum	7.0	10.0	14.0	15.9
p25	15.0	17.0	15.6	18.3
p50	17.0	19.0	18.1	23.9
p75	24.0	30.0	21.1	30.7
Maximum	128.0	207.0	34.1	45.1

[a] Values presented are for 6 child care facilities. Concentrations were recorded every minute for 10 h for five facilities and for four hours and 10 min in one facility for a total of 3250 readings.

[b] One-minute averages refer to particle counts or concentrations for every minute the real-time device was sampling for all measurements taken at all facilities ($n = 3250$ measurements).

[c] An average PM concentration for each facility was calculated for the entire sampling period; reported statistics are based on these facility PM averages.

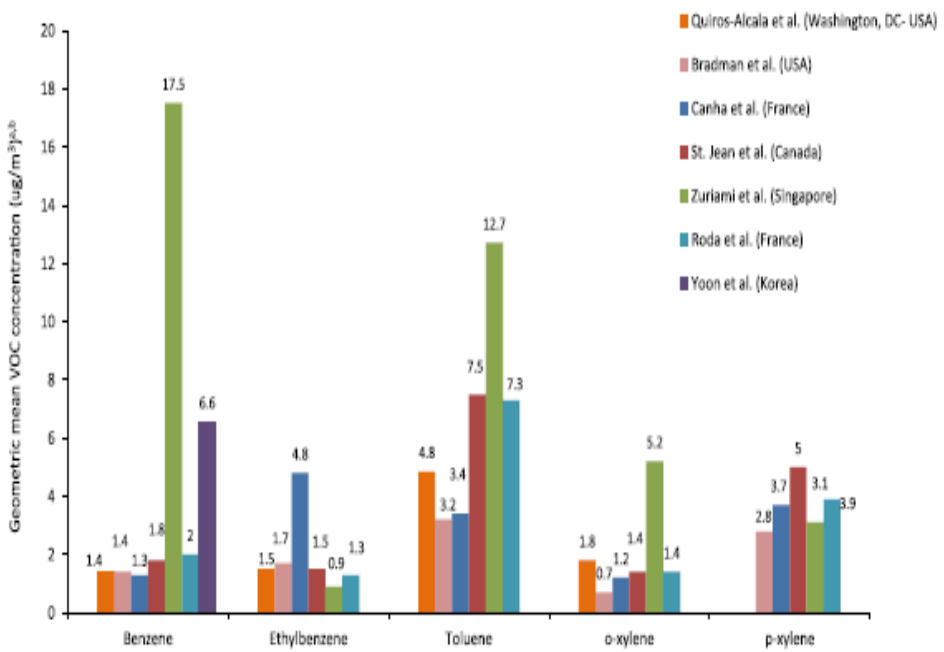

Fig. 1. Geometric mean concentrations for select VOCs measured in childcare centers in the present study and in childcare centers. p-xylene was only detected in one facility in the present study and is thus not presented. Bradman et al. did not report geometric means so the values reported in the figure represent the medians reported for each specific VOC. Roda et al. sampled VOCs in an indoor playroom and bedroom during cold (October–March) and hot months (April–September). Only indoor playroom concentrations are reported in the figure as these more closely resemble the locations sampled in the current study; GM VOC concentrations during cold months were slightly higher than hot months and are represented in the figure.

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