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Pre-clinical Measures of Eye Damage (Lens Opacity), Case-control Study of Tuberculosis, and Indicators of Indoor Air Pollution from Biomass Smoke

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Pre-clinical Measures of Eye Damage (Lens Opacity), Case-control Study of Tuberculosis, and Indicators of Indoor Air Pollution from Biomass Smoke

By

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ABSTRACT

PRE-CLINICAL MEASURES OF EYE DAMAGE (LENS OPACITY) FROM EXPOSURE TO BIOMASS SMOKE AND CASE-CONTROL STUDY OF TUBERCULOSIS AND INDICATORS OF INDOOR AIR POLLUTION EXPOURE

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This dissertation examines the level of two major pollutants in the kitchens from biomass, kerosene and liquefied petroleum gas (LPG) stoves, and two health problems associated with use of biomass and kerosene compared with LPG stove in Nepal. Its chapter a) characterizes the exposure levels of two pollutants-fine particles (PM2.5) and naphthalene from three cookstoves, b) validate exposure questionnaire used in two epidemiological studies with the gold standard, c) showcase method to estimate sample size (duration of measurements) to reliably characterize levels of PM2.5 across rural households from continuously measured PM2.5 data, and d) examines the association of use of biomass and kerosene fuel with pre-clinical damage of lens (lens opacity) and tuberculosis in women.

Divided on 5 chapters, the chapter 1 gives an overview of the dissertation. Chapter 2 provides a detailed background, materials and methods and results of validity study, and measurement results of naphthalene and PM2.5 from passive samplers. Similarly chapter 2 validates method for determining sample size from continuously measured PM2.5 data. Although there exist studies of associations of TB and cataracts from use of biomass fuel but the possible associations of TB and cataracts with the use of kerosene fuel are virtually uninvestigated. Chapter 3 investigates association between biomass and kerosene fuel use and pre-clinical damage of lens (lens opacity) in women and chapter 4 investigates the association between biomass and kerosene fuel use and TB in women. The chapter 5 summarizes the main findings of chapters 2, 3 and 4.

The results of the three main chapters suggest that in Nepal cooks who use unvented biomass cookstoves experience very high mean and peak exposure of PM2.5 compared with kerosene and LPG stoves. By contrast, the cooks experience higher exposure of naphthalene from both kerosene and biomass cookstoves compared with LPG cookstoves. Current and past use of biomass cookstoves is associated with an increase risk of nuclear opacity and use of biomass as a heating fuel and kerosene, either in stoves or in lamps, is a risk factor for TB. Thus, promotion of low-emission biomass stoves, such as semi-gasifier stoves or other cleaner burning fuels (biogas or LPG) for cooking and heating, and promotion of solar lamps or cleaner burning devises for lighting could minimize the risk of lens opacity and TB in women in rural areas of Nepal.

PRE-CLINICAL MEASURES OF EYE DAMAGE (LENS OPACITY) FROM EXPOSURE TO BIOMASS SMOKE AND CASE-CONTROL STUDY OF TUBERCULOSIS AND INDICATORS OF INDOOR AIR POLLUTION EXPOURE

TABLE OF CONTENT

PRE-CLINICAL MEASURES OF EYE DAMAGE (LENS OPACITY) FROM EXPOSURE TO BIOMASS SMOKE AND CASE-CONTROL STUDY OF TUBERCULOSIS AND INDICATORS OF INDOOR AIR POLLUTION EXPOURE

LIST OF TABLES

CHAPTER 2

CHAPTER4

PRE-CLINICAL MEASURES OF EYE DAMAGE (LENS OPACITY) FROM EXPOSURE TO BIOMASS SMOKE AND CASE-CONTROL STUDY OF TUBERCULOSIS AND INDICATORS OF INDOOR AIR POLLUTION EXPOURE

LIST OF FIGURES

CHAPTER 2

CHAPTER3

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I dedicate this dissertation to my wife, Sapana Sharma.

CHAPTER 1

Background

Background

1.0 Introduction

Approximately half of the world's population and up to 90% of rural households in developing countries still rely on unprocessed solid fuels, such as wood, crop residues or dung cake, for cooking and, sometimes, heating the house[1]. In most of the developing countries, solid fuels are burned in an unvented stove in poorly ventilated kitchens or rooms, throughout the year. As the stoves are not energy efficient, fuels are not burned completely. The incomplete combustion releases a complex mixture of organic and inorganic compounds, such as fine particulates (PM2.5), carbon monoxide (CO), poly-cyclic-aromatic hydrocarbons (PAH), formaldehyde (HCHO), naphthalene etc [1-3]. In addition to biomass fuel, many poor households in developing countries also use kerosene for cooking and or lighting. Like biomass, burning of kerosene also emits PM2.5, CO, carbon dioxide, sulphur dioxide, nitrogen dioxide , HCHO, benzene, toluene, hexane and various VOCs (volatile organic carbons)[4, 5]. A number of studies have suggested that exposure to indoor air pollution (IAP) from household solid fuel use increases the risk for several diseases, particularly in women and children, who receive the highest exposures. For example, based on the strength of evidence from meta-analysis[6], Smith et al (2004) have reported strong evidence of association of IAP with acute lower respiratory infection (ALRI in children <5 years), chronic obstructive pulmonary disease (COPD), and lung cancer (from exposure to coal smoke: for women and men $\geq$ 30 years). Although associations with asthma, cataracts (and other eye conditions), adverse pregnancy outcomes (including low birth weight), other cancers, heart disease, and tuberculosis (TB) have been found, they are not as robust as those for ALRI, COPD and lung cancer. In the case of cataracts, and TB, there exist only a few studies of the relationship with indoor air pollution, and they all have important limitations [7, 8]. Although the associations of TB and cataracts from use of biomass fuel have been studied, the possible associations of TB and lens opacity (cataracts) with use of kerosene fuel are virtually uninvestigated.

1.1 Indoor air pollution and tuberculosis: biological plausibility, existing studies and limitations

Pulmonary tuberculosis (TB) is a contagious infection caused by airborne *Mycobacterium tuberculosis* (MTB) bacteria. TB etiology is separated into two main stages -- becoming infected and having latent infection turn into an active case – although people can develop disease at the time of first infection. Worldwide TB kills more than 2 million people a year. In addition to higher prevalence of TB, there has been a coincident rise in the case of multi drug-resistant TB, and the HIV epidemic has made situation more complicated because TB is the chief fatal outcome of HIV infection globally. In 1991, the World Health Organization (WHO) set up the objectives of treating successfully 85 % of the TB cases and of detecting at least 70 % of the smear positive TB cases by introducing the directly observed treatment short-course strategy (DOTs). However, case detection rate for new smear-positive cases in DOTs was estimated at 61% in 2006, which is lower than the anticipated detection rate (70%). Although case findings

(TB) still remain largely passive in many developing countries [9, 10], a global public health effort to fight TB is largely focused on treatment without matching efforts on prevention. The steady rise in TB incidence in developing countries raises the question whether only delivery of treatment programs is sufficient to control this global burden? The public health history of industrialized countries suggests that part of the decline of TB was achieved by improving housing and habitat, better nutrition, decreasing crowding, and introducing better hygiene and sanitation [10, 11]. While society continues to apply new drug combinations and improved treatment programs in developing countries, therefore, it would seem prudent also to more thoroughly explore the roles of environment and socio-economic factors that increases the risk of TB in population. A range of social, environmental, and behavioral factors influence exposure and susceptibility to *Mycobacterium tuberculosis* infection. Thus, identification of TB risk factors and minimizing exposure to them could reduce the TB burden from the world.

Exposure to smoke from both active/passive tobacco smoking and biomass fuel combustion has been shown to have consequences on the human respiratory system in general, and TB in particular [12-14]. Inhalation of fine particles (PM2.5) and chemicals found in smoke from these sources generates an inflammatory response and impairs the normal clearance of secretions from the tracheobronchial mucosal surface. This may allow TB bacteria, to escape the first level of host defenses, which prevent bacilli from reaching the alveoli [15]. Smoke also impairs the function of pulmonary alveolar macrophages, an important early defense mechanism against bacteria [16]. Compared with macrophages from non-smoker, alveolar macrophages isolated from the lungs of smokers have reduced phagocytic ability and a lower level of secreted proinflammatory cytokines[17]. Exposure to wood smoke in rabbits has been shown to negatively affect antibacterial properties of alveolar macrophages, such as the ability to phagocytize bacteria, and intracellular bactericidal processes[18]. Six epidemiological studies have investigated the association between IAP and TB. However, there are important limitations in these studies and the results are also inconsistent [19-24]. Most of the studies had only very limited adjustment for potentially important confounding factors. In addition, exposure information ascertained in all studies is solely based on interviews with standardized questionnaires. None of these studies have validated their questionnaire with either exposure assessment/measurement or a validity study. These limitations clearly suggest that there is a need to confirm the findings of IAP and TB using exposure measures and fully controlling for potential confounders.

1.2 Indoor air pollution and cataracts/lens opacity: biological plausibility, existing studies and limitations

According to WHO estimates, there are 37 million blind and 135 million visually disabled people worldwide who depended on family support or care on a daily basis [25]. About 60% of blindness is due to cataracts and refractive error [25]. Based on the projection of global population and their aging, the WHO has estimated 75 million blind people by 2020 of which 50% blindness would be due to cataracts or opacification of lens. Cataract is a lens opacity that is associated with visual symptoms and some visual disability. And lens opacity is a locus of increased light scattering in the crystalline lens resulting in a decrease of lenticular transparency[26]. The opacities in eye range from minor (not interfering with vision) to major (interfering with total vision loss or blindness).

A causal relationship between exposure to indoor smoke and cataract is biologically plausible. There is an evidence of a dose-response relationship between the cumulative effects of tobacco smoking and the risk of cataracts [27]. Smoke can induce oxidative stress and deplete plasma ascorbate, carotenoids and glutathione, which provide antioxidant protection against cataract formation [28-30]. Tobacco smoke and biomass smoke have many similarities[1, 28] and several studies have indicated that tobacco smoking and fuel smoke condensate enhance the formation of super-oxide radical, which decreases the formation of antioxidants, which, in turn, increases the risk of cataract[29, 31-34]. Six epidemiological studies have found an association between IAP exposure from biomass fuel combustion and cataracts or blindness [7, 35-39]. Although the findings of existing studies of IAP and cataracts/blindness are consistent, each of these studies has important limitations. Like IAP-TB studies, exposure information ascertained in all studies are solely based on interview/questionnaire. None of these studies have validated their questionnaire with either exposure assessment/measurement or a validity study. Similarly none of these studies have examined the association of IAP exposure by anatomical types of cataract at the pre-clinical stages. Identification of risk factors for cataracts at pre-clinical stages would offer prevention strategies for the most common form of blindness in women in developing countries.

1.3 Consideration of measurement error in the epidemiological studies

In environmental epidemiological studies, several issues relating to measurement errors require detailed consideration. Among others, validity or accuracy of the instrument or method used to collect exposure information, selection of exposure agents or pollutants for measurement, and assessment of magnitude of variability of exposure are important and need special consideration. These issues are further discussed below.

1.3.1 Validity and accuracy of measurement instrument

Unbiased results of a study on health hazards can be obtained only if the exposure and outcomes are correctly classified. Existing epidemiological studies investigating the association between solid fuel use and TB or cataracts have used surrogates of indoor air pollution (IAP) exposure such as types of fuel used in the house, kitchen location and ventilation but have not validated the questionnaire or these exposure proxies. Thus, validation of instruments (questionnaire or monitors) before launching an epidemiological study will increase confidence in epidemiologic association between exposure and health outcomes and reduces the bias that arises from exposure misclassifications in the study.

1.3.2 Selection of exposure agents for IAP and lens opacity and TB studies

Exposures to high level of fine particles (PM2.5) in kitchens from biomass or kerosene fuel combustion have been closely related to health effects in several studies. In the kitchen, poor combustion of cooking fuel not only generates PM2.5 but also pollutants like CO, nitrogen oxides and naphthalene. Although fine particulate matter is closely related to health effects, it is

not clear whether it acts alone or it is an indicator of the effect of the mixture of pollutants. For example animal studies have shown an association of cataracts with naphthalene. Biomass and kerosene smokes contain substantial amounts of naphthalene, which has not been measured previously in household's using biomass and kerosene stoves. Thus, development and validation of an affordable passive sampler to measure naphthalene indoors can help to document naphthalene concentration in developing countries where biomass and kerosene combustion is common. Similarly, estimation of mean concentrations of PM2.5 and the naphthalene/PM2.5 ratio across different fuel--stove types can help to decide whether PM2.5 can be used as a proxy for naphthalene concentration. This has not been done before.

1.3.3 Variability of PM2.5 from biomass and kerosene fuel combustion

Indoor air pollution from biomass fuel combustion is known to vary both within and between households. However, the magnitude of variability of air pollution concentrations arising from kerosene and liquefied petroleum gas (LPG) is not known. These variance components and any predictors that influence the variance should be taken into account in exposure assessment to validate the group level exposure as well as to control for the exposures[40, 41]. For example if the variability of exposure is high within certain exposure groups (biomass, kerosene and LPG cookstove users) then a statistically representative sample that can accurately characterize exposure across population or households should be chosen. Till date validation of models and methods to calculate the statistically representative sample to reliably characterize between household differences in IAP levels from continuously measured PM2.5 data has not been undertaken.

1.3.4 Contributions of the dissertation

This dissertation builds on three studies carried out in the Regional Tuberculosis Center (RTC) and Manipal Teaching Hospital (MTH) in Pokhara city, Nepal in collaboration with the Division of Environmental Health Sciences, (School of Public Health) and the School of Optometry of the University of California at Berkeley.

The collaboration focused on characterization of PM2.5 and naphthalene across three stove-fuel groups (unimproved biomass, kerosene and LPG), validation of the exposure questionnaire, and two epidemiological studies: 'use of biomass and kerosene and risk of TB in women' and 'pre-clinical damage of lens (lens opacity) from use of biomass and kerosene cookstoves in women in Pokhara Nepal'.

In chapter 2 of this dissertation, I have provided the methods and results of the validity study. Also chapter 2 documents the level of naphthalene and PM2.5 and within-household and between-household variance of PM2.5 across three groups of cookstoves. In addition, Chapter 2 showcases a method to estimate the needed sample size to reliably characterize IAP level across households in rural areas of Nepal. Building on the validity study, the survey--based questionnaire was used to study the association between use of biomass and kerosene cookstoves and risk of TB. Similarly using the survey--based questionnaire and lens photography, the

association between use of biomass and kerosene cookstoves and risk of pre-clinical damage of lens (lens opacity) in women was investigated.

1.3.5 Dissertation structure

Outcomes of this collaborative study are separately discussed in detail in five different chapters. Chapter 1 presents the relevant background. Chapter 2 has detailed background, materials and methods and results section of validity study, and measurement results of naphthalene and PM2.5 from passive samplers. Chapter 3 has detailed background, materials and methods and results relating to the study of the possible association between use of biomass and kerosene cookstoves and pre-clinical damage of lens (lens opacity) in women. Chapter 4 has detailed background information, materials and methods and results'- of the study of association between use of biomass and kerosene fuel and tuberculosis in women. Chapter 5 has the summary of findings of chapters 2-4.

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CHAPTER 2

Validation of the survey-based exposure questionnaire for indoor air pollution and lens opacity and Tuberculosis study

And

Exposure variability of fine particulate matter (<PM2.5) and measurement of naphthalene indoors

Validation of the survey-based exposure questionnaire for indoor air pollution and lens opacity and tuberculosis study, and exposure variability of fine particulate matter (<PM2.5) and measurement of naphthalene indoors

2.0 Introduction

2.1 Exposure measurement error and its effect in epidemiological studies

Exposure measurement errors are one of the major sources of bias in epidemiological studies. Measurement errors can happen in any phase of the study. Generally, lack of validation of exposure instruments, and lack of consideration of exposure variability causes the measurement errors[1]. Validation of exposure instruments and consideration of exposure variability before starting main study, during data analysis or interpretation phases can minimize the bias. These issues are further discussed below under separate headings.

2.1.1 Instrument validation

Validation is defined as the extent to which a study is actually estimating what it is intended to estimate. Till date none of the epidemiological studies examining the association between indoor air pollution (IAP) and cataracts/lens-opacity or tuberculosis, for example, have validated the exposure instruments (questionnaire or exposure proxies) by either conducting air pollution measurements or actually inspecting or confirming main exposure variables in the study participants' house [2-10].

2.1.2 Validation of naphthalene passive sampler and measurements of PM2.5 and naphthalene concentration indoors

Biomass and kerosene cooking fuel combustion contains high amount of fine particulate matter (PM2.5) and semi-volatile hydrocarbons like naphthalene [11, 12]. The health effects from exposure to fine particulate matter (PM2.5) are well documented but health effects from exposure to naphthalene are not. Along with PM2.5, long or short term exposure to naphthalene can affect both lungs and eyes[13]. For example it has been known for more than a century that an accidental human intake of naphthalene results in lens opacity, cataracts and degeneration of the retina and crystalline deposits in the vitreous body of the human eye [14, 15]. Animal studies have shown association of cataracts with naphthalene [11, 16]. Despite such evidences, very few studies have investigated naphthalene exposure indoors from cooking fuel combustion. Lack of affordable tools to measure naphthalene has been one main reason for the lack of such studies. Thus, development, deployment and validation of an affordable sampler, like passive naphthalene badges would help to investigate the naphthalene concentration indoors in developing countries where combustion of biomass and kerosene is common for cooking, heating and lighting. In addition, such tools can be used in epidemiological studies examining the association between biomass or kerosene fuel use and risk of tuberculosis or cataract.

2.1.3 Magnitudes of exposure variability and its importance in exposure assessment and epidemiological studies

Combustion of biomass and kerosene fuel in the kitchens exhibit high short-term, and different within and between variability of exposure [17, 18]. From health point of view, short-term variability is important as it can potentially affect the health of exposed people [19]. For exposure assessment, exposure control, and epidemiological research, within and between exposure variability information is important. For example if within variability of exposure is greater than between variability of exposure, then grouping participants into a single exposure category increases the chance of exposure misclassification. Whereas, greater between than within variability of exposure increases the probabilities of correctly classifying exposure [20]. Similarly for exposure control, a group level intervention such as replacement of unimproved biomass stove with improved stove or biogas can be suggested, if the exposure variability is greater within than between (ie when between variance: σ^2_{bY} approaches 0).Whereas the focus of intervention should be shifted at individual level, when exposure variability is greater between than within [20-22].

Until now, the magnitudes of variability, both short and long term, and within and between cooking fuel stoves (biomass, kerosene and LPG) have not been studied systematically. Very few studies of IAP [23], and IAP and health have formally examined the question of exposure uniformity by stove groups. These studies are limited to only biomass fuel stove [17]. Thus the question, whether users of typical kerosene or LPG fuel stove also experiences uniform exposures have not been investigated. Kerosene and LPG stoves are common energy technologies in many urban and peri-urban areas of developing countries. Knowing whether or not users of typical biomass, kerosene or LPG stove experiences same mean level of exposure (when σ^2_{bY} approaches 0 or when ${}_bR_{0.95}$[1] ≤ 2) will provide a basis for designing efficient IAP monitoring strategies for IAP-health research.

2.1.4 Exposure measurements (sample size calculation) based on variance information

The presence of exposure variability of IAP also puts a premium upon collecting a large number of measurements or measurements of sufficient duration to reliably characterize IAP across households or persons. For example if there is a higher temporal variability of exposure then multiple measurements or measurements for longer durations are required to accurately characterize exposure[20]. On the other hand if between variance is high and temporal variance is low then few measurements would be sufficient to accurately characterize the exposure. Application of random effects model in repeatedly measured IAP data can generate information of the components of variance including intra-class correlations (ρ). The value of ρ can be exploited to estimate the sample size to optimize exposure assessment strategies. Till date only one study has used the continuously measured IAP data and its variance components to estimate the sample size for indoor air pollution measurements[24]. This one study conducted in India used minute by minute average carbon monoxide (CO) data of five days to estimate the temporal

[1] A ratio of 97.5th and 2.5th percentiles of the log normally distributed exposure concentrations containing 95% of the estimated mean concentration values.

variance of CO, and sample size [24]. Compared with CO, measurements of PM2.5 is very common in both IAP-health studies, and larger IAP monitoring programs in developing countries. Currently with the availability of affordable PM2.5 monitors like UCB, it has become much easier to collect PM2.5 data continuously for a sufficient duration of time [25-27]. However, none of the IAP researchers have utilized the minute by minute average UCB PM2.5 data of one week (covering weekdays and weekends) to characterize exposure, its variance components, and sample size. Thus, development of model to investigate the exposure variability of PM2.5 and confirmation of method to calculate sample size to reliably characterize between household differences of PM2.5 will be valuable in the IAP field.

In order to validate the main exposure proxies: main stove type and ventilation in the kitchen obtained during face to face interviews; investigate the nature of variability of PM2.5; test new naphthalene passive sampler; document weekly mean concentration of naphthalene and PM2.5 in the kitchens, a weeklong measurements of PM2.5 and naphthalene were conducted in Pokhara, Nepal. This study was conducted in the houses of a sub-set of participants of two epidemiological studies: IAP and pre-clinical damage of lens (lens opacity) and TB in women (chapter 3 and 4). The main objectives of the study were;

1. To examine the validity/reliability of exposure information obtained during face-to- face interviews (from questionnaire) at the hospital with data obtained from the indoor air pollution measurements and actual inspection of these features in the houses of sub-set of study participants of IAP-TB and lens opacity studies.

2. To estimate the magnitude of exposure variability of PM2.5 including its variance components (variances between and within three groups of stove: biomass, kerosene and LPG) and document if users of these three stoves have similar exposure profile of PM2.5; and determine required sample size to reliably characterize PM2.5 across households using biomass stove in Pokhara Nepal.

3. To develop and test an affordable passive badges to measure naphthalene indoors and investigate the naphthalene concentrations from three main cookstoves in Pokhara, Nepal.

2.1.5 Chapter summary

This chapter examines the accuracy of survey-based exposure questionnaire used in two epidemiological studies (Chapter 3 and 4) and reports the concentration of PM2.5 and naphthalene from three major cookstoves in Pokhara, Nepal. Using information of week-long PM2.5 measurement data, this chapter examines the contribution of stove and other covariates to the variability of PM2.5 concentration indoors. Using variance information from weeklong measured PM2.5 data, this chapter examines whether users of biomass, kerosene and LPG stoves experience uniform exposure to PM2.5 concentration within stove group. Similarly using variance information, this chapter validates and suggests possible method to calculate the sample size or measurements duration to reliably characterize between household differences of PM2.5

concentrations in households using biomass fuel stove. In the present study, level of nicotine in the kitchen and bedroom was also measured. These results are presented separately in the annex 2.7.

2.2 Methods

2.2.1 Household selection for indoor air pollution and survey based questionnaire validation study

Households for the study were selected from the first twenty-eight participants of the IAP- TB and lens opacity studies conducted in Regional Tuberculosis Center (RCT) and Manipal Teaching Hospital (MTH) in Pokhara sub-metropolitan city of Nepal. Pokhara sub-metropolitan city is a third largest city of Nepal after Kathmandu (capital) and Biratnagar in the east. This city is about 200 km west from Kathmandu and has about 200,000 inhabitants. Agriculture and animal husbandry are the main occupations in this area. Households in Pokhara use wood, kerosene or liquefied petroleum gas (LPG) as their main cooking fuel. Since many households have domestic animals, bio-gas also supplies bulk of energy needs. The houses in the city area are well-ventilated, due to large gaps between the walls and roofs, and open windows and opening doors in the house and the kitchen.

Under IAP monitoring, fine particulate matter (PM2.5), naphthalene and nicotine were measured for one week. PM2.5 and naphthalene were measured in the kitchens, and nicotine (as an indicator of environmental tobacco smoke) was measured in the kitchen and in the bedroom. Since, PM2.5 concentrations had weeklong (minute-by-minute) data, within and between fractions of variances of PM2.5 concentrations were evaluated. To showcase a method to calculate sample size or measurement duration for IAP measurements, minute-by-minute average data of PM2.5 was used. To study the magnitude of exposure variability of PM2.5 from three stove types over time (including its variances-between and within household), the minutes' measurements were converted into an hourly average measurements and used in the analysis (this data somewhat met the normality assumption of residual for log transformed PM2.5 data). Similarly, the covariates influencing the between households variances of PM2.5 was investigated. However, this investigation was limited to households using biomass stove.

Along with the documentation of level of pollutants by major stove type, the accuracy of reported main exposure variables (stove type and ventilation in the kitchen) by participants during face-to-face interviews (at the hospital) were verified at homes during IAP monitoring. The IAP measurements were accompanied by the administration of pre- and post-monitoring questionnaires. These questionnaires documented household characteristics, such as the presence of primary and secondary stove in the house, kitchen type, quantity of fuel used per week, ventilation in the kitchen and sources of other smoke exposures in the house such as tobacco smoking, use of incense and mosquito coil and use of non-electric lamp (kerosene lamps). Instruments, samplers and methods used for these studies are further discussed below.

2.2.2 Measurement of fine particulate matter (2.5 micro meter size)

A UCB particulate matter monitor was used to measure the PM2.5 [25, 26]. The PM2.5 concentrations were measured in the kitchens, where minute-by-minute particle levels were recorded for one week. The UCB particle monitor is a small, light, passive, battery-operated data logger developed at the University of California at Berkeley (UCB)[27]. Along with particles, UCB also continuously records temperature and humidity. The UCB contains two sensor chambers, the photoelectric (optical scattering by airborne particles) and ionization (ion depletion by airborne particles). For the purpose of this study, only data collected using photoelectric chambers were used. The photoelectric chamber is most sensitive to particle size of 2.5 micron (PM2.5). This chamber uses a light-emitting diode with output wavelength of 880 nm and a photodiode that measures the intensity of scattered light at an angle of 45^0 from the forward direction. Each UCB's default particle coefficient (PC) value is 0.0225 but as the light-scattering efficiency is a function of particle size and color, UCBs can mis-report the levels of gravimetric fine particulate matter (gold standard method in air pollution study) if they are not carefully calibrated with appropriate aerosols. For this reason, UCBs were calibrated against combusting aerosols, such as mosquito coils, incense, wood chips, in the lab (Marchant lab at UC Berkeley) against another field-validated instrument; DustTrak before launching them into the field. The DustTrak is a battery-operated laser photometer that gives real-time digital readout of particle concentrations to PM10, PM2.5, PM1.0 or respirable size fractions http://www.raeco.com/products/particulate/tsi_dusttrak.html.

The particle coefficients (PC) for each UCB were derived in the lab running UCBs and the DustTrak simultaneously, where PC was calculated from the slope of the line between the independent variable "mean UCB mass in $\mu g/m^3$ and the dependent variable "mean PM2.5 mass in $\mu g/m^3$ from DustTrak". The experiment was run for about 30 minutes where the average concentration of participles in the chamber obtained through DustTrack was 168 $\mu g/m^3$ (Standard Deviation: 46 $\mu g/m^3$) with minimum and maximum concentration ranging 17 and 229 $\mu g/m^3$, respectively. For this study, the field based particle coefficients were not obtained. The field based experiment conducted for different studies in Guatemala and Mexico have shown >85% correlation between mean UCB PM2.5and gravimetric PM2.5[27]. Thus, in this study average PC generated in the lab were incorporated in each UCB. In the field, all UCBs were zeroed in Ziploc bags for 30-60 minutes before and after monitoring to determine baseline concentration. Particle and temperature coefficients along with the results from zeroing were subsequently used in the data processing. In the kitchens, UCB monitors were placed at 1.5 meter height and about 1 meter from the combustion source. The PCs obtained from different combustion sources and temperature coefficients by UCBs are provided in Annex-2.0.9 table A.0.1. Similarly, Annex 2.7 table A.0.2 has the values of average PC incorporated in each UCB.

The detail information of other sources of emissions in the house and kitchen during monitoring periods, and information, such as whether UCB and other monitors were moved or disturbed during the monitoring period, were documented on pre and post-monitoring questionnaire. Exposure and other sources of emission in the house-related information were used to identify the best predictors for the higher concentrations of PM2.5 and naphthalene in the kitchens.

2.2.3 Naphthalene measurements method

The naphthalene constituents in the kitchens from indoor smoke were collected in passive badges with XAD-4 resin coated quartz filters. Annex 2.7.1 has protocol of XAD coating and extraction of PAH (naphthalene) from the XAD coated filter. These passive diffusion samplers were developed in Dr. Katharine Hammond's lab in UC Berkeley for this particular study, and were analyzed in GC-MS in the same lab. Passive badges were exposed for one-week in the kitchens and were co-located with nicotine passive badges and UCB particle monitors. After completion of the sampling and before analysis, the passive badges were refrigerated in Pokhara and at the lab in UC Berkeley. The sampling rate of the passive monitor for naphthalene in air determined in the lab for these tests, 36 cc/min, agreed well with the theoretically calculated value.

2.2.4 Survey-based questionnaire validation method

The goals of a validity study were; a) to examine the degree of agreement of responses on the exposure information (current stove/fuel type, ventilation) obtained during face-to-face interviews at the hospital with the actual measurements and inspection of these variables at participants' house, and b) to identify the probability of misclassification of exposure from questionnaire based information to gold standard method - actual inspection of these variables in the house. There are several ways to describe the misclassification. In this study, the misclassification was calculated in terms of error rate and accuracy percentage.

2.2.5 Error rate and accuracy percentage calculation method

Equation 1 was applied to calculate the error rate [28]:

$$\text{Error rate} = (NP_i - NP_V) \div (NP_V) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

Where NP_i is the number of stoves reported during face to face interview and NP_V is the number of stoves observed during indoor air pollution monitoring.

Equation 1.1 was applied to calculate the accuracy percentage calculation

$$\text{Accuracy percentage} = (\text{true reports} \div \text{total reports}) \quad \ldots\ldots\ldots\ldots\ldots 1.1$$

2.2.6 Assessment of Inter-vs. Intra household and temporal variability of PM2.5 and sample size (or measurement duration) calculation to reliably characterize IAP level across rural households

A one-way random effects model with restricted maximum likelihood (REML) estimator was used to calculate the variability of PM2.5 for each group of stoves. The detailed methods are further discussed below (section 2.2.8).

2.2.7 Analysis of Variance (ANOVA) test

The differences in mean concentrations of PM2.5 and naphthalene by stove types were compared by ANOVA test. The Proc GLM option in SAS 9.1 was used to test for differences in mean concentrations from three stove types. For naphthalene data, a Kruskal-Wallis and Tukey test (non-parametric ANOVA test) was further carried out to evaluate the difference in mean concentration by stove types.

2.2.8 Inter vs. Intra household and temporal variability of PM2.5 in the kitchens

From the weeklong PM2.5 data, mean and maximum (peak) concentrations and inter, intra household and temporal variation of PM2.5 concentrations were evaluated by stove groups (biomass, kerosene and LPG). A one way random effects model with restricted maximum likelihood (REML) estimator was applied to calculate variability of PM2.5 by stove groups and days [29]. The REML estimates the random-intercept variance taking into account the loss of 1 degree of freedom resulting from the estimation of the overall mean (β). A proc mixed procedure in SAS 9.1 was used for this analysis. The model had following form:

$$Y_{ij} = \ln(X_{ij}) = \mu Y + \beta_i + \varepsilon_{ij} \quad \text{.....2}$$

Where X_{ij} represents PM2.5 level for the i^{th} stove on j^{th} day; μY represents the true fixed logged mean exposure level for the group (stove type)**;** β_i represents the random effects for the $_i^{th}$ stove ($\beta_i = \mu Y_i - \mu Y$); $\boldsymbol{\varepsilon_{ij}}$ represents the random deviation of the observed logged exposure level Y_{ij} on the j^{th} day for stove i ($Y_{ij} - \mu Y_i$). The model 2 assumes that the β_i and εij are mutually independent and normally distributed random variables, with means of zero and variances σ^2_{bY} and $\sigma^2_{wY,}$ representing between and within variances of PM 2.5. Thus, the total variability in logged exposure levels experienced by group is given by $\sigma^2_Y = \sigma^2_{bY} + \sigma^2_{wY.}$

Also in model 2, $Y_{ij} = \ln(X_{ij})$ is normally distributed with mean μY and variance $\sigma^2_{Y.}$ The fixed logged mean exposure level for the group obtained from model 2 was normalized and group level mean (μx) was calculated by applying equation 3 as shown below:

$\mu x = \exp(\mu Y + \sigma^2_Y \div 2)$ ……3

$\sigma^2_x = \sqrt{[\exp(2\mu Y + \sigma\text{^}2Y)][(\exp(\sigma\text{^}2Y) - 1)]}$……3.1

The estimators of μx and σ^2_x have properties that make them preferable to the simple unbiased estimators of population mean (X) and variance (S^2x), which generally have larger variance, for lognormally data[29].

2.2.9 Relative measures of variability of PM2.5 (fold range) by stove groups

Since ranges of exposure levels (between and within variances) indicated by σ^2_{bY} and σ^2_{wY} are in logged scale, to interpret results in simple term, a scale-independent measure of exposure variability, the fold range ($R_{0.95}$) was used [29]. Fold range is a ratio of 97.5th and 2.5th percentiles of the log normally distributed PM2.5 concentrations containing 95% of the estimated mean values [29, 30]. The estimated fold ranges were calculated for each group of stove by substituting values of σ^2_{bY} (between variance) and σ^2_{wY} (within variance) obtained from the random effects model (model 2) using following algorithms: ${}_bR_{0.95} = e^{3.92 * \sigma bY}$ and $wR_{0.95} = e^{3.92 * \sigma wY}$, and overall fold range as ${}_YR_{0.95} = e^{3.92* \text{sqrt } \sigma 2\, bY + \sigma 2 wY}$ respectively [30].

The values of ${}_bR_{0.95}$ suggest how broad the distribution of mean exposures within a given group of stove is, thus, ${}_bR_{0.95}$ was used to define the basis of uniformity of exposure within a stove group. For example if ${}_bR_{0.95}$ is <2, then the group is considered to be uniformly exposed [21].

2.2.10 Application of mixed model to determine the factors/covariates that affects the between stove/household variance of PM2.5

A mixed effects model, with and without fixed effect covariates (such as housing characteristics, burning incense and non-electric lamps during monitoring period etc) was applied to determine the important covariates that influence the between stove variance of PM2.5. Mixed effects model is a generalization of the ordinary least square regression that enables the analysis of data generated from several sources of variation instead of one [31]. A proc-mixed procedure in SAS 9.1 was used in model 2 for this investigation. Before considering the final model, a suitable variance structure was chosen from three variance structures; separate within and between, common within but separate between and common within and between variance structures. A likelihood ratio test was used to compare and choose the variance structures[29]. Only households using biomass stove were included in this analysis. The model had following form:

$Y_{hij} = \ln(X_{hij}) = \mu_{Yh} + \delta_{uhj}C_{uhij} + \beta_i + \varepsilon_{ij}$ ……5.0

Where, h is a group (biomass stove group), i is particular stove ID in a group, j is n_{hi} measurements for stove i in group h and u is a covariate and δ_{uhj} is regression coefficient representing fixed effects of the covariates.

2.2.11 Application of random effects model to determine the sample size (duration of monitoring period) for IAP monitoring

The fundamental statistical principle suggests that variance could be reduced by increasing the number of samples (n) [32]. In indoor air pollution studies, a sample size can be increased by either adding number of houses or number of hours or days of measurements. Thus, the optimal choice of monitoring would depend on the relative magnitude of variance, and an acceptable level of temporal variance that study team feels comfortable with. Partitioning of variance components and estimation of intra-class correlation (ICC or ρ) is possible in repeated measurements data by applying random effects model. The random effects model yields a between stove/households (σ^2_{bYh}) and within stove/households (σ^2_{wYh}) variances and the fraction of the overall variance attributable to between stove/house variability as in equation 6:

$$\rho = \sigma^2_{bYh} \div (\sigma^2_{bYh} + \sigma^2_{wYh}) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 6.0$$

The ICC directly measures the closeness of observations. A higher ICC value also indicates lower temporal variance to the total variance, suggesting that increasing duration of measurements is no longer necessary. Or in other words the duration after which IAP measurements yields no new information as data will be highly correlated [24].

A one way random effects model was applied to PM2.5 dataset of biomass stove group (n=17), which had complete minute by minute data for 7 days (week). The model 2 was applied to moving average values of 1, 3, 15 minutes and 1, 12, 24, 36, 48, 60, 72, 96, 120, 114 and 168 hours, respectively. A PROC EXPAND command in SAS 9.1 was used to calculate the moving averages. A ρ (ICC) of 0.80 was chosen as the acceptable level of variability of PM2.5 in the random effects model. The random effects model and ρ for each moving averages were then plotted against time. Since sample variance decreases with increasing sample size (n), the term σ^2wYh ÷ n can be substituted for σ^2wYh in the equation 6.0. This provides an expression to calculate the sample size or measurement duration required for a given reduction of temporal variability [24].

$$ICC = \sigma^2 bYh \div (\sigma^2 bYh + \sigma^2 wYh \div n) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 7.1$$

$$n = \rho\, \sigma^2 wYh \div \sigma^2 bYh\, (1- \rho) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 7.2$$

2.2.12 Linear Regression

Linear regression is a modeling technique used to describe the relationship between dependent (outcome) variable and a set of an independent (predictor or explanatory) variable. A linear regression model was used to predict quantitative exposures of naphthalene based on concentration by stove type and housing characteristics. Before running linear regression, the concentrations data were observed graphically. On the basis of nature of the data, they were transformed. Regression models were run on transformed data (log linear transformed), which was later normalized (exponentiated). An ordinary least square and stepwise regression model was used to arrive at the most parsimonious model and to assess household characteristics associated with higher concentrations of Naphthalene in the kitchen. Various interactions between predictors were analyzed before considering the final model. A regression model diagnostic was used to check the assumptions of functional form and constant variance of the final regression model. For this purpose a Shapiro-Wilks (Swilk) test was used and a q norm graph was plotted. A large *p* value (>0.05) under the Shapiro-Wilks test indicates that the variance is normally distributed in the model. The model with larger Shapiro-Wilks *p* value was considered as a final model.

2.3 Results

2.3.1 Households characteristics

Participants' demographics and household characteristics are presented in table 2.0.1.

Table 2.0.1 Participants' general household characteristics reported

General characteristics of respondent	Frequency (%) from face to face interview
Age	
20-30 years	9 (32.1)
30-40 years	7 (25.9)
40-50 years	5 (17.9)
>50 years	7 (25.1)
Literacy	13 (46.4)
Can read & write	15 (53.6)
Cannot read and write	
Level of education	
Primary schooling	4 (14.3)
Middle schooling	4 (14.3)
High schooling	3 (10.7)
University/Collage	1 (3.6)
Adult education	1 (3.6)
None of these	15 (53.6)
Area of residency	
Rural	2 (7.1)
Urban & Per-urban	26 (92. 9)

Table 2.0.1 contd. Participants' general household characteristics reported

General characteristics of respondent	Frequency (%) from face to face interview
Current main occupation	
Farming (on family land)	12 (42.9)
Agriculture labor (paid)	2 (7.14)
Government services	1 (3.57)
Commerce/business	2 (7.14)
Industry	1 (3.57)
Housewife	7 (25.0)
Teaching & studying	3 (10.7)
Current cooking status	22 (78.6)
Cook now	6 (21.4)
Not now but cooked in the past	
Days of cooking per week	
4 days	1 (3.70)
7 days	26 (96.3)
Total duration of cooking/day	
1-2 hours	8 (28.6)
2-3 hours	9 (32.1)
3-4 hours	5 (17.9)
>4 hours	6 (21.4)
Main stove type	
Unimproved biomass stove	18 (64.3)
Improved biomass stove	1 (3.6)
Kerosene stove	4 (14.2)
LPG stove	5 (17.9)
Mean years of using present stove	
years	7 (25.0)
1-3 years	1 (3.57)
3-6 years	3 (10.7)
>6 years	17 (60.7)
Kitchen location	
Cook outdoor including (open air)	6 (21.4)
Separate kitchen inside the house	12 (42.9)
Kitchen not separated inside the house	10 (35.7)
Windows in the kitchen	
Yes	26 (92.9)
No	2 (7.1)
Closing door	
Yes	22 (78.6)
No	6 (21.4)

Table 2.0.1 contd. Participants' general household characteristics reported

General characteristics of respondent	Frequency (%) from face to face interview
Current tobacco smoker	
Yes	10 (35.7)
No	18 (64.3)
Annual income	
<Rs 25,000	4 (14.3)
Rs 25,000-50,000	9 (32.1)
Rs 50,000-100,000	9 (32.1)
Rs> 100,000	2 (7.14)
Don't know	2 (7.14)
Refused to answer	2 (7.14)
Ceiling fan/exhaust fan	
Yes	1 (3.7)
No	26 (96.3)

2.3.2 Survey based questionnaire validation study results

Comparisons of exposure variables reported during face-to-face interview and IAP monitoring are presented in table 2.0.2. During the face-to-face interview, 18 participants had reported their main stove as unimproved biomass stove (biomass stove without flue) and 1 participant had reported her main stove as improved biomass stove (biomass stove without flue). All participants (19) were found using stoves as reported during face-to-face interview. Similarly during face to face interview, 4 participants had reported kerosene stove as their main stove but only 3 participants were found using kerosene stoves. In the case of LPG stove, during face to face interview, 5 participants had reported LPG stove as their main stove but 6 LPG stoves were found during monitoring. One person who had reported kerosene stove as her main stove was found using LPG stove. Participants over reported use of LPG stoves and underreported use of kerosene stove. Similarly in the case of kitchen location, participants over reported the separate kitchen inside the house and under reported the kitchen not separated by wall inside. Results are presented in table 2.0.3.

Table 2.0.2 Comparison of exposure variables reported during face to face interview and IAP monitoring

Main stove and kitchen location	Frequency (%) from face to face interview	Frequency (%) from the household inspection
Main stove type		
Unimproved biomass stove	18 (64.3)	18 (64.3)
Improved biomass stove	1 (3.6)	1 (3.6)
Kerosene stove	4 (14.2)	3 (10.7)
LPG stove	5 (17.9)	6 (21.4)
Kitchen location		
Cook outdoor including (open air)	6 (21.4)	6 (21.4)
Separate kitchen inside the house	12 (42.9)	13 (46.4)
Kitchen not separated inside the house	10 (35.7)	9 (32.2)

Table 2.0.3 Error rate of stoves and kitchen location reporting

Stove and kitchen location reported during face-to-face interview at the hospital	Error Rate %
Primary stove reported during face to face interview at the hospital	
Unimproved and improved biomass stove	0
Kerosene stove	33.33
LPG stove	- 20
Kitchen location reported during face to face interview at the hospital	
Cook outdoor including (open air)	0
Separate kitchen inside the house	-8.33
Kitchen not separated inside the house	10

2.3.3 Accuracy percentage

All 19 participants who had reported their main cookstove as being a biomass stove were found to be correct, as were the five reporting use of a LPG stove. One of the four participants who had reported using kerosene stove, however, was found to be using an LPG stove. On that basis, the accuracy (true reports ÷ total reports) of stove reporting was 96%. In the inspection of ventilation characteristics, one participant who had reported not having a window in her kitchen was found to have a temporary outside kitchen with a window-sized opening. Two participants who reported having a window in the kitchen actually did not have a window. Based on these data, the accuracy for reporting ventilation was 89%.

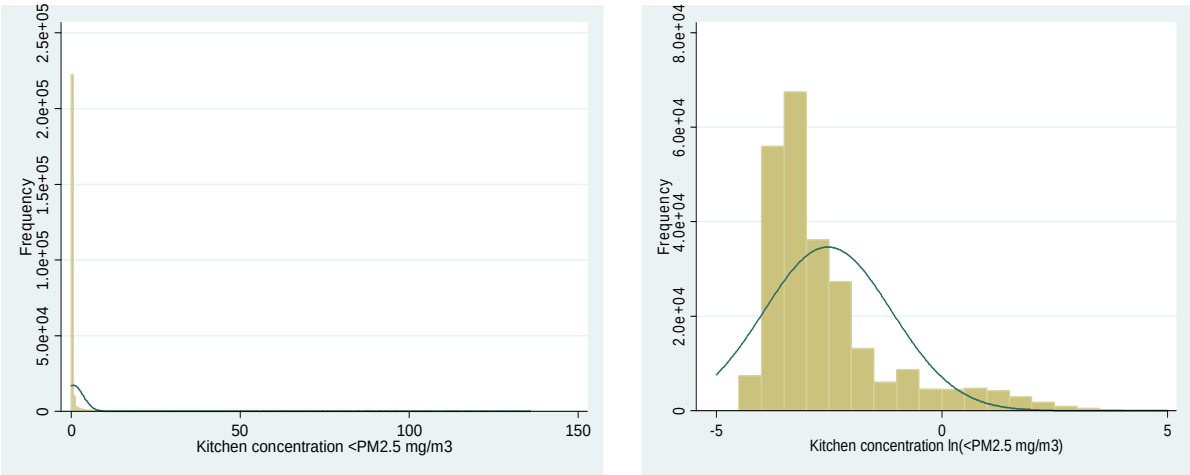
2.3.4 Results of kitchen concentrations of PM2.5

Table 2.0.4 summarizes the PM2.5 concentration for all stoves combined (biomass, kerosene and LPG stoves). Kitchen concentrations are approximately log normally distributed. See figure 2.0.1.

Table 2.0.4 PM2.5 mg/m^3 concentrations for all stoves combined -hourly average PM2.5 data

Moments	Kitchen concentration PM2.5 mg/m^3	ln (Kitchen concentration PM 2.5 mg/m^3)
N	28	28
Mean	0.558	-2.285
Standard Deviation (SD)	1.668	1.544
Coefficient of variation (CoV%)	299.103	-67.565
Minimum	0.171	-4.07
Maximum	27.265	3.306
Std. Error of Mean	0.0264	0.0244
Upper 95% mean	0.506	-2.237
Lower 95% mean	0.610	-2.333

Figure 2.0.1 PM2.5 concentration in mg/m^3 by stove types over a week from an hourly average PM2.5 data



Note: All stoves combined kitchen PM2.5 concentration mg/m^3 (untransformed-left figure and log transformed-right figure)

A mean concentration of PM2.5 was compared across stove types by ANOVA test. A significant differences in kitchen concentrations across different stove categories were found (p = 0.000). See figure 2.0.2 and table 2.0.5.

Figure 2.0.2 One-way analysis of variance of ln(PM2.5 in mg/m^3) by primary stove (ANOVA)

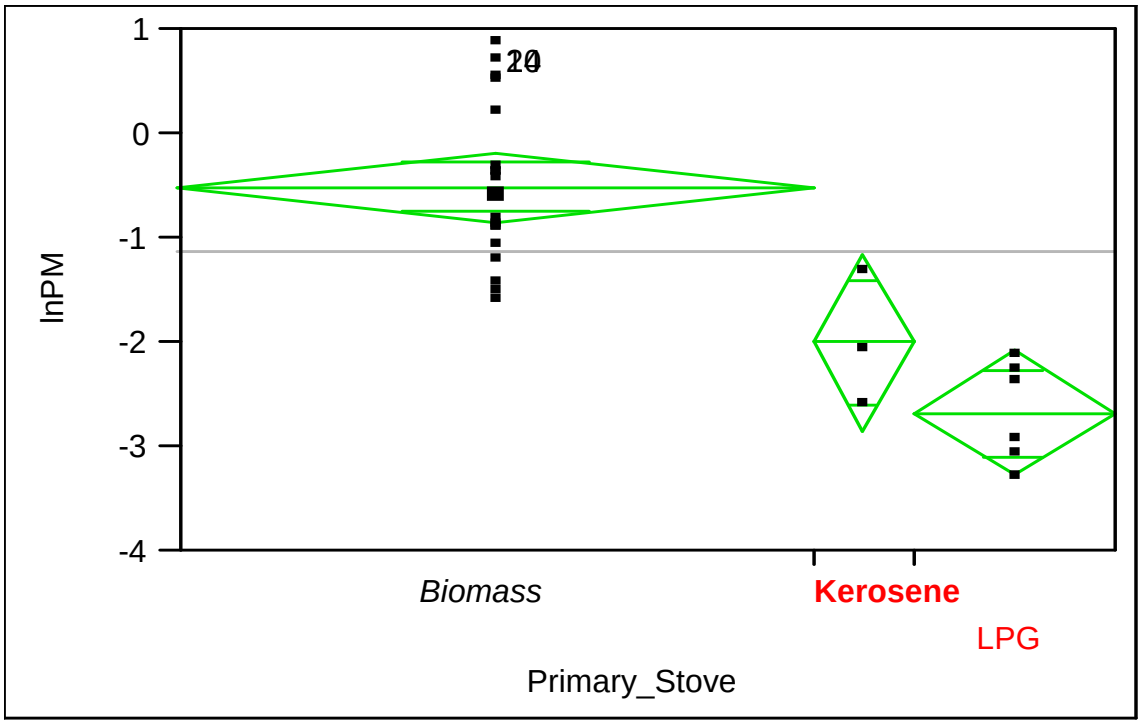


Table 2.0.5 PM2.5 concentration in mg/m^3 by stove types over a week from an hourly average PM2.5 data

Moments	**Biomass stove**	**Kerosene stove**	**LPG stove**
N	19	3	6
Weekly			
Mean (SD)	0.778 (1.99)	0.156 (0.25)	0.076 (0.09)
Coefficient of variation (COV %)	256.12	158.117	119.8
Geometric mean (GSD)			
Minimum	0.122 (5.95)	0.099 (2.22)	0.059 (1.88)
Maximum	0.017	0.046	0.028
Std. Error of Mean	27.27	2.98	1.60
Upper 95% mean	0.038	0.012	0.003
Lower 95% mean	0.85	0.18	0.082
	0.70	0.13	0.07
Hourly			
2nd highest value	24.64	1.63	0.89
3rd highest value	18.39	1.50	0.81
4th highest value	17.55	1.36	0.72

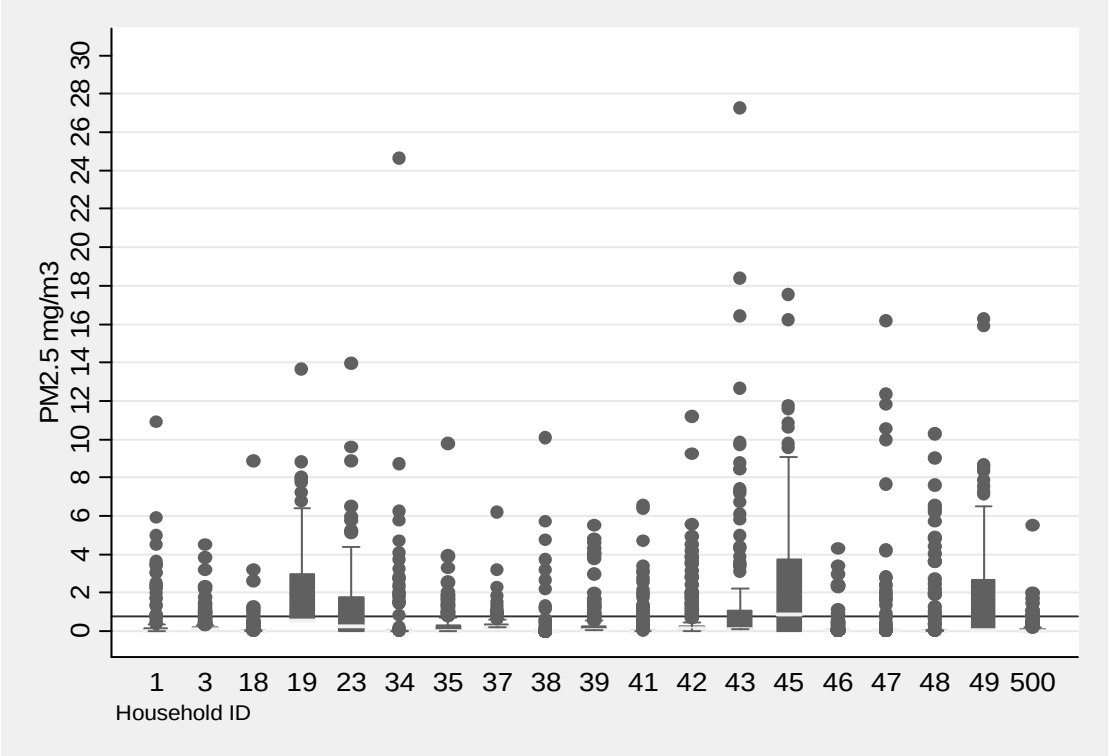
2.3.5 Variability of PM2.5 by days and stove groups

A temporal variability of PM2.5 concentrations was evaluated separately for three stove groups by days, which are discussed in detail below.

2.3.5.1 Temporal variability of PM PM2.5 by days from biomass stove

Figure 2.0.3 summarizes the range of mean concentrations of PM2.5 (mg/m^3) by household IDs over a week from biomass stoves. Table 2.0.6 summarizes the mean PM2.5 concentrations by days, variance components- between, within and total variances ($\sigma^2_{bY}, \sigma^2_{wY}, \sigma^2_{Y}$) and exposures fold ranges-between, within and total ($_bR_{0.95}, wR_{0.95}, _YR_{0.95}$).

Figure 2.0.3 PM2.5 concentrations (mg/m^3) for the ith house from biomass stove during a week period



Note: X axis household IDs and Y axis PM2.5 concentrations (mg/m^3)

Table 2.0.6 Within and between fractions of variation of PM2.5 concentration for biomass stove by days (fold range) from an hourly data

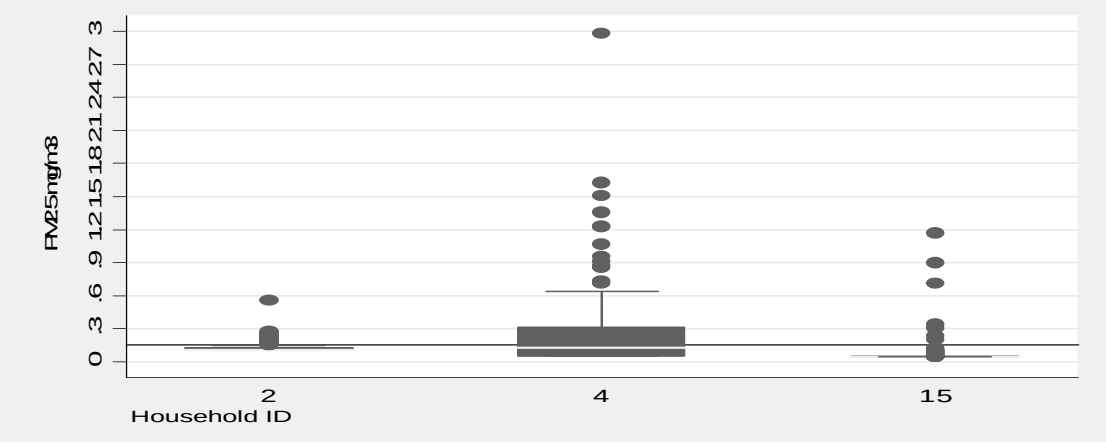
Days	$\mu_X^{\wedge *}$ **(mg/m^3)**	μ_Y^{**} **(mg/m^3)**	σ^2_{bY} $_{(b}R_{0.95)}$	σ^2_{Wy} $(wR_{0.95)}$	σ^2_Y $(_YR_{0.95})$	$\rho = \sigma^2_{bY} / \sigma^2_Y$
Sunday	0.366	-2.304	0.589 (20.277)	2.012 (259.763)	2.601 (556.699)	0.227
Monday	0.825	-1.989	0.837 (36.124)	2.757 (670.660)	3.5938 (1687.949)	0.233
Tuesday	0.568	-2.192	0.937 (44.493)	2.314 (388.740)	3.2513 (1174.143)	0.288
Wednesday	0.803	-2.016	0.678 (25.217)	2.916 (807.287)	3.5937 (1687.775)	0.189
Thursday	0.649	-2.041	0.753 (30.005)	2.464 (470.138)	3.2167 (1130.69)	0.234
Friday	0.709	-1.961	0.629 (22.414)	2.604 (558.732)	3.2333 (1151.362)	0.195
Saturday	0.523	-2.132	0.359 (10.493)	2.609 (561.929)	2.9683 (857.201)	0.121

*EXP ($\mu_{Y +} \sigma^2_Y$ /2): unbiased mean estimates; ** true fixed logged mean exposure

2.3.5.2 Temporal variability of PM 2.5 by days and time from kerosene stove

Figure 2.0.4 summarizes the range of mean concentrations of PM2.5 (mg/m^3) by household IDs over a week from kerosene stoves. Table 2.0.7 summarizes the mean PM2.5 concentrations by days, variance components- between, within and total variances ($\sigma^2_{bY,}$ $\sigma^2_{wY,}$ σ^2_Y) and exposures fold ranges-between, within and total ($_bR_{0.95,}$ $wR_{0.95,}$ $_YR_{0.95}$).

Figure 2.0.4 PM2.5concentrations (mg/m^3) for the ith house using kerosene stove over a week



Note: X axis household IDs and Y axis PM2.5concentrations (mg/m^3)

Table 2.0.7 Within and between fractions of variation of PM2.5 concentration for kerosene stove

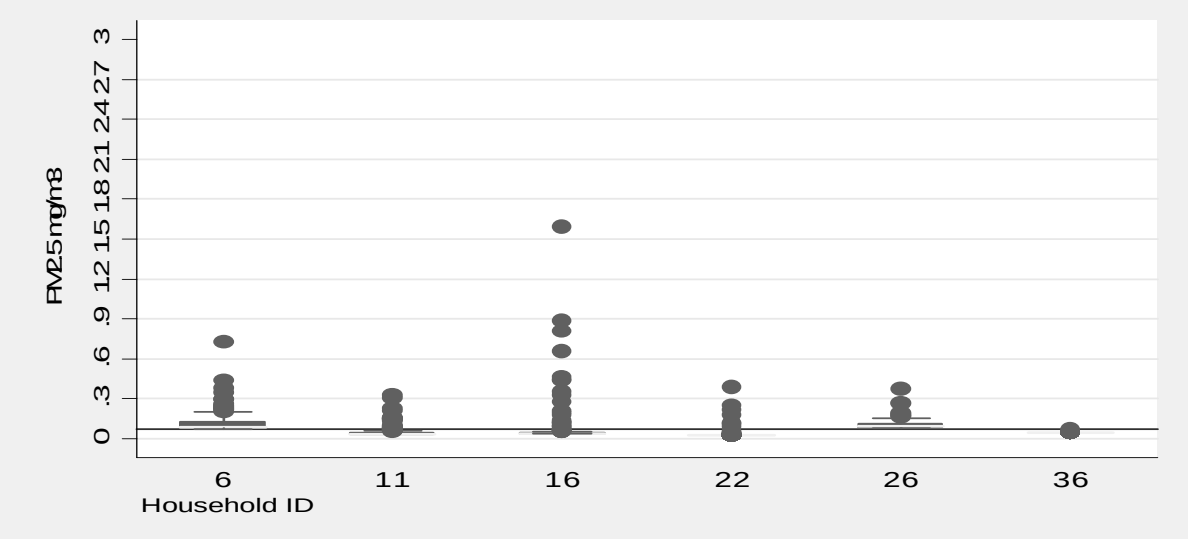
Days	$\mu_X^{\wedge *}$ (mg/m^3)	μ_Y^{**} (mg/m^3)	σ^2_{bY} ($_bR_{0.95}$)	σ^2_{wY} ($wR_{0.95}$)	σ^2_Y ($_YR_{0.95}$)	$\rho = \sigma^2_{bY} / \sigma^2_Y$
Sunday	0.194	-2.144	0.659 (24.112)	0.353 (10.271)	1.012 (51.626)	0.651
Monday	0.108	-2.434	0.2424 (6.889)	0.183 (5.337)	0.425 (12.874)	0.570
Tuesday	0.134	-2.261	0.008 (1.426)	0.486 (15.37)	0.494 (15.728)	0.016
Wednesday	0.199	-2.189	0.459 (14.216)	0.696 (26.314)	1.154 (67.476)	0.397
Thursday	0.179	-2.221	0.205 (5.905)	0.796 (33.036)	1.001 (50.529)	0.205
Friday	0.120	-2.371	0.281 (7.973)	0.221 (6.317)	0.502 (16.059)	0.559
Saturday	0.135	-2.328	0.319 (9.1461)	0.330 (9.512)	0.649 (23.523)	0.492

*EXP ($\mu_Y + \sigma^2_Y$ /2); ** true fixed logged mean exposure

2.3.5.3 Temporal variability of PM 2.5 by days and time from Liquefied Petroleum Gas (LPG) stove

Figure 2.0.5 summarizes the range of mean concentrations of PM2.5 (mg/m^3) by household IDs over a week from (LPG) stove. Table 2.0.8 summarizes the mean PM2.5 concentrations by days, variance components- between, within and total variances (σ^2_{bY}, σ^2_{wY}, σ^2_Y) and exposures fold ranges-between, within and total ($_bR_{0.95}$, $wR_{0.95}$, $_YR_{0.95}$).

Figure 2.0.5 PM2.5 concentrations (mg/m^3) for the ith house using LPG stove over a week



Note: X axis household IDs and Y axis PM2.5concentrations (mg/m^3)

Table 2.0.8 Within and between fractions of variation of PM2.5 concentration for LPG stove by days (fold range) from an hourly data

Days	$\mu_X^{\wedge *}$ **(mg/m^3)**	μ_Y^{**} **(mg/m^3)**	σ^2_{bY} $_{(b}R_{0.95)}$	σ^2_{wY} $(wR_{0.95)}$	σ^2_Y $(_YR_{0.95})$	$\rho = \sigma^2_{bY} / \sigma^2_Y$
Sunday	0.071	-2.848	0.257 (7.281)	0.157 (4.727)	0.414 (12.437)	0.620
Monday	0.067	-2.885	0.239 (6.802)	0.110 (3.674)	0.349 (10.146)	0.685
Tuesday	0.069	-2.871	0.220 (6.296)	0.149 (4.553)	0.369 (10.846)	0.596
Wednesday	0.071	-2.8581	0.266 (7.546)	0.153 (4.627)	0.419 (12.628)	0.635
Thursday	0.077	-2.797	0.221 (6.325)	0.249 (7.074)	0.471 (14.715)	0.471
Friday	0.076	-2.825	0.235 (6.688)	0.259 (7.352)	0.494 (15.724)	0.476
Saturday	0.076	-2.806	0.2083 (5.984)	0.232 (6.596)	0.439 (13.463)	0.474

*EXP ($\mu_{Y+}\sigma^2_Y$ /2); ** true fixed logged mean exposure

2.3.5.4 Inter vs. Intra-household variability of PM2.5 concentrations by stove group

The estimated weekly mean concentration of PM2.5, within and between variance of PM2.5 and exposure fold range (R0.95) for three groups of stove are presented in table 2.0.9. For biomass stove, the estimated weekly PM2.5 mean value of the lognormal distribution, ^μx (0.663 mg/m^3) was smaller than that of the corresponding simple estimates of the mean $\bar{X}$ (0.778 mg/m^3). Whereas for kerosene and LPG stove group, the lognormal distribution, ^μx and simple estimates of mean $\bar{X}$ were very close. The closeness of concentration values of lognormal distribution and simple estimates provides indirect evidence that the underlying exposure distributions of the X_j (or jth concentration received by all members of the observational group) are approximately log normally distributed.

The concentrations of PM2.5 were found varying more within than between stoves/households in biomass and kerosene stove group. However between and within variations of PM2.5 were similar in LPG stove group. The most variable of exposures were evident in biomass stove group (σ^2_Y = 3.306) followed by kerosene and LPG stove group (σ^2_Y = 0.723 and 0.426). The higher within than between variance of exposure in biomass and kerosene stove group suggest that day to day differences in exposure to PM2.5 are more prominent than differences in mean exposures between stoves within a group. The exposures experienced within biomass stove group had 495-fold range (highest value ÷ lowest value), whereas in Kerosene and LPG stove group, the within stove exposure fold ranges were 14 and 6, respectively. Estimates of $_bR_{0.95}$ and $wR_{0.95}$ both covered wide ranges ~6 to ~1200, which suggest many sources of variability operating at both between and within households in all stove groups. The intra-class correlation (ρ) values ranged

from 0.24 to 0.55, which suggests that many repeated measurements will be required to estimate unbiased mean exposure from biomass, followed by kerosene stove compared with LPG stove.

Since uniform exposure occurs when the between variability of exposure approaches 0 (σ^2 bY =0) or bR0.95 is ≤ 2, the higher between variability of exposure in all groups of stoves suggest that exposure to PM2.5 is not homogenous in any stove groups (biomass bR0.95 ~33, kerosene bR0.95 ~8 and LPG fuel stove bR0.95 ~7) in this study population.

Table 2.0.9 Within and between households' variances and fold range of PM2.5during a week period by stove type (hourly average data)

Stove	**X (mean in mg/m^3)**	**^μ_x (mg/m^3) ***	**μ_Y (mg/m^3) ****	**σ^2_{bY} ($_bR_{0.95}$)**	**σ^2_{wY} ($wR_{0.95}$)**	**σ^2_Y ($_YR_{0.95}$)**	**$\rho = \sigma^2_{bY} / \sigma^2_Y$**
Biomass	0.778	0.663	-2.064	0.801 (33.42)	2.505 (494.85)	3.306 (1246.30)	0.242
Kerosene	0.156	0.144	-2.300	0.270 (7.666)	0.453 (13.99)	0.723 (27.99)	0.373
LPG	0.076	0.073	-2.835	0.235 (6.689)	0.191 (5.546)	0.426 (12.92)	0.552

*EXP ($\mu_Y + \sigma^2_Y$ /2): unbiased mean estimates; ** true fixed logged mean exposure

2.3.5.5.1 Normality of predicted random effects and residuals

A normality assumption of model 2, which was applied to generate values for table 2.0.9, was tested for each group of stove. For this test, a random stove effects was estimated by β_i ÷standard error (β_i) and these values were plotted as normal probability plot. Later Shapiro-Wilks W test was applied to test the normality assumption. The normal W test results are presented in table 2.0.10. Probability plots are shown in figures 2.0.6-2.0.8. Models developed for all group of stoves met the criteria of normality as they had p value >0.05.

Table 2.0.10 Assumption of normality of random stove effects (βi) under model 1 for three stove type using the Shapiro-Wilks W statistics to test the (βi/SE βi) – hourly data

Stove type	**N**	**Normality test statistics (W test)**	**p-value of normality of test statistics**
Biomass	19	0.933	0.194
Kerosene	3	0.906	0.406
LPG	6	0.917	0.482

Figure 2.0.6 Probability plot for biomass stove

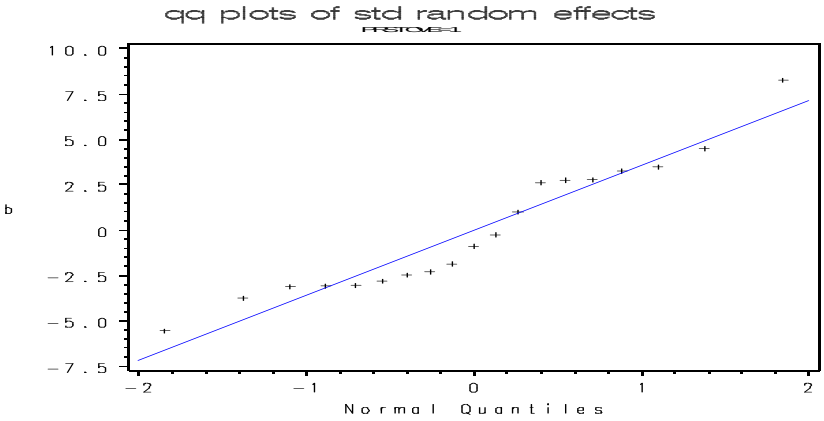


Figure 2.0.7 Probability plot for kerosene stove

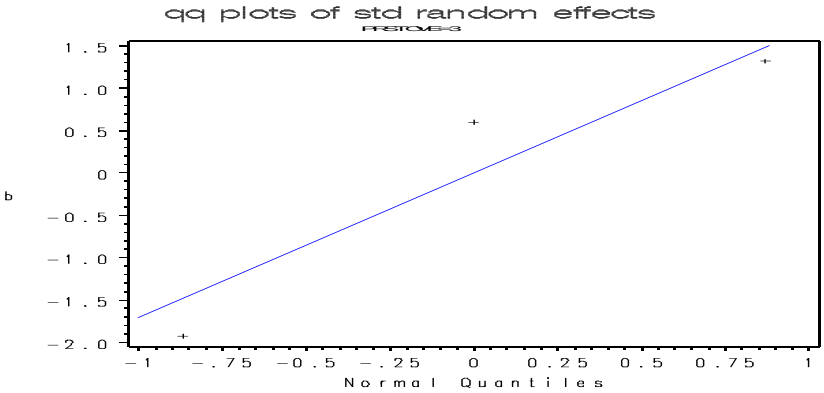
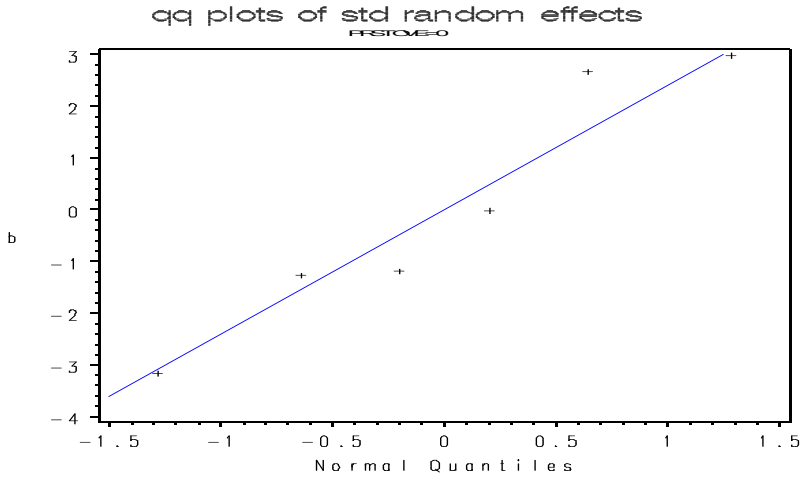


Figure 2.0.8 Probability plot for LPG stove



Similarly a residual (ε_{ij}), specific to stove (i) was tested by plotting a probability plots of residuals. A normality assumption of these plots was tested by normality graphs. Figures, 2.0.9-2.0.11 presents the probability plots of residuals by group of stove. Although *p* values are <0.05 but the visual representation of residual for biomass stoves indicated that residuals are normally distributed. Large residual tail is due to ~3 hours of cooking event generating higher

concentrations of PM2.5 and remaining ~21 hours generating lower concentration or baseline concentration values in a typical day in the kitchens.

Table 2.0.11 Assessment of assumption of normality of εij under model 2 for three stove type using the Shapiro-Wilks W statistics to test the estimated εij –hourly data

Stove type	N	Normality test statistics (W test)	p-value of normality of test statistics
Biomass	19	0.254	0.01
Kerosene	3	0.818	0.001
LPG	6	0.622	0.001

Figure 2.0.9 Probability plots of residuals for Biomass stove -hourly

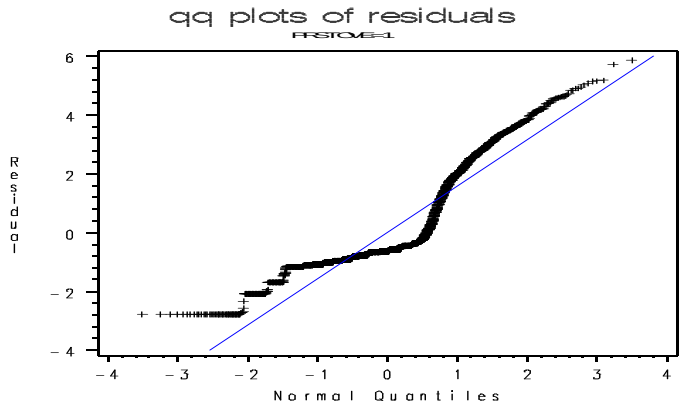


Figure 2.0.10 Probability plots of residuals for Kerosene stove-hourly

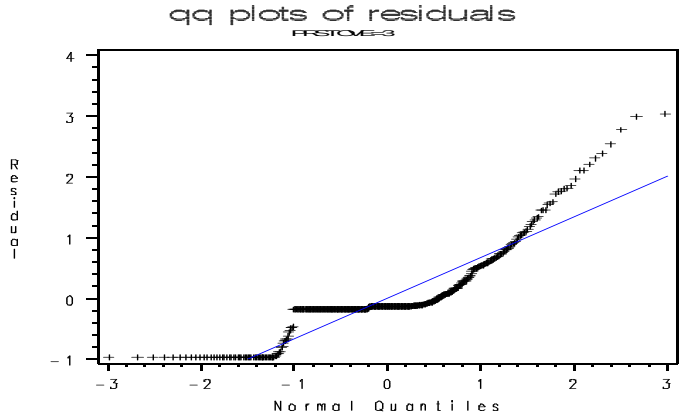
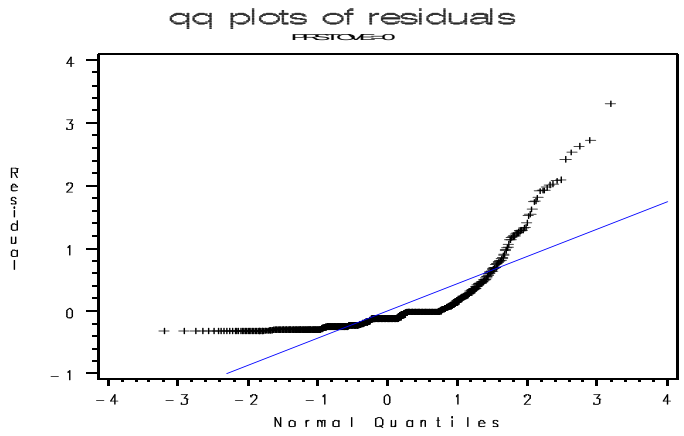


Figure 2.0.11 Probability plots of residuals for LPG stove-hourly



2.3.6 Inter vs. Intra stove and temporal variability of PM2.5 concentrations

The moving averages, standard deviation and maximum values of PM2.5 concentrations (mg/m^3) by stove types for various time intervals are presented in Table 2.0.12. Table 2.0.13 shows a within-household (1-ρ) and between household fractions (ρ) of total measurement variance for biomass stove. Similarly, Figure 2.0.12 shows the ICC (ρ) values from random effects model as a function of moving average and averaging time for biomass stove.

Table 2.0.12 PM 2.5 concentrations (mg/m^3) by stove types evaluated on the basis of moving averages (MA)

Metric	**Biomass stove**			**Kerosene stove**			**LPG stove**		
(MA)	Mean	SD	Max	Mean	SD	Max	Mean	SD	Max
C3-min	0.776	3.33	129.132	0.156	0.391	12.546	0.082	0.200	13.884
C15-min	0.776	2.613	74.936	0.156	0.313	5.583	0.082	0.144	5.780
C1-hr	0.776	2.005	40.948	0.156	0.242	2.979	0.082	0.094	1.596
C12-hrs	0.773	0.811	5.330	0.155	0.129	0.784	0.092	0.088	1.258
C24-hrs	0.770	0.695	4.247	0.153	0.102	0.524	0.100	0.087	0.799
C36-hrs	0.767	0.652	3.461	0.152	0.094	0.474	0.109	0.100	1.120
C48-hrs	0.764	0.624	3.098	0.153	0.089	0.380	0.117	0.111	0.920
C60-hrs	0.760	0.607	3.088	0.155	0.087	0.352	0.125	0.117	0.797
C72-hrs	0.757	0.593	3.025	0.157	0.086	0.326	0.133	0.121	0.971
C96-hrs	0.749	0.567	2.711	0.160	0.085	0.307	0.153	0.147	0.969
C120-hrs	0.741	0.542	2.617	0.165	0.085	0.307	0.171	0.158	0.923
C114 hrs	0.734	0.520	2.511	0.168	0.084	0.301	0.189	0.164	0.874
C168 hrs	0.727	0.498	2.465	0.173	0.085	0.334	0.206	0.173	0.958

Number of samples/measurements: (Biomass stove = 160607 minutes, Kerosene stove = 25902 minutes, LPG stove= 60371 minutes)

Table 2.0.13 Within-household and between household fractions of total measurement variance (1-ρ) for Biomass stove group

Metric (MA times)	**Between household variance (ρ)**	**Within-household fraction of total measurement variance ICC (1-ρ)**
C3-minutes	0.04	0.96
C15-minutes	0.06	0.94
C1-hr (60 minutes)	0.11	0.89
C12-hrs (720 minutes)	0.58	0.42
C24-hrs (1440 minutes)	0.74	0.26
C36-hrs (2160 minutes)	0.79	0.21
C48-hrs (2880 minutes)	0.82	0.18
C60-hrs (3600 minutes)	0.83	0.17
C72-hrs (4320 minutes)	0.84	0.16
C96-hrs (5760 minutes)	0.84	0.16
C120-hrs (7200 minutes)	0.86	0.14
C114-hrs (8640 minutes)	0.86	0.14
C168-hrs (10080 minutes)	0.87	0.13

(Note: Appendix 2.7 table A.0.8 has detailed data)

Figure 2.0.12 Intra-class correlation coefficients (ICC-ρ) for various moving average times for biomass stove

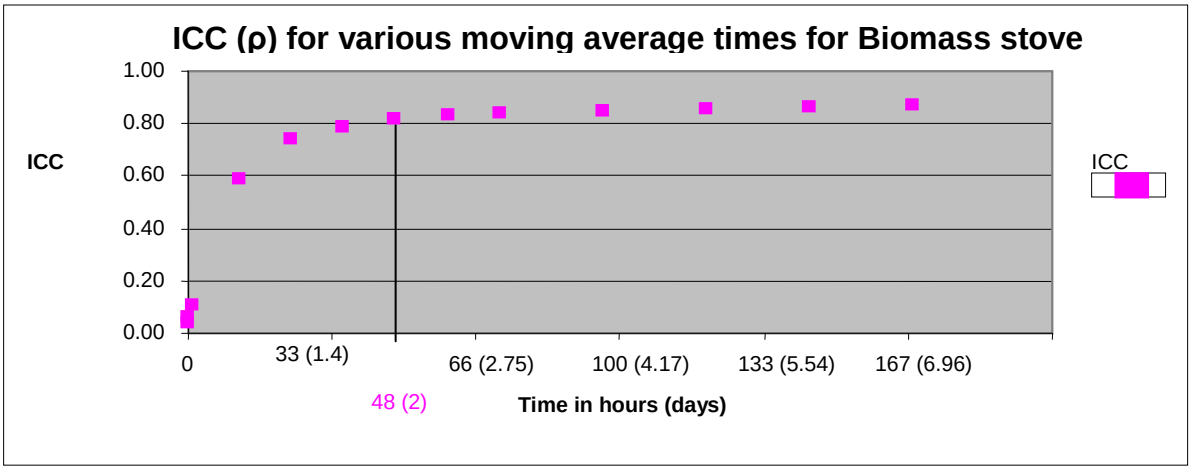


Figure 2.0.12 above confirms the statistical properties that sample variance decreases as sample size (duration of air pollution measurements) increases. Based on the values of table 2.0.13 and figure 2.0.12, an averaging time of 48 hours or more are needed to reliably characterize between household differences in PM2.5 in the present study population with acceptable within household variance (1-ρ) of 0.20. For example, for a group of biomass stove, the 1 hour between and within household variances were 0.444 and 3.633 mg/m^3 respectively (Appendix 2.0.7 table A.0.8). As within-house/stove variance scales with averaging time (n hours), the time needed to optimize the desired ICC (0.80)[2] can be calculated as follows;

$\sigma\varepsilon^2$, n = 3.633/ n

$\rho = \sigma^2 bYh \div (\sigma^2 bYh + \sigma^2 wYh \div n)$
$n = \rho\, \sigma^2 wYh \div \sigma^2 bYh\, (1- \rho)$
$= (0.80*4.077) \div (0.444*0.20)$
$= 36$ hours

[2] ICC (0.80) = 36 hrs

2.3.7 PM2.5 concentration from biomass stove by housing characteristics

Identification of factors that affect exposure or concentration of pollutants indoors is central to the exposure assessment. The uniform exposure to pollutant occurs when the between variability of exposure in an observational group/ stove is small (ie σ^2_{bY} approaches 0 or ${}_bR_{0.95}$ approaches ≤ 2). Based on the estimates presented in table 2.0.9, the value of ${}_bR_{0.95}$ for biomass stove is 33, whereas for kerosene and LPG stove, ${}_bR_{0.95}$ is 8 and 7. These values suggest that the exposure is not uniform and there are many sources of variability within and between houses. To identify the covariates or determinants that influenced the between household variance of exposure, a mixed effects model was run in the dataset of PM2.5 from biomass stove group. The mixed effect model was run with and without fixed effects (model without covariate is random effects model) covariates. The selection of covariates for the mixed effects model was based on the ANOVA test results. Any covariates that significantly influenced the mean concentrations of PM2.5 in ANOVA test were included into the random effects model (one at a time) first. Any covariates that reduced the between households variance in random effects model were then included into the mixed effects model (model 5). A separate within and between variance structures was used as a variance covariance structure in mixed effects model. Table 2.0.14 presents the results of difference in mean concentration of PM2.5 by potential exposure predictors/covariates (ANOVA test). Table 2.0.15 presents the results of between households variance of PM2.5 by exposure predictor/covariates obtained from the random effects model (model 2).

Table 2.0.14 Mean exposure levels of PM2.5 in mg/m^3 by covariates- ANOVA test from hourly data (analyzed using proc GLM model)

Housing characteristics	**Variables**	**Number of household**	**Number of hours of sample**	**Mean mg/m^3**	***P* value**
Stove	Biomass stove	19	2691	0.778	F=74.4
	Kerosene stove	3	434	0.156	*P* value= 0.001
	LPG stove	6	866	0.076	
Kitchen location	Cook outdoor including (open air)	6	867	0.787	F=2.73 *P* value= 0.065
	Separate kitchen inside the house	10	1397	0.713	
	Kitchen not separated inside the house	3	427	0.970	
Secondary stove	Yes		1023	0.889	F= 5.18
	No		1668	0.709	*P* value= 0.023
Housing type	*Pucca* & Semi-*pucca*	4	583	0.633	F=3.93, *P* value=0.048
	Kutcha	15	2108	0.818	
Windows open during monitoring	Yes		2102	0.677	F=20.53
	No		679	1.076	*P* value = 0.001
Incense burn in the house	Yes	6	846	1.196	F= 55.38
	No	13	1845	0.586	*P* value =0.001
Non electric lamp used in the house	Yes	13	1878	0.838	F = 5.73
	No	6	813	0.638	*P* value = 0.017
Observer assigned ventilation status	Good	8	1096	0.511	F = 33.5
	Fair & Poor	11	1595	0.961	*P* value = 0.001

Table 2.0.15 Estimation of between household variance of PM2.5 by exposure predictors in biomass stove group by random effects model (model 2.0) on hourly average data with separate within and between variance components

Predictors	**Mean (mg/m^3)**	**Mean exposure (Biomass stove)**	**Fixed effect *P* value**	**σ^2_{bY} ($_bR_{0.95}$)**	**σ^2_{wY} ($wR_{0.95}$)**	**σ^2_{Y} ($_YR_{0.95}$)**	**Between household variance reduction**
Only-Biomass stove	0.663	-2.064	-	0.802 (33.429)	2.5049 (494.791)	3.306 (1246.301)	-
Kitchen location (ref: kitchen outside and kitchen inside separated by walls	0.685	-2.056	0.969	0.849 (37.108)	2.505 (494.729)	3.355 (1312.658)	+6%
Use of secondary stove (ref: no)	0.685	-2.056	0.962	0.849 (37.109)	2.505 (494.729)	3.355 (1312.658)	+6%
Incense burned in the kitchen (ref: no)	0.552	2.236	0.234	0.778 (31.706)	2.505 (494.79)	3.2825 (1214.416)	-3%
Non electric lamp burned in the kitchen (ref: no)	0.540	-2.280	0.495	0.825 (35.159)	2.505 (494.79)	3.330 (1277.805)	+3%
Observer defined ventilation (ref: good)	0.460	-2.405	0.168	0.753 (30.019)	2.505 (494.79)	3.258 (1182.721)	-6%
Windows opened during cooking or IAP monitoring period in the kitchen (ref: no)*	0.431	-2.371	0.009	0.554 (18.499)	2.505 (494.729)	3.059 (949.423)	-31%
House construction (ref: *pucca* house)	0.683	-2.059	0.991	0.850 (37.116)	2.505 (494.729)	3.355 (1312.798)	+6%

The results in table 2.0.15 show that predictors such as windows opened all the time during cooking, observer assigned good ventilation and burning incense indoors reduce the between household variances of exposure. All these three predictors were included into the multivariate mixed effects model (model 5) to investigate the total reduction of between households variance after their simultaneous inclusion. The result of final model (mixed effect) is shown in table

2.0.16. The multivariate results showed that opening windows all the time during cooking significantly reduces the mean concentration of PM2.5. All three covariates simultaneously reduced the between household exposure fold rage ($_bR_{0.95}$) by 54%.

Table 2.0.16 Multivariate mixed effect model

Predictors	**Estimate**	**Standard error**	**DF**	**t-value**	***P- value***
Biomass stove	-2.5751	0.2556	15	-10.08	0.0001
Ventilation status (Good vs. Poor)	-0.2109	0.4494	15	-0.47	0.6456
Windows in the kitchen (opened all the time vs. some time and not opened)	1.3669	0.4157	15	3.29	0.005
Burned incense (No vs. Yes)	0.866	0.4646	15	1.86	0.082

Table 2.0.17 Comparison of between households' variance of PM2.5concentration from biomass stove by exposure predictors estimated before and after adjustment (an hourly data)

Predictors	**Mean mg/m^3**	**Mean exposure (Biomass stove)**	**σ^2_{bY} ($_bR_{0.95}$)**	**σ^2_{wY} ($_wR_{0.95}$)**	**σ^2_{Y} ($_YR_{0.95}$)**	**ICC**	**Fold range reduction (before and after adjustment)**
Biomass stove (with-out covariates adjustment)	0.663	-2.0640	0.802 (33.429)	2.505 (494.791)	3.306 (1246.301)	0.24	before: 1246.301- After: 876.2979 Reduction= 30%
Biomass stove (after covariates adjustments*)	0.339	-2.5751	0.483 (15.241)	2.505 (494.729)	2.988 (876.298)	0.16	

The normality assumption of the multivariate mixed model (table 2.0.16) was tested by generating standardized random effects. The estimated random effects divided by their estimated standard errors were also tested for normality. The deviations from normality test showed no significant deviations (*p* value 0.194). The normality test results are presented in table 2.0.18. Similarly the random effects divided by their estimated standard errors were plotted in q-q format. Figures 2.0.13-2.0.14 shows the probability plot of residuals.

Table 2.0.18 Assumption of normality of random stove effects (βi) under mixed effect model (table 2.0.16) for biomass stove using the Shapiro-Wilks W statistics to test the (β_i/SE β_i)

Stove type	N	Normality test statistics (W test)	p-value of normality of test statistics
Biomass	19	0.949	0.383
Biomass*	19	0.933	0.194

* mixed effect model without co-variates (table 4.15)

Figure 2.0.13 Probability plot for random effects model-5

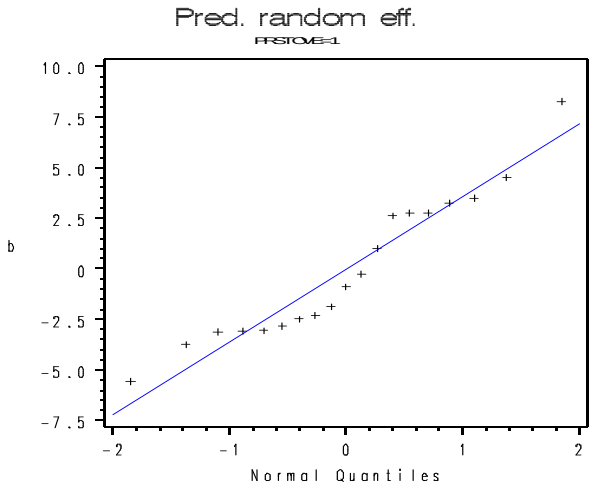
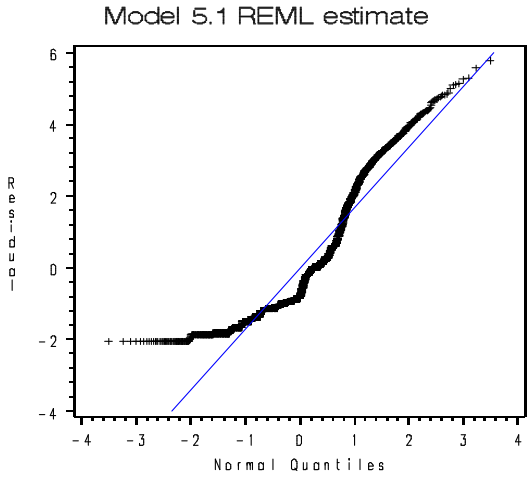


Figure 2.0.14 Probability plots of residuals for random effects model -5



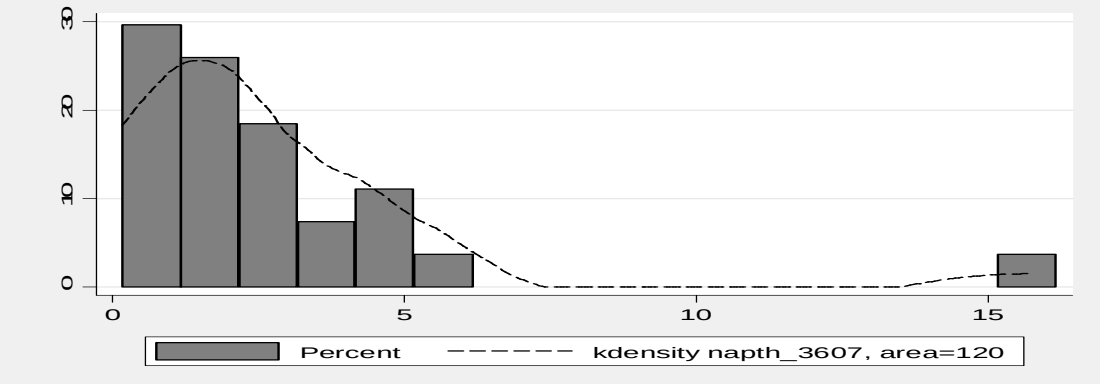
2.4 Results of kitchen concentration of Naphthalene in µg/m^3

Table 2.0.19 summarizes the naphthalene concentrations for all stove types. Kitchen concentrations were approximately log normally distributed. See figure 2.0.15.

Table 2.0.19 Naphthalene concentrations for all stove types in µg/m^3

Moments	Kitchen concentration Naphthalene	ln (Kitchen concentration Naphthalene)
N	27	27
Mean	2.67	0.98
Standard Deviation (SD)	3.02	1.11
COV(%)	113.11	-
Minimum	0.18	-1.71
Maximum	15.76	2.76
Std. Error of Mean	0.58	-0.54
Upper 95% mean	3.86	1.35
Lower 95% mean	1.47	0.39

Figure 2.0.15 Distribution of Naphthalene in µg/m^3 (arithmetic mean) for all stove type



Note: The x axis is mean concentration of naphthalene and y axis is percentage.

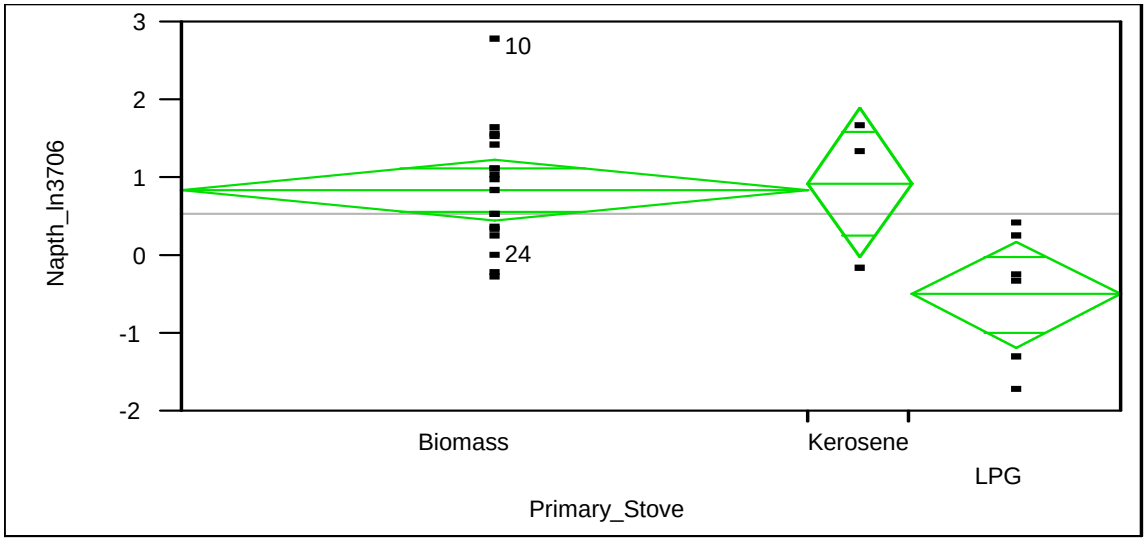
Mean naphthalene concentration was compared across stove groups by ANOVA test with scheffe option (scheffe multiple-comparison test). The mean concentrations between kerosene and biomass stove were not statistically different but the mean concentration of naphthalene was lower in LPG stove than the other two stove types (ANOVA F = 1.56, p=0.23). The difference in mean concentration was observed even after excluding one outlier (ID =19 with biomass stove)

which had a weekly mean concentration of 15.76 µg/m^3. Table 2.0.20 summarizes the kitchen concentrations across different stove types.

Table 2.0.20 Naphthalene concentration in µg/m^3 by stove type

Moments	Biomass stove	Kerosene stove	LPG stove
N	18	3	6
Mean (µg/m^3)	3.20	3.26	0.79
Standard Deviation (SD)	3.42	2.22	0.53
Minimum	0.74	0.84	0.18
Maximum	15.76	5.20	1.51
Std. Error of Mean	0.81	1.28	0.22
Upper 95% mean	1.49	8.77	1.34
Lower 95% mean	4.90	-2.25	0.23

Figure 2.0.16 One-way analysis of variance (ANOVA) ln (Naphthalene µg/m^3) by primary stove



2.4.1 Housing characteristics and Naphthalene concentration from all stove types

The mean naphthalene concentration was evaluated by housing characteristics to see their influence. The mean naphthalene concentrations were evaluated by kitchen location, housing type, observer assigned ventilation status and other sources of emission in the kitchen. Different housing types had significantly different kitchen concentration, whereas kitchen concentrations were not statistically different by kitchen locations. Concentrations were not found statistically different by other housing characteristics and emission sources. Results are shown in table 2.0.21.

Table 2.0.21 Analysis of variance (ANOVA): Naphthalene concentration µg/m^3 by housing characteristics and other sources of emission in the kitchen

Housing characteristics and other emission sources	**Variables**	**N**	**Mean**	**Std. Dev**	**F and p value**
Kitchen location	Cook outdoor including (open air)	6	5.16	5.41	F=3.06
	Separate kitchen inside the house	12	1.85	1.30	P value= 0.07
	Kitchen not separated inside the house	9	2.10	1.76	
Housing type	*Kutcha*	16	3.48	3.56	F= 1.52
	Pucca	4	1.62	2.04	P value=0.24
	Semi-*pucca*	7	1.37	0.97	
Smokers in the house	Yes	8	1.96	1.13	F = 0.61
	No	19	2.97	3.52	P value =0.44
Incense burn in the house	Yes	7	2.93	1.76	F = 0.07
	No	20	2.58	3.39	P value =0.80
Non electric lamp used in the house	Yes	16	2.43	1.42	F = 0.24
	No	11	3.02	4.53	P value =0.63
Observer assigned ventilation status	Very good	3	5.55	8.84	F =1.32
	Good	10	2.36	1.91	P value =0.29
	Fair	10	1.82	1.17	
	Poor	4	3.38	0.60	

2.4.2 Housing characteristics and Naphthalene concentration from biomass stoves

Effect of housing characteristics on naphthalene concentrations were evaluated for households using biomass stoves only. Results are presented in table 2.0.22.

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Table 2.0.22 Analysis of variance (ANOVA): Naphthalene concentration in $\mu g/m^3$ by housing characteristics and other sources of emission in the kitchen.

Housing characteristics and other emission sources	Variables	n	Mean	Std. Dev	F and p value
Kitchen location	Cook outdoor including (open air)	6	5.16	5.41	F=1.62
	Separate kitchen inside the house	10	2.12	1.25	P value= 0.23
	Kitchen not separated inside the house	2	2.69	1.88	
Housing type	*Kutcha*	14	3.60	3.78	F= 0.88
	Semi-*pucca & Pucca house*	4	1.77	0.94	P value= 0.36
Smokers in the house	Yes	7	2.02	1.20	F = 1.37
	No	11	3.94	4.18	P value =0.26
Incense burn in the house	Yes	5	2.81	1.63	F = 0.09
	No	13	3.35	3.95	P value =0.77
Non electric lamp used in the house	Yes	13	2.24	1.21	F = 4.38
	No	5	5.68	5.89	P value =0.05
Observer assigned ventilation status	Very good	1	15.76	0	F = 27.21
	Good	7	2.48	1.72	P value =0.00
	Fair	7	2.09	1.32	
	Poor	3	3.26	0.68	

2.4.3 Multivariate regression for naphthalene concentrations indoors

Before running a multivariate ordinary least square (OLS) to identify the best predictors for higher concentration of naphthalene in the kitchen, a univariate analysis of relationship between PM2.5 and naphthalene and nicotine were evaluated. A linear statistical relationship between PM2.5 and naphthalene concentrations was observed, whereas negative relationship was observed between kitchen and bedroom nicotine concentration and PM2.5 and naphthalene. Appendix-2.7, tables A.0.4-A.0.7 have the estimated coefficients (univariate) and its associated *p* values. Table 2.0.23 has the description of variables included in the multivariate model. The predictors which showed statistically significant difference in mean concentrations in ANOVA test were included in the multivariate model.

Table 2.0.23 Variables included modeling Naphthalene concentration indoors in multivariate regression

Variable name	Description	Values
lnKitchenPM	PM2.5µg/m^3 kitchen concentration (log transferred)	Continuous
lnNaphthalene	Naphthalene concentration µg/m^3 (log transformed)	Continuous
lnKitNicotene	Kitchen nicotine concentration µg/m^3 (log transformed)	Continuous
Stove type	Primary stove type	0= LPG* 1= Biomass stove 2 = Kerosene
Kitchen location	Location of kitchen in the house	1 = Cook outdoor 3 = Separate kitchen inside the house* 4 = Kitchen not separated inside the house
House type	Present construction of house	0 = *Pucca** 1 = Semi *pucca* 2 = *Kutccha*
Ventilation status	Observer assigned ventilation status	1 = Very good 2 = Good 3 = Fair* 4 = Poor
Num_cig_smoked	Number of cigarette smoked during monitoring period	Continuous
Kit_vol	Kitchen volume m^3	Continuous
Hrs_stove used	Average hours of present stove use	Continuous
Dur_incense_burn	Duration of incense burn in minutes	Continuous
Dur_lamp_burn	Duration of non-electric lamp burned in minutes	Continuous
Dur_garbage burn	Duration of garbage burn outside in minutes	Continuous

* Reference category

Table 2.0.24 shows the results of final OLS model for Naphthalene kitchen concentration (µg/m^3). This model includes all three stove categories where LPG stove is a reference category. The stepwise regression dropped house type (*kuttcha* and *pucca* house), duration of garbage burned outside the house (in minutes), PM2.5 concentration, kitchen nicotine concentration, ventilations in the kitchen, kitchen volume, duration of incense burned in minutes, number of cigarette smoked, and kitchen inside the house not separated by walls. The model had R^2 of 0.66, suggesting that around 66% of variation on kitchen concentration is explained by the model. The Shapiro- Wilk test *p* value of final model was 0.45, which suggest that it will be reasonable to accept the null hypothesis that data for this model are normally distributed. Residual vs. fitted

values and q-norm plot of regression model (table 2.0.24) are presented in figures 2.0.17 and 2.0.18.

Table 2.0.24 Final regression model for Naphthalene kitchen concentration

Naphthalene ug/m^3	Coefficient	Std error	t	p value	95% CI
Biomass stove	3.37	1.48	3.08	0.01	1.45-7.79
Kerosene stove	3.05	2.08	1.52	0.15	0.64-14.55
Kitchen outdoor including open air	2.85	1.47	2.72	0.02	1.25-6.49
Duration of non electric lamp burned	1.01	1.00	2.32	0.04	1.00-1.02
Hours of stove used	0.95	1.02	-2.68	0.02	0.90-0.99
Constant	1.38	1.42	0.92	0.37	0.66-2.89
n=21, R^2 = 0.66 adj = 0.54					

Figure 2.0.17 Residual vs. fitted values of regression

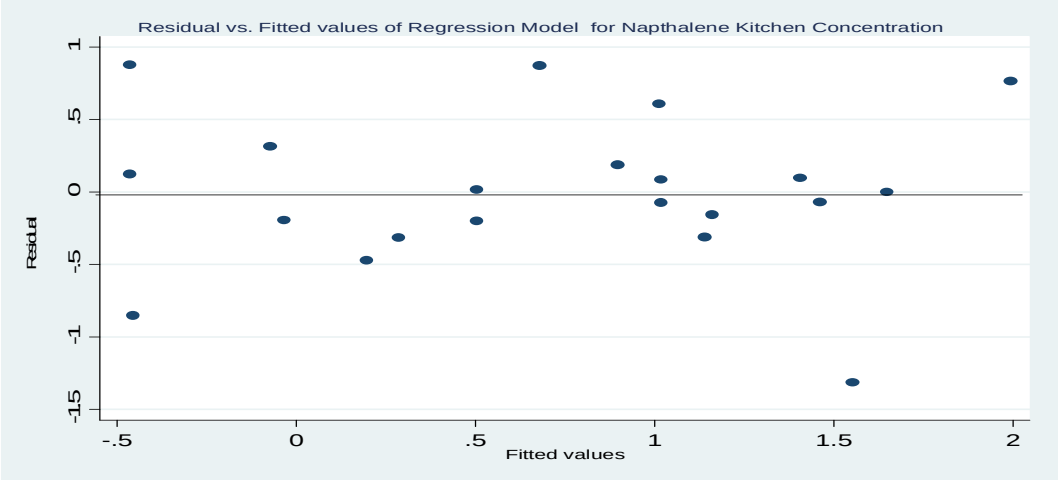
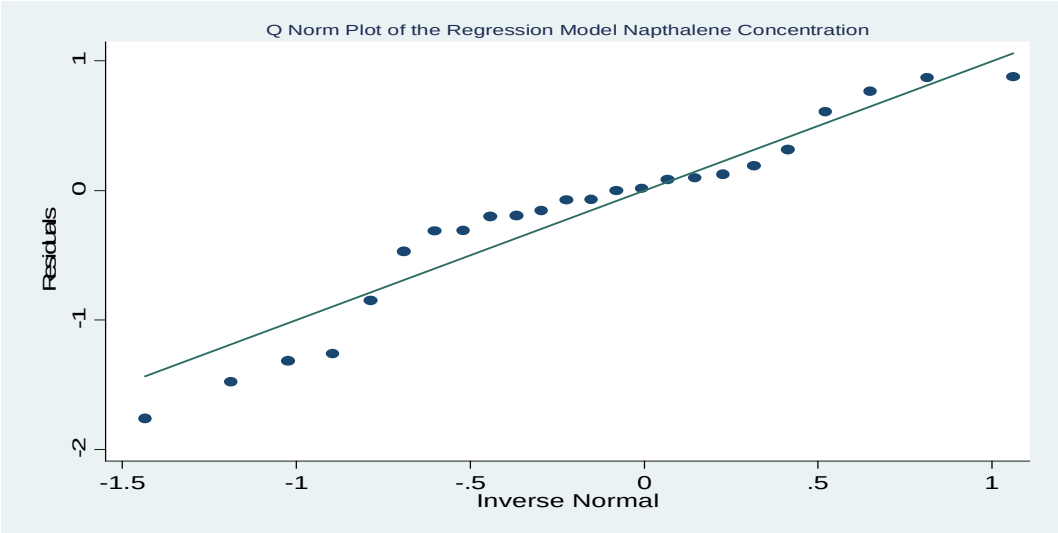


Figure 2.0.18 Q norm plot of the regression model



2.5 Discussion

Out of 28 participants in this study, 18 had unimproved and 1 had improved biomass stove (with chimney), 4 had kerosene and 5 had LPG stoves. All participants who had reported their main cookstove as being a biomass cookstove were found to be correct, as were the five reporting use of a LPG stove. One of the four participants who had reported using kerosene stove, however, was found to be using an LPG stove. On that basis, the accuracy (true reports ÷ total reports) of stove reporting was 96%. In the case of ventilation characteristics, one participant who had reported not having a window in her kitchen was found to have a temporary outside kitchen with a window-sized opening. Two participants who reported having a window in the kitchen actually did not have a window. Based on these data, the accuracy of reporting ventilation was 89%. The results suggest that the questionnaire used during face-to-face interview at the hospital was adequate and reliable to collect main exposure information (stove type and ventilation in the kitchen) from study participants of indoor air pollution and lens opacity (cataracts) and tuberculosis (TB) in Pokhara.

A significantly different mean PM2.5 concentration was found between three groups of stove. Mean concentration of PM2.5 for all averaging times were higher from biomass stove followed by kerosene and LPG stoves. However, at higher averaging times (from 5-7 days moving average) the PM2.5 concentrations were higher from LPG stoves compared with kerosene stoves (0.206 vs 0.173 mg/m^3). In the LPG stove group, one household had the above normal mean concentration of PM2.5. The higher concentration in this particular house could be due to other sources of emission indoors. However, due to small sample size (n=6), the main covariate influencing this high value could not be determined.

The PM2.5 concentrations were highly variable during the days based on the cooking pattern. The concentrations showed diurnal pattern, higher in the morning (between 6-10 AM) and

evenings (between 7-9 PM) and lower in the afternoon. The variability of PM2.5 concentration in terms of coefficient of variations (CoV), which is defined as the ratio of standard deviation to mean (a measure of the variability of data relative to its mean) were higher for biomass stove (mean CoV = 2.53; range: 2.32-2.80) followed by kerosene (mean CoV=1.28; range:0.76-2.05) and LPG stove (mean CoV=1.04; range:0.72-1.80). However, the fluctuations of CoVs were mostly observed in LPG and kerosene stoves. In biomass stove group, although CoVs were higher than kerosene and LPG stoves but they were similar throughout weekdays as well as weekends. The ratios of highest to lowest CoVs in seven days in kerosene, LPG, and biomass stoves were 2.70, 2.51 and 1.20, respectively. Similarly the 15 minutes peak concentrations of PM2.5 was highest in biomass stove (75 mg/m^3).

The widest ranges of exposures were evident in biomass stove group followed by kerosene and LPG stove. The overall exposure fold range (ratio of 97.5^{th} and 2.5^{th} percentiles of the log normally distributed concentrations of PM 2.5) in biomass, kerosene and LPG stove groups were 1245, 28 and 13. The overall exposure fold ranges by days were also high in biomass stove group (557 on Sunday and 1688 on Wednesday). In biomass and kerosene stove group, the exposure varied mostly within than between households/stove groups. Whereas in LPG stove group, total variance of PM2.5 explained by within stove was 45% compared with 55% of variances caused by differences between the stoves. The ratio of within and between variances (λ) were 3.13, 1.68 and 0.812 for biomass, kerosene and LPG stoves. The higher within compared to between variability of exposure in biomass and kerosene group suggests that day-to-day differences in exposure to PM2.5 are more prominent than differences in mean exposure between stoves.

Similarly, the uniform exposure of PM2.5 was not observed in any stove groups (biomass ${}_bR_{0.95}$ =33, kerosene ${}_bR_{0.95}$ =8 and LPG fuel stove ${}_bR_{0.95}$ =7), this suggests that it will be helpful and important in future studies to assess the uniformity of exposure hypothesis before making any assumption. On the basis of acceptability of temporal variability (within-house variability of PM2.5 level) of 20%, the estimated variance components of between- and within-households suggested that averaging times of 48 hours are needed to reliably characterize between-household differences in PM2.5 levels. As high intra-class correlation (ICC) indicates lower temporal variance relative to total variance, and suggests increasing the duration of measurements is no longer necessary, 82% of the variability in 48-hours average PM2.5 concentrations was explained by between-household differences, while only 11% of the 1 hour concentration variability was explained by between-household factors. The ICC values were similar (~0.82) for moving averages of 60 hours and more and approached 0.90 on seventh days.

In the households using biomass stove, housing characteristics such as housing type, kitchen location, use of secondary stove, windows opened all the time, burning of incense and non-electric lamps influenced the PM2.5 concentrations (ANOVA test results). However, when these covariates were included in the random effects model for univariate analysis, the between household variance of PM2.5 concentrations decreased significantly for only one covariate – 'windows opened all the time during cooking period compared with windows not opened or opened half of the time (-31% reduction)'. Other predictors such as observer assigned ventilation and burning of incense indoors reduced the between- household variances by 6 and 3% respectively but they were not statistically significant. On the other hand, covariates such as

burning of secondary stove (Kerosene stove for making tea and other day activities), non-electric lamp and poor housing quality (*kutchha*) increased between household variability of PM2.5 by 6%, 6% and 6% respectively. When all three predictors, which reduced the between stove/household variances were included into the multivariate mixed effects model (model 5), the between household exposure fold rage ($_bR_{0.95}$) was reduced by 54%. Thus specific households/kitchen characteristics, which affect the between households difference of variance could be considered in developing a criteria for defining uniformly exposed groups of people in future epidemiological studies. The weekly mean PM2.5 concentrations in the kitchens that used biomass (778 µg/m^3), kerosene (156 µg/m^3) and LPG stoves (76 µg/m^3) were 78, 16 and 8 times higher than the WHO annual air quality guidelines value (10 µg/m^3).

The PM2.5 concentrations found in this study is similar to the results of other indoor air pollution monitoring study conducted in Nepal and in other developing countries. In the study conducted near Kathmandu valley, Shrestha et al [33] has documented one day mean concentrations of 2418 µg/m^3 (range 505-8078 µg/m^3) of PM10 from biomass fuel stoves and 793 µg/m^3 (range 355-1698 µg/m^3) from kerosene stoves. In the co-location study conducted near Kathmandu valley using photometric devises, similar to the PM monitor used this study (UCB), Kurmi et al [34] has documented one day mean concentrations of 792 µg/m^3 (range 136-2610 µg/m^3) of PM2.5 from biomass fuel stoves. In Guatemala, Naeher et al [35] has documented 636 µg/m^{3}of PM 2.5 in 22 hours of monitoring from open fire and 174 µg/m^{3}of PM 2.5 from *Plancha* (improved biomass stove). Similarly, Balakrishnan et al has documented maximum 24 hours PM2.5 level of >3000 µg/m^{3}from biomass based fuel combustion in India [36].

The higher within variance of PM2.5 compared with between found in the present study is comparable to other studies. In an indoor air pollution study conducted in Kenya, Boleij et al (1989)[37] has reported 69% of total variance of PM2.5 explained by within household variances compared with 31% variances caused by differences between the houses.

The 15 minutes peak concentration of PM2.5 documented in the present study (75 mg/m^3) is very high compared with other studies of indoor air pollution in developing countries. For example, in a study conducted in rural part of Kenya, Ezzati et al[17] has documented highest level of suspended particulate matter ~ 50,000 µg/m^3 from an unimproved biomass stove. In Costa Rica, Park et al[18] has documented highest peak concentration of PM2.5 as 18900 µg/m^3 from *fogon* stove (a type of biomass stove). In an hour long measurement of particle concentrations, Regalado et al [38] has documented peak concentration of PM 10 as 3000 µg/m^3 from unimproved biomass stove in rural Mexico.

The naphthalene concentrations in majority of samples were above the limit of detection. The weekly mean concentrations of naphthalene between kerosene and biomass stoves were not statistically different but the mean concentration was lower in LPG stoves than other two stove types (ANOVA F = 1.56, p=0.23). The difference in mean concentration of naphthalene between biomass and LPG stoves was observed even after excluding one outlier (the mean concentration of 15.76 µg/m^3 from biomass stove).

The housing characteristics and presence of other exposure covariates in the kitchen did not influence the mean naphthalene concentrations. For example, better ventilation in the kitchen did not reduce the naphthalene concentration. The highest concentration of naphthalene was found in the separate kitchen outside the house (n=1). The smokers in the house and use of incense did not add naphthalene concentration indoors. However, duration of use of non electric lamp and duration of use of stove were found to be good predictors of naphthalene in the multivariate regression model. The naphthalene to PM ratio was 0.004, 0.02 and 0.01 for biomass, kerosene and LPG stove. Similarly the ratios of naphthalene to kitchen nicotine concentrations in the biomass, kerosene and LPG stove using households were 23, 22 and 2 respectively. The naphthalene badges used in this study can thus be deployed in other studies in future.

The weekly mean concentration of naphthalene from biomass stove and kerosene stove in this study is higher than the ambient naphthalene concentration found in developed countries but lower than the reported mean concentration indoors. For example ambient monitoring of naphthalene in the 11 US urban/suburban areas have documented concentration ranging 0.4 to 170 $\mu g/m^3$, with a median concentration of 0.94 $\mu g/m^3$ (0.0002 ppm). In the US, the measured concentrations of naphthalene in ambient air in urban areas have been found four times higher than in rural areas. The highest concentration of naphthalene has been found near industrial and hazardous waste sites. For example the average concentrations of naphthalene near five hazardous waste sites in New Jersey were in the range of 0.42-4.6 $\mu g/m^3$. Near the creosote impregnation plant, Bouchard et al [39] has documented 3.79 $\mu g/m^3$ of naphthalene. In the occupational setting, very high naphthalene concentration has been found in the charcoal making plant using wood fuel [12]. In a preliminary quantitative assessment, Kato et al [12] has documented 11.5 (SD: 1.54) $\mu g/m^3$of naphthalene from charcoal making plant in Brazil. Authors have also reported monotonic increase in the level of urinary metabolites of naphthalene (2-napthol) among non smokers charcoal makers with the level of exposure to wood smoke. In the indoor environment setting, Viau et al [11] has reported mean concentrations of 28.7±23.4 $\mu g/m^3$ (standard deviation) of naphthalene from 8-12 hours of sampling from biomass fuel stove in Burundi.

Exposure to naphthalene has become an environmental and occupational concern worldwide. The National Toxicology Programs (NTP's) *11th Report on Carcinogens* has listed this compound as *"reasonably anticipated to be a human carcinogen"*[40]. The California EPA has considered naphthalene as a toxic air contaminant and a substance that causes cancer and has calculated a unit risk of 3.4×10^{-5} per $\mu g/m^3$[41]. For non-cancer effects, EPA has set a reference concentration of 3 $\mu g/m^3$ (0.67 ppb) and reference exposure level of 9 $\mu g/m^3$[41, 42]. Similarly, OSHA has set a PEL (permissible exposure limit) of 10 ppm (50 $\mu g/m^3$) for naphthalene and ACGIH has set TLV (threshold limit value) of 10 ppm (50 $\mu g/m^3$) and STEL (short-term exposure limit) of 15 ppm (75 $\mu g/m^3$) for naphthalene, which is similar to NIOSH's REL (recommended exposure limit) and STEL values[13].

The concentration of naphthalene from biomass and kerosene stoves in this study is slightly higher than the reference concentration set by California EPA but lower than the ACGIH TLV values of 50,000 $\mu g/m^3$. As inhalation is the main route of exposure of naphthalene followed by dermal routes [43], the average daily intake of naphthalene for women who cook with biomass fuel and kerosene in the present study site is about 62 micro gram. This value is about three

times higher than the naphthalene intake by general public in the US, considering median naphthalene concentration of ~1 µg/m^3 in urban and suburban area with inhalation rate of 20 m^3/day.

In this study, the nicotine concentrations in some samples were below the limit of detection. The mean concentration of nicotine was higher in the bedroom (0.29 µg/ m^3) than in the kitchen (0.20 µg/m^3). The maximum nicotine concentration was found in the kitchen compared with bedroom (1.87 µg/m^3 vs. 1.53 µg/m^3 in the bedroom). However, the mean concentrations of nicotine in the bedroom and kitchen were not statistically different. The higher nicotine concentrations were found in the house with smoking family members compared with non smoking family members in the house (0.63 vs. 0.11 µg/ m^3 in the kitchen and 0.65 vs. 0.06 µg/ m^3 in the bedroom). Housing characteristics did not influence the mean concentration but the housing type was exception. The highest concentration was found in the *pucca* house compared with *semi-pucca* and *kutcha* house. The ratio of kitchen nicotine and PM2.5 concentration was 0.0004. Appendix 2.7.2 has nicotine study results in detail.

The mean concentration of nicotine found in this study is lower than the nicotine concentration found in the homes of smokers (1-3 µg/m^3) [44] and work places where smoking is allowed (2.14 µg/m^3) in the US [45]. The mean concentration of nicotine found in this study was lower than the mean concentration that is generally observed in the homes of smokers (1-3 µg /m^3) and work places where smoking is allowed (2.14 µg/m^3) in the US. However, the median concentration of nicotine was higher than the median nicotine concentration documented in the homes with smokers in Asia (0.15 vs. 0.09 µg/m^3) and lower than that of median nicotine concentrations documented in the homes with smokers in Nepal (~0.4 µg/m^3) as reported in the multi-country second-hand smoke study conducted in 31 countries (in 1284 houses) of Latin America, Asia, Eastern Europe and Middle East [46] The nicotine concentrations in multi-country second hand smoke study were measured by passive nicotine badges for one week similar to our study. The lower concentration could be due to several openings/ventilation in the houses in Nepal.

2.6 References

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Appendix 2.7

Table A.0.1 List of UCBs and temperature and particle coefficients

SN	UCB ID	Temperature Coefficient (Average)	Particle Coefficient obtained from mosquito coil, woodchips, incense (outside) and , incense (inside)			
			Mosquito coil	Woodchips	Incense (outside)	Incense (inside)
1	P3b00303	-0.6465	0.0424	0.0334	0.0567	0.0862
2	P3b00351	-2.5598	0.0453	0.0328	0.0899	0.0862
3	P3b00352	-0.6453	0.0303	0.0265	0.0511	0.0710
4	P3b00353	-1.1406	0.0280	0.0262	0.0406	0.0775
5	P3b00417	-0.8918	0.0264	0.0249	0.0405	0.0697
6	P3b00333	-0.7478	0.0299	0.0316	0.0447	0.0809
7	P3b00392	-0.8682	0.0365	0.0319	0.0575	0.0842
8	P3b00413	-0.6914	0.0471	0.0359	0.0718	0.0857
9	P3b00402	-0.80365	0.0392	0.0444	0.0568	0.1150
10	P3b00400	-0.60695	0.0332	0.0376	0.0455	0.0991
11	P3b00416	-0.8572	0.0226	0.0219	0.0344	0.0584
12	P3b00376	-0.7385	0.0266	0.0266	0.0415	0.0738
Average values			**0.0340**	**0.0311**	**0.0526**	**0.0823**

Note: Incense (inside) means one incense stick is placed inside chamber and burnt. This produces high concentrations of particles

Incense (outside) means two incense sticks are placed outside the chamber and burnt together. The smoke is channeled through a funnel and tube inside the chamber.

Table A.0.2 List of UCBs with average particle coefficients (PCs) and standard deviation

SN	UCB ID	Temperature Coefficient (Average)	Average values	Standard Deviation (SD)
1	P3b00303	-0.6465	0.0547	0.0231
2	P3b00351	-2.5598	0.0636	0.0288
3	P3b00352	-0.6453	0.0447	0.0206
4	P3b00353	-1.1406	0.0431	0.0238
5	P3b00417	-0.8918	0.0404	0.0208
6	P3b00333	-0.7478	0.0468	0.0237
7	P3b00392	-0.8682	0.0525	0.0239
8	P3b00413	-0.6914	0.0601	0.0227
9	P3b00402	-0.80365	0.0639	0.0349
10	P3b00400	-0.60695	0.0539	0.0306
11	P3b00416	-0.8572	0.0343	0.0170
12	P3b00376	-0.7385	0.0421	0.0223

Table A.0.3 Weekly PM 2.5 concentration in mg/m^3 by stove type from minutes average data

Moments	**Biomass stove**	**Kerosene stove**	**LPG stove**
N	19	3	6
Mean	0.776	0.156	0.082
Standard Deviation (SD)	3.629	0.418	0.241
Coefficient of variation (COV %)	467.7	267.9	293.9
Geometric mean (GSD)	0.088 (5.333)	0.0867 (2.230)	0.0588 (1.876)
Minimum	0.017	0.046	0.028
Maximum	135.78	14.01	28.70
2^{nd} highest value	53.37	5.49	4.87
3^{rd} highest value	51.68	3.47	2.74
4^{th} highest value	41.47	3.35	2.36
Std. Error of Mean	0.009	0.003	0.001
Upper 95% mean	0.793	0.161	0.084
Lower 95% mean	0.758	0.151	0.080

Table A.0.4 Ratios between PM2.5, Naphthalene and Nicotine irrespective of stove type

Ratios	**Values**
Kitchen Nicotine/PM	0.0004
Naphthalene/PM	0.005
Naphthalene/Kitchen nicotine	13.1
Naphthalene/Bedroom nicotine	9.20

Table A.0.5 Univariate and adjusted linear regression between naphthalene and PM2.5

Pollutant	**PM 2.5 concentration**			**PM 2.5 concentration adjusted for kitchen nicotine concentration**			**PM 2.5 adjusted for bedroom nicotine concentration**		
	Coef	R^2	P value	Coef	R^2	P value	Coef	R^2	P value
Naphthalene	0.002	0.22	0.01	0.002	0.24	0.02	0.002	0.19	0.06

Table A.0.6 Univariate and adjusted linear regression between naphthalene and nicotine

Pollutant	**Kitchen nicotine concentration**			**Kitchen nicotine concentration adjusted for PM 2.5 concentration**			**Bedroom nicotine concentration adjusted for PM 2.5 concentration**		
	Coef	R^2	P value	Coef	R^2	*P* value	Coef	R^2	*P* value
Naphthalene	-0.84	0.01	0.59	-0.20	0.24	0.89	0.50	0.19	0.77

Table A.0.7 Univariate and adjusted linear regression between nicotine and PM2.5

Pollutant	**PM 2.5 concentration**			**PM 2.5 concentration adjusted for Naphthalene concentration**		
	Coef	R^2	P value	Coef	R^2	P value
Kitchen nicotine	-0.0001	0.03	0.39	-0.0001	0.03	0.50
Bedroom nicotine	-0.0002	0.09	0.20	-0.0002	0.10	0.21

Table A.0.8 Within and between households fraction of variance and ICC values by measurements time

Time	**Between household variance**	**Within household variance**	**Total variance**	**Intra-class correlation (*p*)**
3 minutes	0.444	10.718	11.163	0.04
15 minutes	0.445	6.439	6.884	0.06
1hour	0.444	3.633	4.076	0.11
12 hours (0.5 days)	0.415	0.295	0.709	0.58
24 hours (1 day)	0.392	0.139	0.530	0.74
36 hours (1.5 days)	0.370	0.098	0.469	0.79
48 hours (2 days)	0.351	0.077	0.429	0.82
60 hours (2.5 days)	0.336	0.069	0.405	0.83
72 hours (3 days)	0.322	0.063	0.385	0.84
96 hours (4 days)	0.296	0.055	0.350	0.84
120 hours (5 days)	0.273	0.046	0.319	0.86
144 hours (6 days)	0.252	0.039	0.292	0.86
168 hours (7 days)	0.229	0.035	0.264	0.87

Appendix 2.7.1

XAD Coating of Quartz Filters

Hammond Lab, UC Berkeley

Cleaning Aluminum foil glassware

Clean aluminum foil by baking at 400°C for least 30 minutes, or by using a 1: 1: 1 mixture of hexane, methanol, and dichloromethane. Clean all glassware using established protocols for organic-free glassware. (For new and XAD contaminated glassware, UCB soaks the inside of each piece with alcoholic KOH; and rinses 5 times with de-ionized water; followed by air drying; rinse with solvent just before use. For glassware previously cleaned with KOH and not contaminated with XAD, it is not necessary to use KOH in subsequent cleaning.

Baking the Quartz Filters

Place a piece of quartz fabric in a muffle furnace. Arrange pre-cut quartz filter individually on the quartz fabric by using clean forceps. Hold the edge of the filter without damaging it. A second layer can be placed over the first; slightly offset so that all filter will be exposed to enough oxygen to remove organic contaminants from them. Cover the assembled filters with piece of quartz fabric. Bake filters at 700-800 °C for 4 hours. Turn the furnaces off and once the furnace is cooled, transfer the baked filters to a clean glass container. The filter should be kept away from contact with ambient air until the coating process starts.

Preparing the slurries for coating filters

1. To coat seventy-five 37 mm quartz filters or sixty 47 mm quartz filters, add 1.8 g ground and cleaned XAD4 resin in clean 400 ml beaker. Add 275 ml n-hexane into the beaker and cover the slurry with a clean aluminum foil. Sonicate for at least 30 minutes in the hood. The slurry can be sonicated up to 4 hours.
2. Turn the sonic bath off and allow the slurry to settle for 15 seconds. Decant above 75% of the slurry (About 200 ml) into a clean 250 ml beaker.
3. Sonicate the optimized slurry briefly. Divide the slurry equally into two clean 150ml beakers. Pour about 30 ml into the first beaker and the next 30 ml into the second beaker. And then again pour above 30 ml into the first beaker and 30 ml into the second beaker. Keep doing this until the slurry is divided equally. Cover the both slurry with clean aluminum foil.

Preparing QC filters

Prepare four baked quartz filters. Scratch very gently backside of each filter without making a hole. (i.e. Scratch once for QC filter#1, Scratch twice for QC filter#2, etc.)Weigh the four filters and second their weight in the lab book.

Coating the filters

1. Sonicate the first slurry briefly and put the second slurry aside in the hood. After the sonication, take a baked quartz filter with a pair of clean forceps and hold the edge without damaging. Dip the filter ten times into the filter coating slurry. Place the coated filter on clean aluminum foil. Coat the second filter in the same way. Keep track of the order in which filters are coated. Sonicate the slurry briefly to resuspend the XAD every 10 filters. Coat a pre-weighed QC filters every 10 or 15 filters. Continue this procedure until all of the backed filters are coated. Let the coated filters dry in the hood (set temperature at 30 to 40°C).
2. Sonicate the second slurry (about 30 minutes).Once the second slurry is ready and the filters are dry, repeat the coating procedure in reverse order. (Start coating with the filter that was most recently coated). After the second coating is complete, let the filters dry in the hood.
3. Once the filters are dry, remove excess XAD from the filters. Add 100ml n-hexane into a clean 150ml beaker. Dip the coated filter ten times into the hexane and place it on clean aluminum foil. Rinse the second filter in the same way. Keep track of the order in which filters are rinsed. Continue this procedure until all of the filters are rinsed once. Add 100ml n-hexane into another clean beaker and repeat rinsing procedure in reverse order. Let the filters dry in the hood. Once the filters are dry, weigh the four QC filters and record weight in the lab book. Calculate the difference between pre-weight and post-weight.
4. Transfer all of the filters to a clean glass amber jar as soon as the filters are dry. Put label on the jar (i.e.date, name, #of filters, etc.). Keep the filters away from ambient air until the filters are used for sample collection.

Extraction of PAH from XAD-coated Quartz Filter

Hammond Lab, UC Berkeley

Purpose: To extract PAH from XAD- coated quartz filters without contaminating the sample; filter and concentrate to 0.20 to 0.30 ml.

This procedure describes the extraction of individual XAD- coated quartz filter, 37 mm and 47 mm, for determination of polycyclic aromatic hydrocarbon (PAH)

Laboratory equipment and special facilities

1. Laboratory hood with adequate ventilation and vacuum, for location the filtration apparatus and sanitation.
2. Ultra pure N_2 (dry, hydrocarbon-free) for evaporation the sample extract.
3. Sonicator.
4. All – glass vacuum filtration device, suitable for 25-47 mm diameter filters, preferably designed to collect the filter directly into a pear- shaped flask with a 24/40 standard taper (for rotary evaporation) or beaker.

Supplies needed

1. 50 ml pear- shaped flask for filtration apparatus (about half a dozen), with 24/40 standard tapers Teflon filter for filtering extract – Millipore FHUP, 47-mm diameter, 0.5 micrometer pore size (unlaminated). If alternate filter is used, check that it can remove particle as small as 0.5 micrometer in diameter from liquid.
2. Filter holder with vacuum sidearm for #3 (as described in laboratory Equipment, #4.)
3. 150 ml beaker (6)
4. 500 ml beaker
5. Glass funnels (2)
6. Clean aluminum foil (bake at 400 C^0 for 30 min)
7. Graduated cylinder, 1 l
8. 2 ml vials with narrow neck and Teflon lined cap for holding filtered evaporated extract (one per extract)
9. Extraction solution, Dichloromethane
10. Internal standards (deuterated fluoranthene, deuteated phenanthrene, in hexane)
11. Ring stand and clamp or rod
12. Tweezers (blunt end) for handling the Teflon filters
13. Labels and marking pens for glassware
14. log sheet laboratory note book
15. Syringes or glass micro pipette for adding known volume of internal standards.
16. Syringes (250 µl and 500 µl) for measuring the extract final volume, preferably with blunt-tipped needles
17. Powder-free gloves such as Microgrip Boxed-Ambi-Nitrile(VWR40104-348)

Cleaning

Clean all glassware using established protocols for organic-free glassware. (For new and XAD contaminated glassware, UCB soaks the inside of each piece with alcoholic KOH; and rinses 5 times with de-ionized water; followed by air drying; rinse with solvent just before use). For glassware previously cleaned with KOH and not contaminated with XAD, it is not necessary to use KOH in subsequent cleanings.

1. Clean aluminum foil by baking at 400 C^0 for 30 min. Wrap Teflon caps in clean aluminum foil. When dry place bottles (not Teflon caps) in muffle furnace for 4 hours at 500 C^0. When bottles are at room temperature seal with clean Teflon caps.
2. Prepare a clean flat area of the laboratory hood to locate the ring stand glassware. Invert the beakers on a clean surface or cover them. Cover the openings of the filtration apparatus with clean aluminum foil.

Extraction

1. Change to nitrile gloves. For extraction of 6 filters, measure approximately 150 ml of DCM in a loosely covered 250 ml beaker.
2. Record the first numbers and sample identification on the appropriate log sheet and in the laboratory notebook.
3. Using a syringe add appropriate amounts of internal standards to the filter. Add 50uL PAH 1 (~150 ng D10-Phenanthrene and 50ng D10-Fluoranthene)
4. Place the filter in a 150 ml beaker and 5mL DCM using a glass pipette and bulb.
5. Sonicate for 15 minutes.
6. Transfer the liquid to a 50 ml beaker.
7. Add another 5 ml DCM to the filter.
8. Sonicate for 15 minutes.
9. Transfer the liquid to the same 50 ml beaker, rinse the filter 3x with DCM and add to the beaker.
10. Assemble a vacuum filter system with a new Millipore FHUP filter and apply vacuum to rinse with 30 ml of DCM. Discard the rinse, change to a clean collection flask (50-mL pear shaped flask). Pass the extract through the filter into the 50 ml pear shaped flask. For quantitative transfer, rinse the beaker 3x with DCM and filter
11. Transfer the filtrate to a clean 14mL centrifuge tube. Rinse the 50 ml pear shaped flask 3x and add the rinse to the centrifuge tube.
12. Concentrate up to 6 filter extracts in centrifuge tubes using ultra-pure N2 and a gas block.
13. When the contents of a tube reach ~300 µl, turn off the N2 flow to that tube.
14. Using a clean syringe (rinse 20x with DCM before each new sample), take up the contents of the centrifuge tube. Measure and record the final volume of the sample.

15. Transfer the syringe contents to a clean 2-ml vial with a glass insert and Teflon septum. Label the vial with appropriate identification and place a mark on the bottle at the bottom of the solvent meniscus. Run on the GC/MS or store the vial in a freezer at -20 C^0.

Filter blank

1. Each day that filter samples are extracted, a blank XAD coated filter should be extracted as well.

QA/QC

1. Each time a new batch of solvent is made, save 150 ml and evaporate the sample to 0.5 to 1 ml as in step 10, Extraction, above. Cool flask to room temperature. Measure with a syringe and record in log sheet the amount of extract and color. Transfer to a clean 2 ml brown bottle with narrow neck and Teflon lined cap. Label the bottle with appropriate identification. Store the bottle in a freezer at-20 C°.

Determination of PAH

Run standards and samples using the GC/MS

Appendix 2.7.2

2.7.2.1 Nicotine measurements method

There is an evidence of a dose-response relationship between the cumulative effects of active tobacco smoking and the risk of cataracts[47] and tuberculosis (TB) and passive smoking increasing the risk of TB in children[48-50]. Environmental tobacco smoke (ETS) is emitted either as side stream smoke or the exhaled mainstream smoke of cigarettes. ETS is a complex mixture of over 4500 chemicals found in both particulate and gas phases, but nicotine [3-(1-methyl-2-pyrrolidinyl)-pyridine] is a marker of ETS, as it is unique to tobacco [51, 52]. Nicotine is a major constituent in the smoke and comes with enough environmental concentrations compared with other constituents and it is also relatively easy to measure. Since approximately 95% of ETS nicotine is in vapor phase[53], passive samplers can easily collect these compounds.

A passive diffusion sampler developed by Dr. S. Katherine Hammond's lab in UC Berkeley[53] was used to monitor nicotine concentration in the kitchen and bedroom. The passive monitor works on a principle of passive diffusion (Ficks law), where nicotine is diffused to a filter treated with sodium bisulfate. The sodium bisulfate compound was treated in a teflon-coated glass fiber filter. The nicotine passive samplers were exposed for one week and were co-located with UCB particle monitor and Naphthalene passive samplers. After exposing filters for one week, the samplers were refrigerated at 3^{0} C before processing them in the lab. In the lab, the adsorbed nicotine in the filter was desorbed from the filters and analyzed by gas chromatography for total nicotine.

2.7.2.2 Results of Kitchen and bedroom concentration of nicotine (ug/m^3)

Table A.0.9 summarizes the kitchen and bedroom nicotine concentrations. Kitchen and bedroom nicotine concentrations are approximately lognormally distributed. See figures A.0.1 and A.0.2.

Table A.0.9 Kitchen and bedroom concentration of Nicotine (µg/m^3) from all houses

Moments	**Kitchen nicotine concentration**	**ln (kitchen nicotine concentration)**	**Bedroom nicotine concentration**	**ln (Bedroom nicotine concentration)**
N	27		21	
Mean	0.20	-1.07	0.29	-0.91
Standard Deviation (SD)	0.40	0.58	0.48	0.66
CoV	200%	-	166%	-
Geometric mean	0.08	0.06	0.12	0.12
Minimum	0.00	-2.00	0.00	-2.00
Maximum	1.87	0.27	1.53	0.18
Std. Error of Mean	0.08	0.12	0.10	0.16
Upper 95% mean	0.36	-0.83	0.51	-0.58
Lower 95% mean	0.05	-1.31	0.07	-1.23

Figure A.0.1 Distribution of Kitchen Nicotine (arithmetic mean)

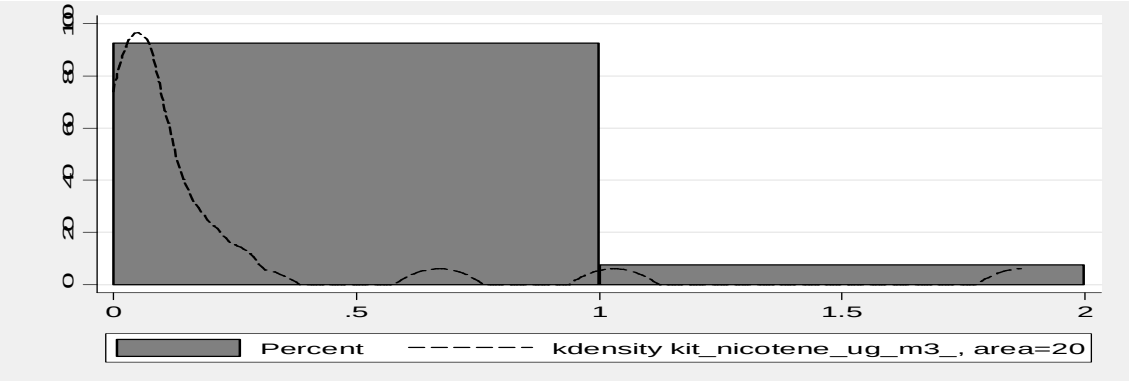
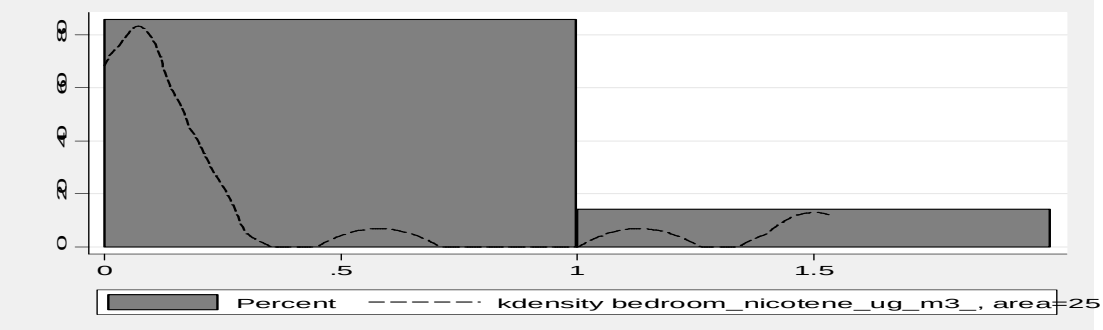


Figure A.0.2 Distribution of Bedroom Nicotine (arithmetic mean)



A mean difference in the kitchen and bedroom nicotine concentration was compared by using both parametric (t test) and non parametric test (sign ranked test). The mean concentration was not significantly different across these two places. Results are shown in table A.0.10.

Table A.0.10 The comparison of means of kitchen and bedroom nicotine concentration

Place category	t test * Mean nicotine concentration			Wilcoxon signed rank test Mean nicotine concentration			
	N	Mean	Std. Error	Sign	Observed	Sum ranks	Expected
Kitchen	20	0.24	0.10	Positive	10	103	104.5
Bedroom	20	0.30	0.11	Negative	9	106	104.5
p value = 0.33				Zero	1	1	1
				All	20	210	210
				Unadjusted variance	717.5		
				Adjustment for ties	-0.38		
				Adjustment for zeros	-0.25		
				Adjusted variance	716.88		
				p value	0.96		

* Note: Bartlett's test performed on cells with positive variance: 10 single-observation cells not used

To distinguish the mean concentration of nicotine in the kitchen and bedroom and to identify the best proxy of nicotine concentration in the houses, participants' houses were divided as smokers and non-smoker's house and by number of smokers in the house. Although a trend of higher mean nicotine concentration was seen in the house where large number of people smoked but results were not statistically significant. Tables A.0.11-A.0.14 summarizes the results.

Table A.0.11 Kitchen nicotine concentration ($\mu g/m^3$) by smokers and non smokers in

Moments	**Participant is smoker but no other family members smoke (n=5)**	**Participant is not smoker but other family members smoke (n=6)**	**Both participant and other family members smoke (n=5)**	**Neither participant nor other family members smoke in the house (n=11)**
Mean	0.09	0.12	0.63	0.11
Std Dev	0.07	0.09	0.81	0.20
Median	0.05	0.08	0.15	0.04
Highest	0.21	0.29	1.87	0.67
Lowest	0.01	0.04	0	0
Standard Error	0.033	0.04	0.36	0.06
Lower 95% CI	-0.006	0.02	-0.37	-0.02
Upper 95% CI	0.18	0.21	1.63	0.24

Table A.0.12 Kitchen nicotine concentration in µg/m^3 according to (indoor air pollution) pre and post monitoring questionnaire response•

Moments	Did any body smoke cigarette during monitoring period? [3]		Did you (study Participant) smoke cigarette?		Did other family members smoke cigarette?		By total number of smokers in the house including participant		
	Yes	No	Yes	No	Yes	No	0	1	2
	(n=8)	(n= 19)	(n= 10)	(n= 17)	(n=11)	(n=16)	(n=11)	(n=7)	(n=9)
Mean	0.21	0.20	0.36	0.11	0.35	0.10	0.11	0.08	0.41
Std Dev	0.34	0.43	0.61	0.16	0.58	0.17	0.20	0.06	0.63
Median	0.10	0.05	0.10	0.05	0.11	0.05	0.04	0.07	0.15
Highest	1.03	1.87	1.07	0.67	1.87	0.67	0.67	0.21	1.87
Lowest	0	0	0	0	0	0	0	0.01	0
Standard Error	0.12	0.10	0.19	0.04	0.17	0.04	0.06	0.02	0.21
Lower 95% CI	-0.07	-0.01	-0.08	0.03	-0.04	0.02	-0.02	0.03	-0.8
Upper 95% CI	0.49	0.41	0.80	0.20	0.74	0.19	0.24	0.14	0.90
ANOVA (p value)	F ratio =0.005 (0.9		F ratio =2.51 (0.13)		F ratio =2.62 (0.12		F ratio =1.92 (0.17)		
Tukey (p value)	Q * Not significan		Q * Not significant		Q * Not significan		Q * Not significant		

[3] Information collected under post monitoring questionnaire during indoor air pollution monitoring study

Table A.0.13 Bedroom nicotine concentration ($\mu g/m^3$) by smokers and non smokers in the house

Moments	Participant is smoker but no other family members smoke (n=4)	Participant is not a smoker but other family members smoke (n=6)	Both participant and other family members smoke (n=5)	Neither participant nor other family members smoke in the house (n=6)
Mean	0.21	0.28	0.65	0.06
Std Dev	0.25	0.43	0.78	0.09
Median	0.11	0.16	0.1	0.01
Highest	0.58	1.13	1.53	0.22
Lowest	0.05	0	0.05	0
Standard Error	0.13	0.17	0.35	0.04
Lower 95% CI	-0.19	-0.17	-0.32	-0.04
Upper 95% CI	0.61	0.72	1.61	0.15

Table A.0.14 Bedroom nicotine concentration in µg/m^3 according to indoor air pollution pre and post monitoring exposure questionnaire response

Moments	Did any body smoke cigarette during monitoring period?		Do you (study Participant) smoke cigarette?		Does other family members smoke cigarette?♦		By total number of smokers in the house including participant		
	Yes	No	Yes	No	Yes	No	0	1	2
	(n=8)	(n= 13)	(n= 9)	(n= 12)	(n=11)	(n=10)	(n=6)	(n=6)	(n=9)
Mean	0.25	0.31	0.45	0.17	0.44	0.12	0.06	0.36	0.40
Std Dev	0.50	0.48	0.62	0.31	0.61	0.18	0.09	0.42	0.63
Median	0.07	0.16	0.1	0.06	0.16	0.05	0.01	0.18	0.1
Highest	1.47	1.53	1.53	1.13	1.53	0.58	0.22	1.13	1.53
Lowest	0.02	0.0	0.05	0	0	0	0	0.05	0
Standard Error	0.18	0.13	0.21	0.09	0.18	0.06	0.04	0.17	0.40
Lower 95% CI	-0.17	0.02	-0.02	-0.03	0.03	-0.009	-0.04	-0.09	-0.09
Upper 95% CI	0.66	0.61	0.93	0.37	0.85	0.25	0.15	0.81	0.88
ANOVA (p value)	F ratio =0.09		F ratio = 1.96		F ratio =2.62		F ratio =1.01		
Tukey (p value)	(0.77)		(0.18)		(0.12)		(0.39)		

2.7.2.3 Housing characteristics and Kitchen nicotine concentration

The kitchen nicotine concentrations were evaluated by kitchen location, housing type and availability of closing door in the kitchen. Nicotine concentrations were found higher in *pucca* houses (house made with good material, cement and brick) compared with *kutcha* house (house made with poor quality material, mud and brick). Other predictors did not show any difference in kitchen nicotine concentration. Results are summarized in table A.0.15.

♦ Information collected during face to face interview

Table A.0.15 Analysis of variance: Kitchen nicotine concentration µg/m^3

Housing characteristics	Variables	N	Mean	Std. Dev	F and p value
Stove type	Biomass stove	17	0.14	0.24	F = 1.81
	Kerosene stove	3	0.15	0.07	P value = 0.19
	LPG stove	5	0.52	0.80	
Kitchen location	Cook outdoor including (open air)	5	0.038	0.02	F=0.75
	Separate kitchen inside the house	11	0.24	0.32	P value= 0.49
	Kitchen not separated inside the house	8	0.33	0.63	
Housing type	*Kutcha*	16	0.09	0.07	F= 3.86
	Pucca	3	0.70	1.01	P value= 0.04
	Semi-*pucca*	5	0.38	0.45	
Smokers in the house	Yes	7	0.24	0.35	F = 0.03
	No	18	0.21	0.44	P value =0.87
Incense burn in the house	Yes	7	0.37	0.66	F = 1.32
	No	18	0.16	0.27	P value =0.26
Non electric lamp used in the house	Yes	15	0.14	0.25	F = 1.37
	No	10	0.34	0.57	P value =0.26
Observer assigned ventilation status	Very good	2	0.03	0.014	F = 0.27
	Good	9	0.17	0.21	P value =0.84
	Fair	10	0.26	0.57	
	Poor	4	0.33	0.47	

Table A.0.16 Analysis of variance: Kitchen nicotine concentration in the house using biomass stove

Housing characteristics	**Variables**	**N**	**Mean**	**Std. Dev**	**F and p value**
Kitchen location	Cook outdoor including (open air)	5	0.038	0.02	F= 0.87
	Separate kitchen inside the house	9	0.22	0.32	P value=0.44
	Kitchen not separated inside the house	2	0.13	0.03	
Housing type	*Kutcha*	14	0.10	0.08	F= 8.31
	Pucca	-		0.70	P value= 0.01
	Semi-*pucca*	2	0.53		
Closing door in the kitchen	Yes	12	0.10	0.08	F=3.38
	No	3	0.38	0.56	P value= 0.09
Smokers in the house	Yes	6	0.27	0.37	F = 2.72
	No	10	0.08	0.08	P value =0.12
Incense burn in the house	Yes	5	0.10	0.04	F = 0.20
	No	12	0.16	0.29	P value =0.66
Non electric lamp used in the house	Yes	12	0.15	0.28	F = 0.05
	No	5	0.12	0.12	P value =0.82
Observer assigned ventilation status	Very good	1	0.04	0	F = 1.76
	Good	6	0.11	0.11	P value =0.20
	Fair	7	0.07	0.04	
	Poor	3	0.41	0.53	

CHAPTER 3

Pre-clinical measures of eye damage (lens opacity) from exposure to biomass smoke in Nepalese women

Pre-clinical measures of eye damage (lens opacity) from exposure to biomass smoke in Nepalese women

3.0 Introduction

The World Health Organization (WHO) has defined blindness as the inability to count fingers at a distance of 3 meters or having visual acuity of 3/60 or less with best possible correction [1]. According to WHO estimates, there are 37 million blind people worldwide, and 135 million are visually disabled and depend on family support or care on a daily basis [2]. It has been estimated that, annually, about 1-2 million people become blind, and cataracts and refractive error accounts for more than 60% of this blindness [2]. Cataract is a lens opacity associated with visual symptoms and some visual disability. Lens opacity is defined as a locus of increased light scattering in the crystalline lens resulting in a decrease in lenticular transparency [3]. The opacities in eyes range from minor (not interfering with vision) to major (interfering with total vision loss or blindness).

Currently there is no clear agreed definition of cataracts. This lack of definition has hampered comparisons of cataract burden across countries. However, the existing global blindness statistics show that there is a marked difference in cataract burden between developing and industrialized countries [4]. Also, data from developing countries show that the prevalence of blindness is higher in rural areas than in urban areas and a higher proportion of females than males are blind because of cataracts [5]. A meta-analysis based on published population-based surveys has reported the age-adjusted odds of blindness in females to be higher than in males in many developing countries (39% higher in Africa and 41% higher in Asia) [6, 7]. Epidemiological studies have established certain risk factors for lens opacity and cataract formation, but these accounts for a relatively small percentage (about 15%) of potential causes. At present, understanding of risk factors and biochemical and structural events leading to the formation of cataracts or lens opacity is quite incomplete [1].

Cataracts are generally classified as congenital, infantile, or age-related (“senile”). Cataracts are also divided into four distinct anatomical, pathologic, and clinical entities: nuclear sclerosis, posterior sub-capsular (PSC), cortical and mixed types. Nuclear sclerosis or nuclear opacity is the most common type of cataract found in the nucleus of the lens. Posterior sub-capsular cataracts form as a granular layer of cells between the back of the lens and its encircling capsule. Cortical cataracts occur at the periphery of the lens in a spoke-like manner. Any combination of the three types of cataract is designated as mixed cataract [8]. Since 1980s, epidemiological studies of cataract have focused on three main classes of age related cataracts: nuclear, cortical and posterior sub-capsular. Differentiation of type is important in the study of cataracts because risk factors appear to be different for each type. At present, nuclear opacity dominates the cataract categories in developing countries but the causes are not understood [9]. However, studies have indicated that environmental and genetic factors probably play a role [10].

3.1 Risk factors for cataract: epidemiological evidences and biological plausibility

Senile or age related cataracts have multiple etiologies. Epidemiological studies have indicated the following possible risk factors associated with cataract formation;

Exposure to sunlight/UV-B radiation [9, 11-20]
Cigarette smoking [20-25]
Diabetes [26-28]
Severe diarrhea and malnutrition [29-31]
Lower socioeconomic status, lower education and occupation [32-36]
Exposure to indoor smoke from solid fuel [32, 37-41]

Epidemiological studies support the association between tobacco smoking and cataracts. Tobacco smoke and biomass fuel smoke have similar properties but there are some differences also[42]. Currently nearly half of the world's population cooks over biomass fires (solid fuel) producing high levels of indoor air pollution (IAP) [42]. Although there are several studies of the association of cataracts and IAP from cooking, there have been none that examined the association with pre-clinical cataracts**.** Identification of risk factors for cataracts at pre-clinical stages could not only offer an earlier medical intervention but also help identify prevention strategies for the most common form of blindness in women in developing countries. The main aim of this study was to investigate whether cataracts at the pre-clinical stage are associated with the use of solid fuel (biomass fuel) for cooking in households.

3.1.1 Exposure to indoor smoke from solid fuel and cataracts-existing studies and limitations

Approximately 90% of rural households in the poorest developing countries rely on unprocessed biomass-based solid fuels (wood, dung and crop residues) for cooking and heating. Most of the stoves are not energy efficient and fuels in such stoves are not burned completely. The incomplete combustion of biomass releases a complex mixture of inorganic and organic compounds. Cigarette smoke and solid fuel smoke has many similarities but there are many differences also. In the study of Shalini et al [43] plasma emission spectroscopy revealed that fuel smoke condensate (FSC) contains Mn, Cu, Fe, Co, Pb and Ni and cigarette smoke condensate (CSC) contained the same metals. Besides metals, biomass-based solid fuel burning also produces poly-cyclic aromatic hydrocarbons like naphthalene, and aldehydes, like formaldehyde [42, 44-46].

During cooking, particularly with biomass fuels in an unimproved stove, air has to be blown into the fire from time to time and this causes considerable smoke exposure to the person who is responsible for cooking. There could be two possible routes by which elements present in the smoke reach the eye lens and cause damage: through the cornea or by the systemic circulation. Bruce et al [47] suggests that toxins from biomass fuel smoke are absorbed systemically and accumulate in the eyes (which are also absorptive surfaces) and result in its opacity [47]. Wegener et al [48] suggest that the tear film covering the corneal and conjunctival surfaces

forms a lipid/water interface into which chemicals, vapors and suspended particles can dissolve. Once a chemical reaches a sufficient concentration in the tear film, they can either penetrate the cornea directly or be absorbed by the conjunctiva. Or if a compound reaches the naso-lacrimal duct and is absorbed into the blood through the nasal mucosa or is ingested into the gastrointestinal tract together with the nasal mucosa, this may lead to the systemic distribution of a chemical. Compounds may reach the eye through a combination of both routes, provided the substances succeed in crossing the blood-ocular barriers. However, according to Shalini et al [43], a direct absorption mechanism is less likely because the eye's lens is protected entirely by the capsule, aqueous humor and cornea. They postulate that inhaled smoke reaches the lens through circulation and then causes the damage. Shalini et al [43] have also investigated the in vitro effects of firewood smoke condensate (FSC) on isolated intact eye lenses and lens cell membranes. In this study, investigators monitored the changes that occurred in vitro in the lenses of pigmented Copenhagen rats. After oral feeding of FSC daily for eight weeks, the results indicated that FSC contain factors that cause oxidative stress in the lens. Similar to CSC (cigarette smoke condensate) it is believed that FSC also enhances the formation of super oxide radicals, decreases the formation of antioxidants and causes oxidative stress, which ultimately reduces the important antioxidants in the aqueous humor, such as glutathione and glucose-6-phosphate dehydrogenase (G6PD). Glutathione and glucose-6-phosphate dehydrogenase (G6PD) are important antioxidants, that protect against cataract formation [49].

The epidemiological study of Mohan et al [32] supports the hypothesis that glutathione peroxidase and G6PD reserves protect human eyes from cataract formation. In a hospital-based case-control study of 1441 patients with age-related cataracts and 549 controls, in New Delhi, India, the investigator measured blood biochemistry (serum levels of calcium, zinc, and copper and plasma level of proteins, ascorbic acid and vitamin E) on 207 (38%) cataract-free controls and 928 (64%) cases. Investigators also measured nutrient-dependent red blood cell enzymes: glutathione reductase (riboflavin dependent), transketolase (thiamine-dependent), aspartate aminotransferase (pyridoxine-dependent) and glutathione peroxidase (selenium-dependent) and erythrocyte levels of G6PD. From the measured blood biochemistry variables, they created three antioxidant indexes (AOs). The AO1 comprised of glutathione peroxidase and ascorbic acid; AO2 comprised of glutathione peroxidase, ascorbic acid, and vitamin E and AO3 comprised of glutathione peroxidase, ascorbic acid, vitamin E, and G6PD. The study found a higher level of AO3 in serum to be associated with reduced risk of posterior sub-capsular (OR: 0.23; 95%CI: 0.06-0.88) and combined posterior sub-capsular-nuclear cataracts (OR: 0.12; 95%CI: 0.03-0.56). This study also found use of cow dung and biomass-based fuels to be associated with increased risk of cataract (adjusted odds ratio of 1.61; 95%CI: 1.02-2.50) for nuclear, cortical, and mixed cataracts, but not posterior sub-capsular cataracts.

Besides study of Mohan et al [32], other five epidemiological studies have found an association between IAP and cataracts or blindness. This is further discussed below. The main findings are presented in Table A.

3.1.1.1 Exposure to indoor smoke from solid fuel and risk of cataracts-epidemiological evidence

To identify the etiology of senile cataracts in people between 40 and 60 years, Sreenivas et al [40] conducted a community based case-control study in two rural areas of Calcutta (West Bengal) and Angamally (Kerala) in India. The study included 258 cases and 308 controls from Angamally, and 301 cases and 591 controls from Calcutta. In the study, a questionnaire was used to collect information from all participants about their socio-demographic details (religion, education, and monthly family income), exposure to sunlight and protection from sunlight exposure, exposure to fire/dust, type of cooking fuel used in the house, tobacco smoking and tobacco chewing habits, alcohol drinking habit, nutrition intake and blood pressure. In the multivariate model, the risk of senile cataract was associated with usage of cheap cooking fuels - wood & cow dung for both sexes (men and women) in Calcutta (OR=1.82; 95% CI 1.13;2.92). Whereas, in Angamally, the risk of cataracts was found significant for only 2 hours per day exposure to fire/dust (OR = 1.85; 95% CI 1.43 ; 2.40). Authors did not report the multivariate OR for use of cheaper cooking fuels in Angamally. However, the univariate OR was 0.37 (95% CI 0.02 ; 6.65). A separate analysis of data from Calcutta also showed a marked difference of OR by gender. The OR for risk of cataracts from the use of cooking fuels-wood & cow dung was 1.80 (*p* 0.02) for men and 2.2 (*p* 0.002) for women. The sex specific estimates for use of cooking fuels were not reported from Angamally (Kerala).

Mishra et al [50] analyzed the relationship between the type of cooking fuel and the prevalence of partial and complete blindness in India, using data on 173 520 persons age 30 and over from the 1992-1993 National Family Health Survey. In this survey, a question relating to blindness was ‘Does anyone listed (in the household listing) suffer from blindness?’ Response categories were ‘yes, partial’, ‘yes, complete’, and ‘no’. The partial blindness was defined as blindness in one eye, partial cataract, night blindness or other eye problems resulting in seriously impaired vision. The analysis yielded an adjusted odds ratio for reported partial or complete blindness of 1.32 (1.16; 1.50) in respect of persons mainly using biomass fuel compared with other fuels, after adjusting for socio-economic, housing and geographical variables.

Zodpey & Ughade [38] conducted a group-matched case-control study at the Government Medical College in Nagpur, India, to investigate the association between exposure to cheaper biomass-based cooking fuel (solid fuel) and age-related cataract. The study included 223 female cases of age-related cataract and an equal number of controls matched for age and sex. The risk of age related cataract associated with exposure to cheaper cooking fuels was two times higher among solid fuel users compared to liquid fuel users (OR: 2.37; 95% CI: 1.44; 4.13), after adjusting for socio-economic status.

Table A Studies that have investigated the association between solid fuel use relative to cleaner burning fuel or electricity and risk of cataracts and blindness

Authors	Study type and location	Outcome	RR (95% CI) and prevalence %	Exposure Metric used
Mohan et al (1989)[32]	India-US case control study	Cataracts	1.61(1.02;2.50)	Exposure questionnaire *(main cooking fuel currently use)*
Sreenivas et al (1999)[40]	Case-control study, Calcutta, India	Cataracts	1.82(1.13;2.92)	Exposure questionnaire *(wood/ cow-dung as cooking fuel)*
Zodpey et el (1999)[38]	Case-control, India	Cataracts	2.37(1.44;4.13)	Exposure questionnaire *(type of fuel household use mainly for cooking)*
Mishra et al (1999) [50]	Cross sectional, India	Blindness	1.32(1.16;1.50)	Exposure questionnaire *(Clean fuel stove, solid fuel improved stove and solid fuel unimproved stove)*
Pokhrel et al (2005) [51]	Case-control, Nepal- India	Cataracts	1.90(1.00;3.61)	Exposure questionnaire *(Fuel stove used-wood, coal dung vs. LPG) Duration/number of years of use of stove*
Haq et al (2009) [41]	Cross sectional, Aligarh India	Cataracts	Prevalance (solid-fuel vs. LPG)- 24.9% vs. 14%	Exposure questionnaire *(Fuel used-wood, coal dung vs. LPG)*

Pokhrel et al (2005) conducted a cataract case-control study in an area of the Nepal-India border where cooking with solid fuels in unvented indoor stoves is a common practice. In this study, the presence or absences of cataracts (yes vs. no) in study participants (all women) were confirmed by slit lamp examination in a regional eye hospital. This study found evidence that the use of solid fuel in an unvented indoor stove is associated with increased risk of cataract in women who do the cooking. Compared with using a liquid and gaseous-burning-fuel stove (biogas, liquefied petroleum gas, or kerosene), the adjusted odds ratio (OR) for using a vented solid-fuel stove was 1.23 [95% CI: 0.44; 3.42], whereas use of an unvented solid-fuel stove had an OR of 1.90 (95%

CI: 1.00; 3.61). Lack of kitchen ventilation was an independent risk factor for cataract (OR 1.96; 95% CI: 1.25; 3.07).

Haq et al (2009) conducted a community based cross-sectional prevalence survey of ocular morbidities (cataracts, refractive errors, glaucoma and corneal opacities) and its socio-cultural correlates in Aligarh, India. The study included all adults above 20 years old (n=226) residing in the field practice areas of the urban and rural health training centers of the Department of Community Medicine, Jawaharlal Nehru Medical Center in Aligarh. The participants were interviewed and asked questions about socio-demographic characteristics, including types of fuel mainly used (firewood, coal, cow dung, LPG) in the house. The cataracts were defined as lens opacity accompanied by or capable of causing some level of visual loss, which was based on the results of visual acuity and slit lamp examination. The overall prevalence of cataracts was 21.7%. The bilateral cataract was present in 16.9% and unilateral cataract was present in 4.8%. In the univariate test (Mantel-Haenszel chi-square test), cataract was significantly associated with the use of solid fuels (prevalence 24.9%) compared with use of LPG (prevalence 14.0%) with *p* 0.031.

Although the findings of existing studies of IAP and cataracts/blindness suggest an association, these studies have important limitations. The first study to find an association between cataract and indoor smoke exposure by Mohan et al. (1989) was not specifically investigating this association. They regarded it as an incidental finding likely to have been a result of confounding by socioeconomic factors. The second study by Sreenivas et al (1999) also concluded that the association found for cheap cooking fuel was likely to be related to poor socio-economic status.

The third case-control study, by Zodpey and Ughade (1999), which also found an association between cataract and cheaper cooking fuels (coal, cow dung, and wood), presented limited data on potential confounding factors, other than age and socioeconomic status. In particular, there was no information on kitchen characteristics or ventilation, dietary habits/practices, history of diarrhea, exposure to sunlight, or smoking habits, which might confound the relationship. The fourth study, by Mishra et al (1999), with a cross-sectional design, used data from the 1992-93 Indian National Family Health Survey. This study found an association between biomass fuel use and partial and complete blindness after adjustment for a number of potentially confounding factors. However, information was not available on smoking or on type of blindness, which was determined through self-reporting.

In the fifth study of Pokhrel et al (2005), the risk factors for cataracts were not analyzed by type of cataract. Also there was some concern related to selection bias. In the study, controls were recruited from the refractive error clinic from the same hospital. Although there do not exist any studies showing an association between IAP and refractive error, there exist (inconclusive) evidence of the association of refractive error with education (indicator of higher socio-economic status) [52-57] [58-60]. Thus there is a possibility that people with refractive error may have different life styles or exposure patterns and lower odds of exposure to solid fuel smoke than cataracts cases [61]. The sixth study of Haq et al has only reported a univariate association (higher prevalence) of cataracts with solid fuel use, leaving open the possibility of confounding of this association.

These concerns regarding existing studies suggest that there is a need to confirm the association of IAP and cataracts by applying a high-quality objective cataract classification system, which quantifies opacities on a continuous scale from the earlier stage, and following a different study design that reduces selection bias. Conducting the study in a different geographical setting would also add credibility to the findings. In addition, identification of cataracts at the pre-clinical stage using such a classification system and identification of risk factors associated with the most common forms of cataract could offer an earlier intervention opportunity for populations at risk.

To identify risk factors associated with progression of opacification, with a specific focus on exposures to IAP in women, a hospital-based, cross-sectional study on lens opacity was conducted between July-November 2006 at the Ophthalmology Department of Manipal Medical College, Pokhara, Nepal

3.1.1.2 Hypothesis of lens opacity study

"Cooking with traditional biomass stoves without a chimney increases the severity of lens opacity in women compared with cooking using gaseous fuels".

3.2 Research design and methods

3.2.1 Recruitment of participants

Human subject's approvals were obtained from the institutional review boards at the University of California, Berkeley, and Nepal Health Research Council. Women between 20-65 years were recruited from the Ophthalmology and Outpatient Department (OPD) at Manipal Teaching Hospital, Pokhara, Nepal. To avoid the possibility of recruiting only cooperative participants by an interviewer in the hospital, throughout the study period, only the first five patients present at the OPD between 9-10 am each morning were recruited. Participants who reported being pregnant, diabetic, infected with HIV-AIDS, or on chemotherapy for any form of cancer, or having had previous diagnosis or surgery for cataracts, macular degeneration or history of penetrating eye trauma were excluded.

3.2.2 Ocular examination

After oral consent, and recruitment, all participants' visual acuities were measured at 3 meters with an illuminated Bailey and Lovie visual acuity chart with the room lights off [62]. Visual acuity of the right eye was measured by asking -participants - to read down the chart from the first line while closing their left eye and the visual acuity of the left eye was similarly measured with the right eye covered. The procedure was followed by refraction and measurement of intra-ocular pressure (IOP in mm Hg). This was followed by examination of the eye brows, eyelids,

conjunctiva, sclera, cornea, anterior chamber, iris, pupils and crystalline lens first by diffuse illumination and then with a slit lamp. After the completion of general eye examination, the questionnaire concerning the participants' demographics and environmental exposures were administered. After completion of the interview, participants' pupils were dilated to 7 mm or more with a mixture of 1% Tropicamide and 10% Phenylephrine. Crystalline lenses of these patients were then examined under slit-lamp first with diffuse illumination, then with a slit beam and later with retro-illumination. The height and breadth of slit beam were maintained at 5 millimeter (mm) and 1-2 mm. The slit beam was narrower than 2 mm, especially for the retro and the cross sectional photos. The slit lamp had an inbuilt digital camera (TOPCON Model SL-D) and lens photographs were taken when the pupil diameter reached 7 mm or more. It took about 90 minutes to complete the procedure in one participant.

For photography of nuclear opacity, vertical height of the slit beam was extended to maximum and breadth of the slit beam was fixed at minimum. The slit beam was directed at 45^{o} from left of the biomicroscope and the nuclear opacity was photographed. For photography of cortical and posterior sub-capsular opacities, the height of the slit beam was fixed at 5mm and the breadth of the slit beam was fixed at approximately 1-2mm. The slit beam was placed at the junction of the right margin of the patients' pupils in both eyes. The cortical and posterior sub-capsular opacities were then photographed against the red background. First the cortical opacities were focused using the joystick of the slit-lamp and photographed, then the posterior sub-capsular opacities were focused and photographed. Altogether six photographs of the cross-sectional view and the retro-illumination view were collected from 144 participants. After photography one participant was found with congenital cataracts, this participant was excluded from the risk factor analysis.

After completion of the study, the photographs were sent electronically and on compact disk to the School of Optometry at the University of California, Berkeley (UCBSO). At UCBSO two graders provided scores for each photograph by comparing with the reference photographs of LOCS III system [63]. The LOCS III system is a standardized, validated and widely used system of grading age-related opacification in the human lens. Compared with the subjective classification of cataracts such as 'yes' vs. 'no' or 'immature, 'mature' and 'hyper mature', the LOCS III system quantifies opacities on a continuous scale (0.1) and by its type (nuclear opacity, nuclear color, cortical, posterior sub capsular)[64] [63]. The scale ranges from the lowest value of 0.0 to the highest value of 6.9. The LOCS III system uses 3 series of reference photographs, with each series arranged in order of severity and numbered. There are 6 reference photographs in the series that show progressively more severe nuclear changes, with 5 ordered severity levels for cortical cataract and posterior sub-capsular cataract. With these sets of reference photographs, the cataract grader assigns scores using decimalization interpolating between the ranked levels of severity and extrapolating beyond the sample showing least severity,(number 1) and the sample showing most severity (number 6 for the nuclear series, and number 5 in the cortical cataract or PSC series). For the series illustrating the severity of nuclear change, there are two different qualities that are graded, nuclear opalescence (NO) and nuclear color (NC). For each, the range of possible scores is from 0.0 to 6.9. For both the cortical cataract (C) and the posterior sub-capsular cataract (PSC), the range of possible scores is from 0.0 to 5.9.

The nuclear opacity was graded on a decimal scale of 0-6.9 based on optical density and the nuclear color (NC) was similarly graded as 0-6.9. A lens given a LOCS III NC grade of 0 is colorless. A lens with a LOCS III NC grade of 3.0 has a lemon-yellow color (brunescence). A lens given a LOCS III NC grade of 6.9 is reddish-brown nuclear color. Similarly, Cortical opacity was graded on a decimal scale of 0-5.9 according to the opacity that obscured the light reflex on retro-illumination, and posterior sub-capsular opacity (PSC) was graded on a decimal scale of 0-5.9. Besides LOCS III scores, the results were also expressed as secondary score and as % area opaque for Cortical (C) and posterior sub-capsular (PSC) opacities. However, results of secondary scores are not presented in this chapter.

3.2.3 Definition used for ocular morbidities in the study

Vision loss

Normal vision	95-110	(Visual Acuity Rating-from Bailey and Lovie chart) 20/25 or better
Mild vision loss	75-94	(Visual Acuity Rating-from Bailey and Lovie chart) 20/63 or better
Moderate vision loss	55-74	(Visual Acuity Rating-from Bailey and Lovie chart) 20/160 or better
Severe vision loss	35-54	(Visual Acuity Rating-from Bailey and Lovie chart) 20/400 or better
Profound vision loss	15-34	(Visual Acuity Rating-from Bailey and Lovie chart) 20/1000 or better
Near blindness	0-14	(Visual Acuity Rating-from Bailey and Lovie chart) 20/2000 or better
Blindness	No light perception	

Intra ocular pressure (IOP)

IOP between 10 and 20 mmHg was considered as normal.

Cataracts

Lens opacity for nuclear opalescence (NO) ≥2.0, nuclear color (NC) ≥2.0, Cortical (C) ≥2.0 and Posterior sub-capsular (PSC) ≥2.0

Participants were characterized as having age-related cataracts if any type of opacity had scores equal or greater than (≥) 2. The grade ≥2 cutoff is close to a grade 2 or greater' in the LOCS II system, which was also the definition of cataracts adopted by some major population-based cataract studies [65, 66].

Refractive error

Emmetropia (no error of refraction): Spherical equivalent between -0.50 and +0.50 diopter sphere [DS]

Hypermetropia (far-sightedness): Spherical equivalent between > +0.50 diopter sphere [DS]

Myopia (near-sightedness): Spherical equivalent between < -0.50 diopter sphere [DS]

Astigmatism: Cylindrical error more than 0.50 diopter cylinder (DC) in any axis. Astigmatism was further classified as simple hyperopic and simple myopic, compound hyperopic or compound myopic and mixed astigmatism.

3.2.4 Exposure variables under this study (questionnaire)

Questions were asked about the participants' (main) present, previous and past fuel stove type (gaseous fuel stove, kerosene fuel stove, solid fuel in improved stove and solid fuel in unimproved stove). They were asked if they remembered changing fuels or stoves anytime before and after their marriage and types of fuel and stoves they had changed or switched to and duration of their use. Information about the duration of use of fuel-stoves from the age they had started cooking actively (before or after marriage) till they stopped cooking (if they had stopped cooking during the time of interview) was used to study the exposure response relationships between duration of cooking with biomass and kerosene fuel/stoves and the risk of lens opacity.

Participants were also asked the number of hours they spent while cooking or helping in the kitchen and the kitchen location and presence of windows in the kitchen. Since a cook's personal exposure to indoor air pollution depends on the overall ventilation character, in which kitchen locations and opening windows in the kitchen play an important role, information on kitchen location and opening windows in the kitchen were combined to create a composite dichotomous variable for ventilation. "Fully/partially ventilated kitchens" included open-air kitchen + kitchen inside & outside + separate kitchen outside with opening window. "Un-ventilated kitchens" included separate kitchen outside without windows and partitioned kitchens inside without windows and non-partitioned kitchen inside the house (in the bedroom).

In addition, information was also collected on other sources of emission indoors such as type of fuel used to heat the house; source of light in the house, practice of burning mosquito coil or incense indoors. A detailed histories of tobacco smoking and alcohol consumption, exposure to environmental tobacco smoke, exposure to sunlight (time work outside in the sun every day and number of years of work)[4], protection from sunlight exposure was collected. And questions related to socio-economic status (annual family income, level of education, area of residency,) and dietary practices were also asked and documented.

[4] (hours/day x 6 day/week x 52 week/year x # year)

Some exposure variables such as number of tobacco products (cigarette and *bidi*) participants smoked every day and years of smoking was combined to calculate the pack-years of smoking. The number of pack-years smoked was calculated as the average number of cigarettes smoked per day multiplied by the duration in years of smoking, divided by 20, assuming that a pack contains 20 cigarettes or *bidis*. The median pack-years were used to access the risk of lens opacity by pack-years. The standard questionnaire developed by National Cancer Institute was used to document tobacco- related information.

Since nutritional epidemiological studies have shown higher frequencies of consumption of vegetables and meat or protein rich diets to be associated with reduced risk of cataract formation, questions related to the frequencies of consumption of meat & fish, eggs and milk per week and month were also asked.

3.2.5 Statistical analysis methods and risk modeling

3.2.5.1 Statistical analysis of lens opacity scores

The preliminary analysis of the data included participants' demographic information and the descriptive statistics of opacity scores provided by two scorers[5] for the right and the left eye. The opacity scores were summarized as means, medians, standard deviations, percentiles, and measures of skewness and kurtosis. To check for approximate normality of distribution of scores, a skewness-kurtosis test was used. The *p* values of <0.05 for skewness and kurtosis was used to reject the null hypothesis of normality assumption. The difference in mean opacity scores of the left and the right eye were evaluated with the two sample Student's t test, which tests the hypothesis that the population means opacity score in the right and left eyes are equal. The *p* value of <0.05 rejects the null hypothesis of equality.

To investigate if there was a systematic bias in opacity scoring on the same eye by two scorers, the patterns of agreement or disagreement was investigated. The statistical tools like correlation coefficient, regression or Kappa statistics are commonly used to investigate the patterns of agreement or disagreement[67]. However, studies have shown that the values/results of two continuous measurements may be highly correlated but there could be substantial differences in the two values/results across their range of measurements[68]. Thus, along with correlation and regression, the patterns of agreement or disagreement on opacity scores were investigated by the Bland-Altman plot (BAP). The basic concept of Bland-Altman's plot is to visualize the difference of the measurements results made by the two methods (scorers in this study) by plotting the differences or the bias, on Y-axis versus the mean of the two methods/score on X-axis[68]. This plot helps to visually check if the magnitudes of the differences are essentially constant throughout the range of measurements. When there is no systematic bias, then the differences are symmetrical around zero. However, if one scorer is usually higher than the other by a consistent amount, then the mean will be far from zero but the confidence interval will be narrow. On the other hand if the scorers tend to disagree, but without consistent pattern or one giving higher score than the other, the mean will be near zero but the confidence interval will be

[5] (scorer 1 = RD; scorer 2 = IB)

wide. To further investigate if the mean difference of scores increase or decrease with increasing level of opacity scores, a linear relationship line was drawn between the paired difference and the paired average on the BAP. The linear trend suggests lack of agreement at higher opacity scores. The Stata software (Stata version 10; Stata Corp LLC, College Station, TX, USA) was used in all analysis including Bland Altman Plot. Also on the BAP test results, the *p* value of 0.00 for Pitman's correlation implies that the variances of two correlated samples are different.

On the basis of the results of descriptive statistics and patterns of agreement or disagreement of opacity scores, all four scores (two score for each eye given by two scorers) for each opacity type were included in the statistical analysis including risk modeling. Since observations of opacity scores for the same individuals are highly correlated, this gives a biased estimate of variance if the underlying correlation structure is not taken into account in the analysis. This problem was addressed by using a cluster option in the risk models, which adjusted within individual-cluster correlation and gave robust variance estimate and unbiased 95% confidence intervals [69, 70].

3.2.5.2 Identification of confounders and covariates for the risk model

A combination of causal diagrams, prior knowledge of potential confounders for cataracts, and a data-driven approach were taken to identify potential confounders of the relationship between lens opacity and the use of biomass fuel stove for cooking. After identification of potential confounders through causal diagrams, analysis of variance (ANOVA) tests were conducted a) to investigate the distributions of mean opacity scores across confounders/covariates and b) to determine whether some covariates significantly affected the mean opacity scores. ANOVA measured overall test of significance (F-test) between the means of opacity by confounders / covariates. In addition to known confounders, other covariates that significantly affected the mean opacity scores (*p* value <0.05) were considered a candidate for an adjustment in the multivariate risk model.

3.2.5.3 Risk models

The observed opacity scores ranged from 0 to 4.2. Two types of modeling approach were used to estimate the risk of opacity in populations by the exposure of interest. First, the population means of different types of opacities were estimated by the main exposure of interest using an ordinary multivariate least square regression models with the cluster option. For this model, opacity scores on a continuous scale were used. However, as there were zero scores for all types of opacities, they could not be adequately modeled by the log transformation. As a consequence, the opacity scores were also grouped into five bins (ordinal categories) as 0 '0', 1 '0-<=0.9', 2 '>0.9- <=1.9', 3 '>1.9 - <=2.9' & 4 '>2.9'. The bins included no opacity, some opacity, moderate opacity, high and severe opacity. The risks of severity of opacities were then estimated by an ordered logistic regression model with the cluster option. The ordered logistic regression model estimated the odds ratio for a one-category increase in severity of opacities, given that the other variables in the model were held constant[71].

3.2.5.4 Visual acuity

The preliminary analysis of the data included the descriptive statistics of visual acuity on both eyes and prevalence of refractive errors (Emmetropia, Hypermetropia, Myopia and Astigmatism) in the study population.

3.3 Results

3.3.1 Participants age, area of residence and marital status

Participants' mean, minimum and maximum age (standard deviation: SD) were 45 (SD: 12), 20 and 65 years. Majority of participants were Hindus (95.14%), married (95.83%) and urban residents (84.72%). Table 3.0.1 summarizes participants' age in 10 years band, their marital status and area of residency.

Table 3.0.1 Participants age in 10 years band, religion, marital status and area of residence

Age, marital status and locality	**Number**	**Percentage (%)**
Age in 10 years band	11	7.64
>= 20 & <=29	35	24.31
>29 & <=39	45	31.25
>39 & <=49	6	4.17
>49 & <=59	47	32.64
>59		
Religion		
Hindus	137	95.14
Buddhists	6	4.17
Christian	1	0.69
Marital status		
Married	138	95.83
Unmarried	6	4.17
Locality		
Rural	20	13.89
Urban	122	84.72
Peri-urban	2	1.39

3.3.2 Education

Equal number of participants reported that they can and cannot, read and write in Nepali. The highest level of education was college degree (4.17%). Table 3.0.2 summarizes participants' literacy and level of education.

Table 3.0.2 Participants level of literacy and education

Education	Number	Percentage (%)
Level of literacy	72	50.0
Can read and write	72	50.0
Cannot read and write		
Level of education		
Adult education (6 months of informal education)	12	8.33
Primary school (1-3 grades)	21	14.58
Middle school (3-7 grades)	19	13.19
High school (7-10 grades)	14	9.72
College (10-14 grades)	6	4.17
None of these education levels (illiterate)	72	50.00

3.3.3 Occupation

Like in many developing countries, in Nepal, women generally have the primary responsibility to care or feed family by cooking food and taking care of livestock's. Apart from these responsibilities, women are also actively involved in farming or business or commerce (in city areas). In this study, 44% of participants were housewives and 31% worked on their own farm. About 10% of participants were involved in commerce and business related activities.

A question was asked about the number of years they have been working in the current main occupation or employment. The mean years of employment in current occupation was 26 years (SD: 14 years), with maximum and minimum years of 0.25 and 58 years, respectively. Table 3.0.3 summarizes the distribution of participants' main occupation and the mean duration of employment (in years) in the present main occupation.

Table 3.0.3 Participants present main occupation and years of employment

Main occupation	Number	Percentage (%)	Mean years (SD) of employment	Minimum and maximum years of employment
Farming on own land	45	31.47	29 (13)	2 and 58
Agriculture labor (paid)	6	4.20	28 (10)	16 and 42
Laborer (non-agriculture)	7	4.90	8 (13)	0.75 and 37
Government services	5	3.50	10 (11)	0.25 and 22
Commerce/business	14	9.79	13(12)	0.33 and 42
Housewife	63	44.06	30 (12)	9 and 53
Teacher and student	3	2.10	16 (6)	10 and 22

3.3.4 Cooking practices, kitchen location and ventilation

Cooking is one of the main activities of women in Nepal. They start working in the kitchen as young girls by either helping their mother or other elders at home. Thus, women are exposed to cooking fuel smoke at an earlier age than men. After marriage, cooking becomes one of their main activities. In this study, participants' mean age of starting cooking actively was 12 years (SD: 5 years). During the interviews, about 90% of participants reported that they cook regularly, whereas 10% of participants reported that they cook sometimes and only one participant reported she had never cooked. Irrespective of stove types, the mean duration of cooking in the morning, afternoon and evening, respectively, were: 1.29 (SD: 0.46 hours), 0.54 (SD: 0.37 hours) and 1.29 (SD: 0.48) hours. The mean duration of cooking in a day was 3.11 hours (SD: 1.14).

About 68% of participants had a separate kitchen inside the house and 96% of these kitchens had openings- windows and a door. Similarly 5% of participants had a ceiling fan in the kitchen and 9% had an exhaust fan in the kitchen. Table 3.0.4 summarizes participants' reported present cooking practices, kitchen location and status of ventilation in the kitchen.

Table 3.0.4 Present cooking practices, kitchen location and ventilation in the kitchen

Present cooking practices, kitchen location and ventilation in the kitchen	Number	Percentage (%)
Present cooking practices		
Cook regularly	128	88.9
Cook sometimes	15	10.4
Never cooked	1	0.69
Present kitchen location		
Open-air kitchen	2	1.39
Separate kitchen outside	13	9.03
Kitchen both outside and inside	3	2.08
Semi-enclosed kitchen	3	2.08
Separate kitchen inside	98	68.1
Separate kitchen inside not partitioned by walls	25	17.4
Overall ventilation in the kitchen (definitions on page 84)		
Fully ventilated	112	77. 8
Unventilated	32	22.2

3.3.5 Participants' current main fuel-stove and duration of use

The predominant fuel-stove type was an LPG stove, followed by unimproved biomass stoves. Nobody reported using an improved biomass stove (biomass stove with flue). However, about 10% of participants had biogas and 4% had kerosene stove as their main fuel-stove. Table 3.0.5 presents the distribution of main fuel-stove types and duration of use of present main fuel stove at the time of interview.

Table 3.0.5 Main fuel-stove type at home and duration of use of present main fuel-stove

Fuel-stove type	Number	Percentage (%)	Mean years of use (SD)	Minimum and maximum years of use
Biomass-unimproved stove	57	39.6	24.7 (11.8)	1 and 49 years
Kerosene pump stove	4	2.78	11.5 (9.88)	4 and 26 years
Kerosene wick stove	1	0.69	4	-
LPG stove	68	47.2	7.36 (4.10)	0.25 and 19 years
Biogas stove	14	9.72	9.93 (5.03)	3 and 20 years

3.3.6 Stove change pattern

To evaluate the stove change pattern, a question was asked whether there had ever been a change in fuel-stove type used. This question was followed by a question about the stove and fuel type before the present one and type of fuel-stove during their childhood or while they lived at their

parents' house and when they started cooking. About 66% of participants reported that they had had changed stove in the past. About 93% reported that they had cooked with an unimproved biomass stove while at their parents' home, before marriage or during their childhood. Table 3.0.6 summarizes participant's previous and past fuel-stove types.

Table 3.0.6 Previous and past (at parents house) fuel-stove used by participants

Fuel-stove type	Previous		At parents house (during childhood)	
	Number	Percentage (%)	Number	Percentage (%)
Biomass - unimproved stove	69	47.9	134	93.1
Kerosene pump stove	13	9.03	2	1.39
Kerosene wick stove	4	2.78	0	0.00
LPG stove	2	1.39	1	0.69
Biogas stove	6	4.17	Currently living at parents house but had following fuel-stove: LPG stove : 6	
Electric stove	1	0.69	Biogas stove: 1	4.86
No other stove used	49	34.0		

3.3.7 Duration of use of various cooking fuel stove over active cooking life

To evaluate an exposure-response pattern of lens opacity by duration of exposure to cooking fuel smoke during active cooking life (age they started cooking actively till they stopped cooking, if they were not cooking at the time of interview), durations of exposure to fuel-stove by their types were calculated. For this calculation, kerosene pump and wick stoves were combined, and LPG and biogas fuel stoves were combined. Table 3.0.7 summarizes participants mean duration of use of various cooking fuel stove.

Table 3.0.7 Mean duration of exposure to smoke from various fuel-stove types during active cooking life

Fuel-stove type	**Mean years of exposure (SD)**	**Minimum and maximum years of exposure**
Biomass unimproved stoves	24.5 (13.5)	0 and 55 years
Kerosene pump stoves	1.61 (5.06)	0 and 26 years
Biogas and LPG stoves	4.79 (5.37)	0 and 20 years

3.3.8 Main heating and lighting fuel used in the present house

A question was asked about the heating fuel and main source of light used in the present house. 60% (n=86) of participants used wood as a source of heating fuel in the house. And 98% had

electricity in the present house and only 2 participants used a kerosene lamp. Table 3.0.8 summarizes the results.

Table 3.0.8 Main heating and lighting fuel in the present house

Heating and lighting fuel	Number	Percentage (%)
Heating fuel type		
Wood/Biomass	86	59.7
Coal	1	0.69
Electricity	2	1.39
No heating fuel used	55	38.2
Main source of light in the house		
Electricity	141	97.9
Kerosene lamp	2	1.39
Solar lamp	1	0.69

3.3.9 Tobacco smoking

Tobacco smoking is a known risk factor for cataracts. About 33% (n=47), participants reported they had smoked. However, of them only 38% (n=18) were current smokers. Among the ever smoked group, 89% reported they smoked cigarettes, 9% reported they smoked *bidis* and 2% reported they smoked a *hukka* regularly.

Participants' were asked about their smoke inhalation practice/habit. Majority of participants, who smoked, reported that they inhale smoke up to the chest (62%).

Smoker participants' were asked about the types of tobacco product they smoke generally. 28% reported they smoke unfiltered tobacco product, 30% reported they smoke filtered and 42% reported they smoke both filtered and unfiltered tobacco products equally. Table 3.0.9 summarizes participants' smoking practices.

Table 3.0.9 Ever vs. never smoked cigarette, *bidi* or *hukka*, tobacco inhalation method and mean duration of tobacco smoking

Ever vs. never smoked	**Number**	**Percentage (%)**	
Ever vs. never smoked			
Ever smoke	47	32.64	
Never smoke	97	67.36	
Smoke inhalation method			
Mouth only	5	10.64	
Mouth and up to chest	13	27.66	
Chest	29	61.70	
Tobacco product mainly smoked		Mean years of smoking (SD)	Minimum and maximum years of smoking
Cigarettes	42	26.40 (12.38)	1 and 51 years
Bidis	4	18.67 (10.60)	9 and 30 years
Hukka	1	13	-

3.3.9.1 Pack years of smoking

The average number of cigarettes smoked per day was recorded for all current smokers. The number of pack-years smoked was calculated as the average number of cigarettes smoked per day multiplied by the duration (years) of smoking, and divided by 20. The median pack-years of smoking among smokers were 8.7, with the mean of 11 pack-years (SD: 10 pack-years). This variable was further categorized as, 0 pack-year smoked, ≤9 pack-years smoked and >9 pack years smoked. Table 3.0.10 summarizes the results.

Table 3.0.10 Pack-years of smoking

Pack years of smoking	**Number**	**Percentage (%)**
0 pack-years of smoking	97	67.36
>0 and ≤9 pack-years of smoking	24	16.67
>9 pack-years of smoking	23	15.97

3.3.10 Number of smokers in the house and relationship with participant

Participants were asked if there were other family members who smoked inside the house; 58 (40.3% of total) participants reported other family members smoked inside the house. Some participants had two or more than two family members who smoked inside the house. This information was combined and the total number of smokers in the house was calculated. Table 3.0.11 summarizes the results.

Table 3.0.11 Number of smokers in the house other than the participant

Smokers in the house and participant's relationship	Number	Percentage (%)
Participants relationship with the smoker		
Husband	37	63.8
Father	5	8.62
Father-in-law	3	5.16
Mother-in-law	5	8.62
Son	3	5.16
Mother	3	5.16
Brother-in-law	2	3.44
Total number of smokers in the house		
No other family member smoke	97	67.4
One family member smoke	39	27.1
Two family members smoke	6	4.17
Three family members smoke	1	0.69
Four family members smoke	1	0.69

3.3.11 Use of mosquito coil and incense indoors

Mosquito coils are generally burned indoors between March and September. However, their use is intense when the population of mosquitoes peaks, during or after the monsoon (rainy season). In this study, 37% participants reported that they burned mosquito coils in their houses for two-three months.

Incense is burned while worshipping in the morning and evening every day. Majority of participants (91%) reported they burned incense indoors. The mean frequency of burning incense was 6.95 days (SD: 0.62 days) per week. The incense burning practice was analyzed by religion. About 92% of Hindus and 83% of Buddhists reported they burned incense indoors. A question was asked about the place where they burn incense mostly. 44% reported they burned incense in the kitchen and 31% reported they burn incense in the bedroom. Table 3.0.12 summarizes the results.

Table 3.0.12 Burn mosquito coil and incense indoors

Burn mosquito coil and incense indoors	Number	Percentage (%)
Use mosquito coil		
Yes	53	36.8
No	91	63.2
Burn incense indoors		
Yes	131	91.0
No	13	9.03

3.3.12 Alcohol consumption

Participants were asked if they regularly (every day) consumed alcohol. Only 7 participants (4.86%) reported they regularly consumed alcohol. Out of these 7, only 5 reported that they still consume alcohol; Participants were asked their age when they started drinking alcohol and age when they stopped drinking. The mean age when they started drinking was 33 years (SD: 15 years) and the mean duration of drinking alcohol was 17.14 (SD: 15.77) years.

Table 3.0.13 Alcohol consumption

Ever consumed alcohol	Number	Percentage (%)
Yes	7	4.86
No	137	95.1

3.3.13 Diet and nutritional intake

Nutritional epidemiological studies have shown higher frequencies of consumption of fruits, vegetables and meat or protein rich-diets, and milk, to be associated with reduced risk of cataracts. Participants were asked about their present food habits. About 92% of participants were non-vegetarian, and 8% were vegetarian. Among vegetarians, all but one participant had stopped eating meat or fish for more than 12 years. The mean years of not eating meat or fish was 41 years (SD: 19 years) and some were lifelong vegetarians. Since frequency of consumption of meat, milk or eggs depends on the socio-economic conditions, participants were further asked a question about the frequencies of consumption of vegetables, meat/fish, egg and milk. Table 3.0.14 summarizes the results.

Table 3.0.14 Dietary practice and frequency of consumption

Dietary practice and frequency of consumption	Number	Percentage (%)
Vegetarian or non-vegetarian		
Vegetarian	11	7.64
Non-vegetarian	133	92.4
Frequency of consumption of meat		
Once per week	73	50.7
Once per month	58	40.3
Daily	1	0.69
Never	12	8.33
Frequency of consumption of egg		
Once per week	46	31.9
Once per month	19	13.2
Daily	2	1.39
Never	4	2.78
Rarely	73	50.7
Consume green leafy vegetables everyday		
Yes	140	97.2
No	4	2.78
Consume milk everyday		
Yes	79	56.8
No	28	19.4
Sometimes	37	25.7

3.3.14 Exposure to sunlight and sunlight protection

Exposure to UV-B rays is a known risk factor for certain types of cataract. About 64% (n=92) of participants reported they work outside in the sun every day for more than one hours. The mean duration of exposure to sun every day was 4 hours (SD: 1.90) with minimum and maximum of 1 and 8 hours, respectively. Those who reported that they work outside regularly were further asked numbers of years they had been working outside. The mean years of working outside in the sun was 26.5 years (SD: 13.5 years) with minimum and maximum of such work being 2 and 53 years.

A question was asked if they protected their eyes from sun exposure with a veil, hat or sunglasses while working or walking outside. No one reported using a hat. However, 88 (61%) particpants reported they use a veil to protect themselves, but only 4 (2.78%) reported they use sunglasses. Table 3.0.15 summarizes the results.

Table 3.0.15 Work outside in the sun every day and protection from the UV-B exposure

Work outside in the sun every day and protection from UV-B exposure	**Number**	**Percentage (%)**
Work outside in the sun every day		
Yes	92	63.9
No	52	36.1
Use protection		
Veil	88	61.11
Sunglass	4	2.78
No protection used	52	36.11

3.3.15 Socio-Economic status

Studies have linked cataracts with poverty. A series of questions related to socio-economic status (SES) were asked to participants. The questions related to SES were annual family income in Nepalese rupees (NRs), land ownership and means of personal transportation owned by the household. Table 3.0.16 summarizes the results.

Table 3.0.16 Participants socio-economic status

Participants' socio-economic status	**Number**	**Percentage (%)**
Family annual income in NRs		
<25,000	29	20.14
25,000-50,000	49	34.03
50,000-100,000	39	27.08
>100,000	21	14.58
Declined to give answer	6	4.17
Own any land?		
Yes	103	71.53
No	41	28.47
Type of personal transportation in the house		
Car, jeep or van	7	4.86
Motorcycle	22	15.28
Bicycle	5	3.47
None of the above	110	76.39

Note: 3 participants who had car, jeep van also had motorcycle thus only highest form of personal transportation was kept

Ocular Morbidities

3.3.16 Intra ocular pressure, refractive error & visual acuity

3.3.16.1 Intra ocular pressure (IOP)

The mean and standard deviation of IOP was 14.04 ±2.90. The mean and standard deviation of IOP for right and the left eyes were 14.26 ±3.01 and 13.82±2.79, respectively. The mean IOP were in the normal range (10-20 mm Hg)

3.3.16.2 Refractive error

Table 3.0.17 presents the results of prevalence of refractive error in the right and left eyes in study participants. The majority of the participants had no error of refraction (emmetropia; 72.5% in the right and 75.2% in the left eye). About 16 % had far-sightedness (hypermetropia) in the right eye and 14% in the left eye. The prevalence of myopia (near-sightedness) was 1.41% in the right and 1.42% in the left eye. The prevalence of astigmatism was 10.5% in the right eye and 9.2% in the left eye.

Table 3.0.17 Prevalence of refractive error

Refractive error type	Right Eye Number (%)	Left Eye Number (%)
Astigmatism	0	1 (0.71%)
Astigmatism Compound Hypermetropic	4 (2.82%)	3 (2.13%)
Astigmatism Mixed	1 (0.70%)	1 (0.71%)
Astigmatism Simple Hypermetropic	7 (4.93%)	6 (4.26%)
Astigmatism simple myopic	3 (2.11%)	2 (1.42%)
Emmetropia	103 (72.5%)	106 (75.2%)
Hypermetropia	22 (15.5%)	20 (14.2%)
Myopia	2 (1.41%)	2 (1.42%)

3.3.16.3 Visual acuity

Table 3.0.18 summarizes the prevalence and range of vision loss in the study participants. The majority of participants had normal vision (55.3% in the right eye and 59.9% in the left eye). Following correction, there was only a minor change in the distribution across the people with normal vision but the visual acuity score (VAS) improved slightly after correction in people with mild and moderate vision.

Table 3.0.18 Visual acuity score (VAS) and prevalence of vision loss

Ranges		**VAS (letter count-Right eye) n=141**	**VAS (letter count-Left eye) n=142**
(Near-) Normal vision	Range of normal vision VAS: 95-110 (20/25)	78 (55.3%)	85 (59.9%)
	Mild vision loss VAS: 75-94 (20/63)	46 (32.6%)	41 (28.9%)
Low vision	Moderate vision loss VAS: 55-74 (20/160)	17 (12.1%)	16 (11.2%)
	Severe vision loss VAS: 35-54 (20/400)	0	0
	Profound vision loss VAS: 15-34 (20/1000)	0	0
(Near-) Blindness	Near blindness VAS: 0-14 (20/2000)	0	0
	Blindness No perception of light	0	0

VAS=Visual Acuity Score

Ocular Opacities (Lens Opacity Classification System III-LOCS III)

3.3.17 Nuclear opacity (NO)

3.3.17.1 Summary statistics of nuclear opacity scores

Figures 3.0.1 and 3.0.2 summarizes the frequencies and distribution of NO scores for right and the left eye from scorers 1 and 2. The intra correlation of scores between the right and left eyes were slightly higher for scorer 1 than scorer 2 (correlation: 0.90 vs. 0.83- see table 3.0.19), but when the scores for the right and left eyes were compared, the correlation was higher for the left than the right eye (correlation 0.72 vs. 0.64). However, the mean difference of scorers was slightly higher for left than the right eye. See the Bland Altman Plot (BAP) in table 3.0.20. The BAP also showed that the 4.20% and 5.63% of values on the right and the left eyes were outside the limits of agreement. The differences between the scores slightly increased with the increasing level of opacity. In terms of variability, the variance of scores was higher for scorer 1 than 2 (Pitman's $p < 0.05$).

Table 3.0.19 LOCS III- Nuclear opacity scores from scorers 1 and 2 (n=143)

Moments	**Scorer 1 Right Eye**	**Scorer 1 Left Eye**	**Scorer 2 Right Eye**	**Scorer 2 Left Eye**
Mean	1.64	1.65	1.58	1.56
Standard	0.89	0.84	0.59	0.60
Deviation	0.80	0.71	0.35	0.36
Variance	1.6	1.6	1.6	1.6
Median	0	0	0.3	0
Smallest value	4.2	4.1	3.9	3.6
Largest value	0	0	0.3	0.3
1% percentile	1	1.1	1.2	1.2
25% percentile	2.3	2.1	1.9	1.8
75% percentile	3.9	3.8	3.4	3.4
99% percentile	0.39	0.34	0.51	0.55
Skewness	2.63	2.79	4.47	4.25
Kurtosis				
Correlation (r^2)	0.90		0.83	
t test (p value)	-0.41 (0.68)		0.80 (0.42)	

Table 3.0.21 has the descriptive statistics of combined scores - four of each of the lens opacity grades for each subject (2 eyes, 2 graders). The combined mean scores was 1.61 with standard deviation (SD) of 0.74. The distribution statistics indicated that the nuclear opacity scores are not normally distributed (skewness (p=0.00) and kurtosis (p=0.06)). Figure 3.0.3 is the frequency graph of NO without transformation.

Figure 3.0.1 Frequencies and distribution of nuclear opacity (NO) scores for right and the left eyes from scorer 1

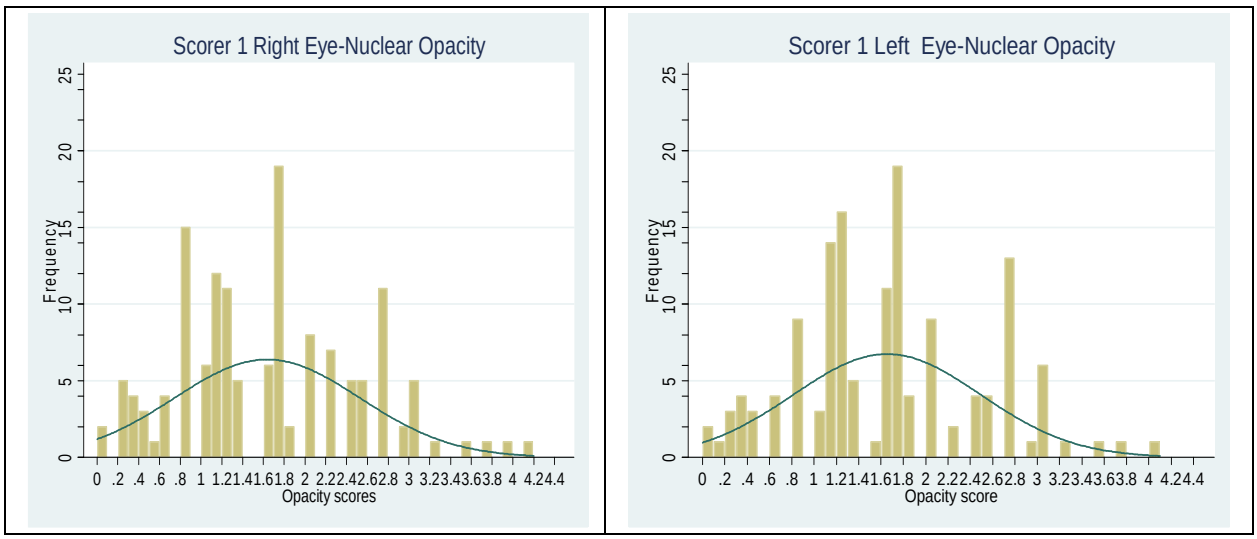


Figure 3.0.2 Frequencies and distribution of nuclear opacity (NO) scores for right and the left eyes from scorer 2

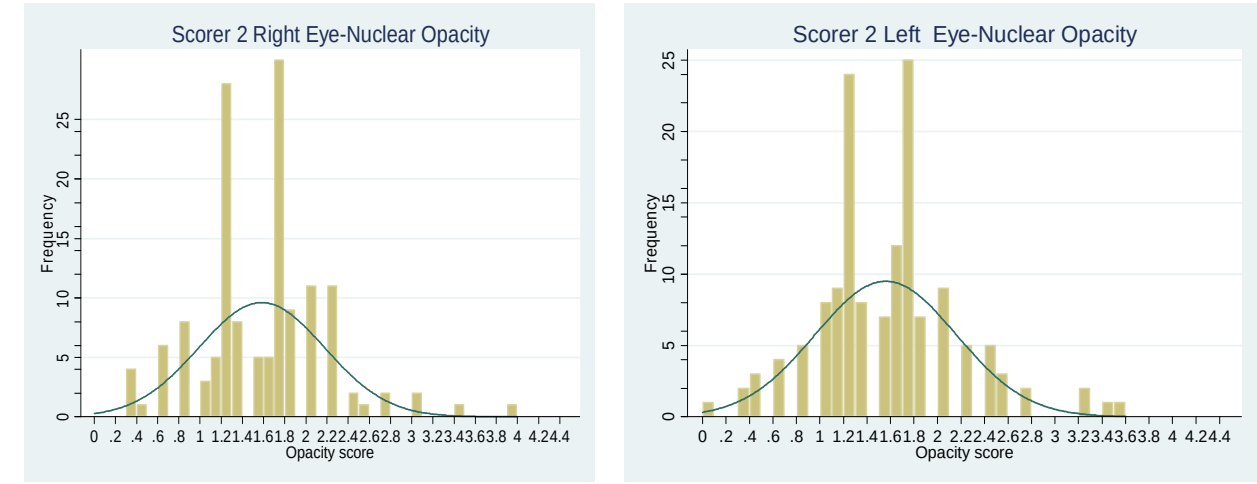


Table 3.0.20 Comparison of nuclear opacity scores for right and the left eyes (BAP and regression)

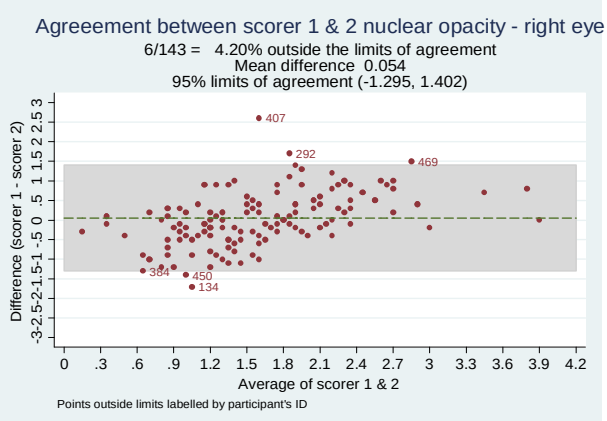
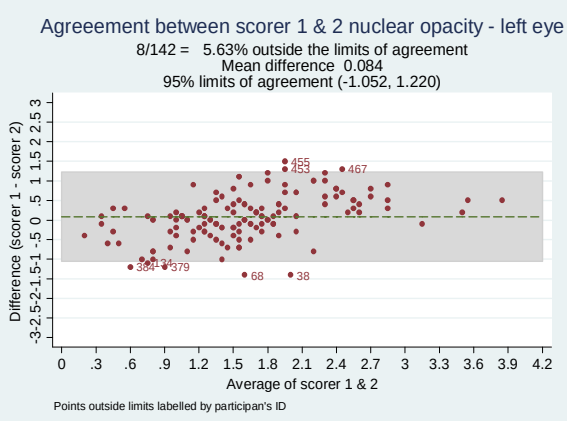
Scorer 1 Vs. Scorer 2 Bland Altman plot- right Eye	**Scorer 1 Vs. Scorer 2 Bland Altman plot- Left Eye**
	
Bland Altman regression graph- right eye	Bland Altman regression graph- left eye
	
Limits of agreement (Reference Range for difference): -1.322 to 1.430 Mean difference: 0.054 (CI -1.295 to 1.420) Range : 0.150 to 3.900 Pitman's Test of difference in variance: r = 0.479, n = 143, p = 0.000	Limits of agreement (Reference Range for difference): -1.075 to 1.243 Mean difference: 0.084 (CI -0.012 to 0.180) Range : 0.200 to 3.850 Pitman's Test of difference in variance: r = 0.467, n = 142, p = 0.000

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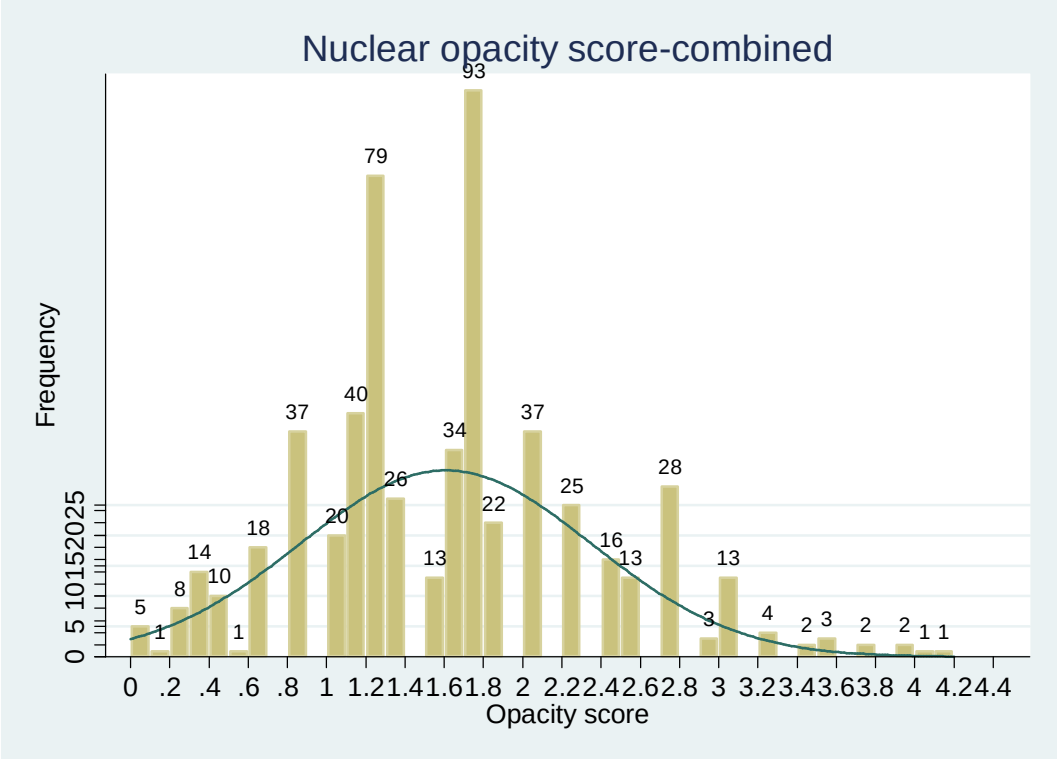
Table 3.0.21 LOCS III- All Nuclear opacity scores (n=571)

Moments	Mean NO
Mean	1.61
Standard Deviation	0.74
Variance	0.55
Median	1.6
Smallest value	0
Largest value	4.2
1% percentile	0
25% percentile	1.1
75% percentile	2.0
99% percentile	3.8
Skewness	0.47
Kurtosis	3.42
Skewness/Kurtosis tests for normality	
Pr (Skewness)	0.00
Pr (Kurtosis)	0.06
Adjsted Chi-square (2)	20.11
Pr Chi-square	0.00

Figure 3.0.3 Frequencies and distribution of nuclear opacity (NO) scores (n=571)



3.3.18 Nuclear color (NC)

3.3.18.1 Summary statistics of nuclear color scores

Figures 3.0.4 and 3.0.5 summarizes the frequencies and distribution of NC scores for right and the left eyes from scorers 1, and table 3.0.22 summarizes scores from scores 1 and 2. The intra correlation of scores between right and the left eyes were slightly higher for scorer 2 than 1 (correlation: 0.92 vs. 0.88) but when the scores for right and the left eyes were compared, the correlation was higher for the left than the right eye (0.74 vs. 0.79). The mean difference of scores was slightly higher in the left than the right eye (0.135 vs. 0.006). The BAP showed 4.90% and 4.20% of values outside the limits of agreement on the right and left eyes. In terms of variability, the variance of scores were similar for scorer 1 and 2 (Pitman's p >0.05). Table 3.0.24 has the descriptive statistics of combined scores- four of each of the lens opacity grades for each subject (2 eyes, 2 graders). The combined mean score was 1.70 with standard deviation (SD) of 0.76. The distribution statistics indicated that the nuclear color scores are normally distributed (skewness (p=0.64) and kurtosis (p=0.07)). See figure 3.0.6.

Table 3.0.22 LOCS III- Nuclear color scores from scorers 1 and 2 (n=143)

Moments	**Scorer 1 Right Eye**	**Scorer 1 Left Eye**	**Scorer 2 Right Eye**	**Scorer 2 Left Eye**
Mean	1.73	1.76	1.72	1.61
Standard	0.80	0.75	0.76	0.74
Deviation	0.64	0.57	0.58	0.54
Variance	1.7	1.7	1.8	1.7
Median	0	0.1	0.2	0
Smallest value	3.8	3.9	3.7	3.7
Largest value	0.1	0.2	0.3	0.2
1% percentile	1.2	1.3	1.1	1.1
25% percentile	2.2	2.2	2.3	2.1
75% percentile	3.7	3.8	3.5	3.3
99% percentile	0.047	0.12	0.02	-0.03
Skewness	2.67	2.91	2.40	2.61
Kurtosis				
Correlation (r^2)	0.88		0.92	
t test (p value)	-1.014 (0.31)		4.19 (0.00)	

Figure 3.0.4 Frequencies and distribution of nuclear color (NC) scores for right and left eyes from scorer 1

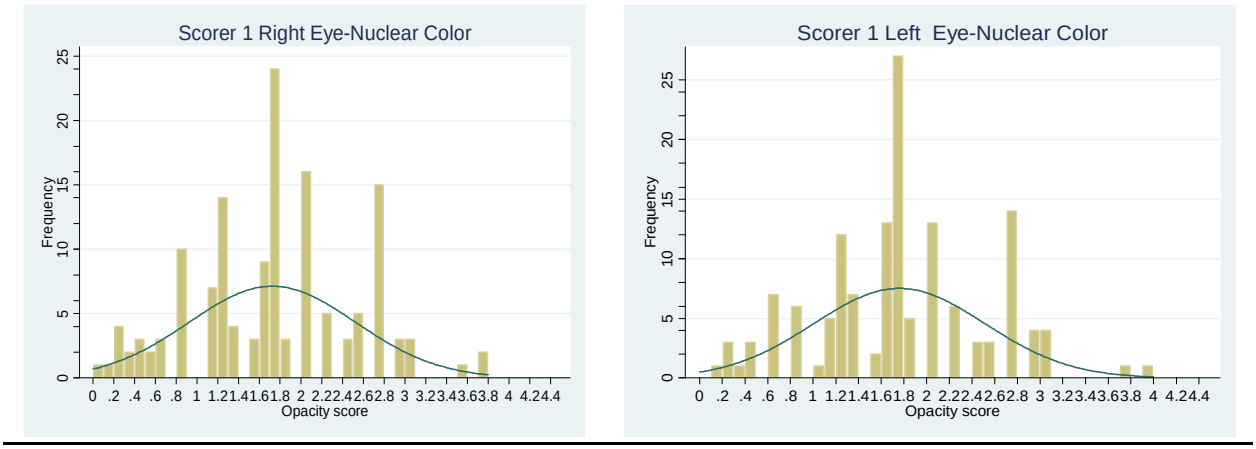


Figure 3.0.5 Frequencies and distribution of nuclear color (NC) scores for right and left eyes from scorer 2

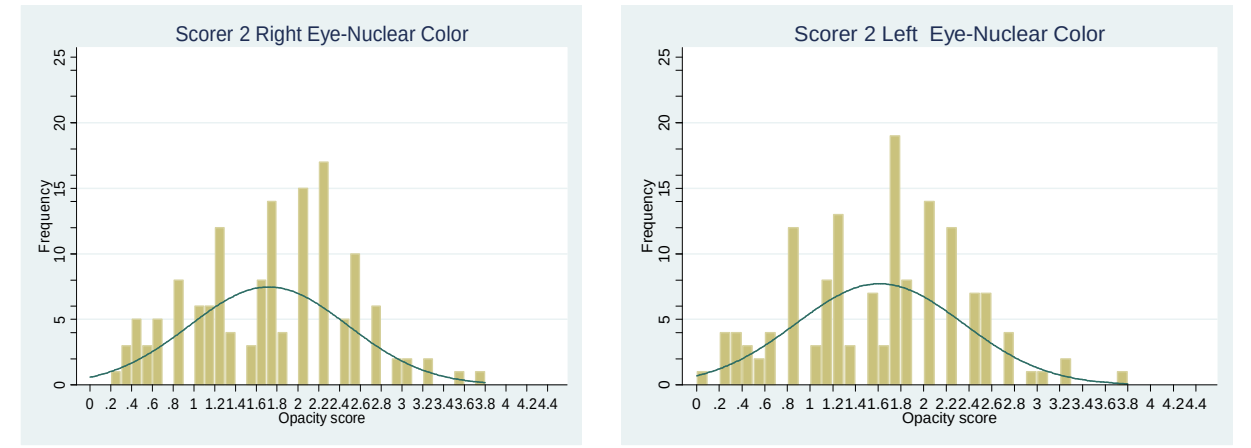


Table 3.0.23 Comparison of nuclear color scores for right and left eyes (BAP and regression)

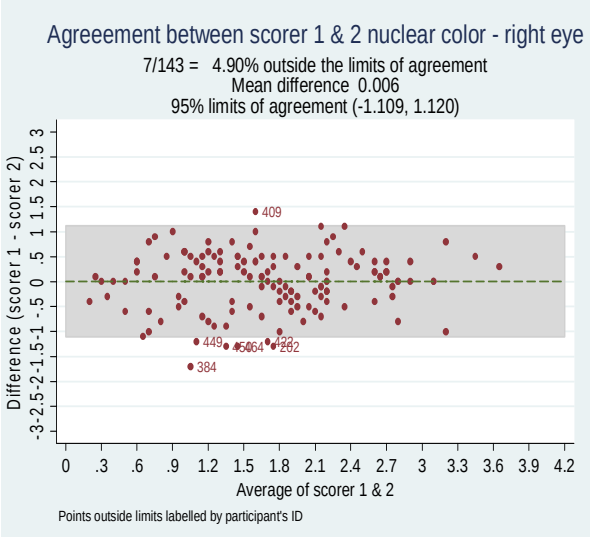
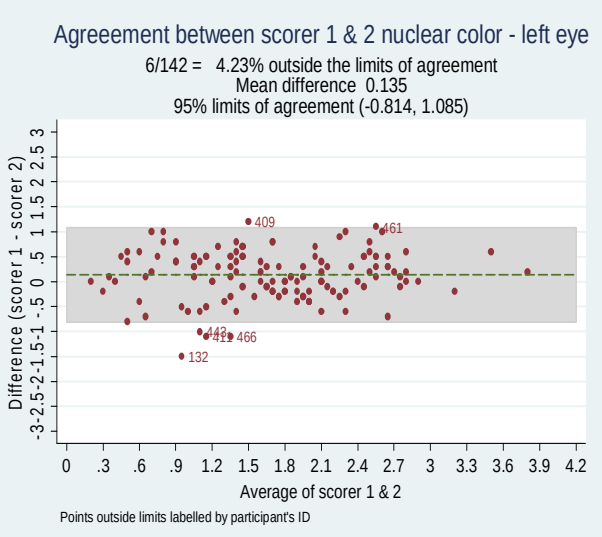
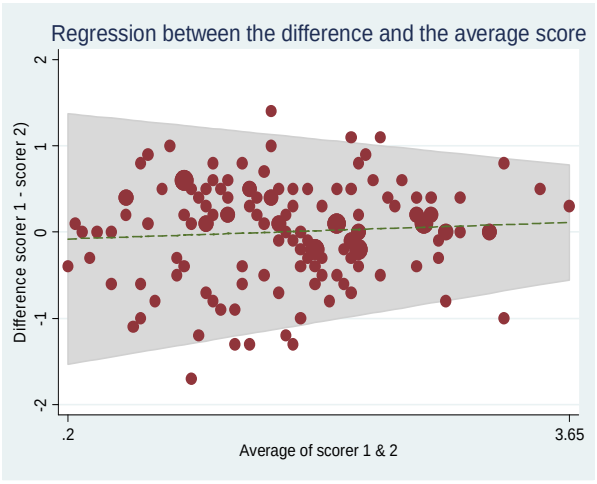
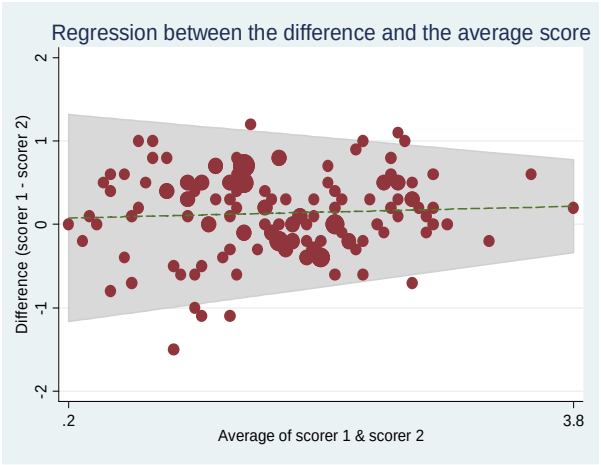
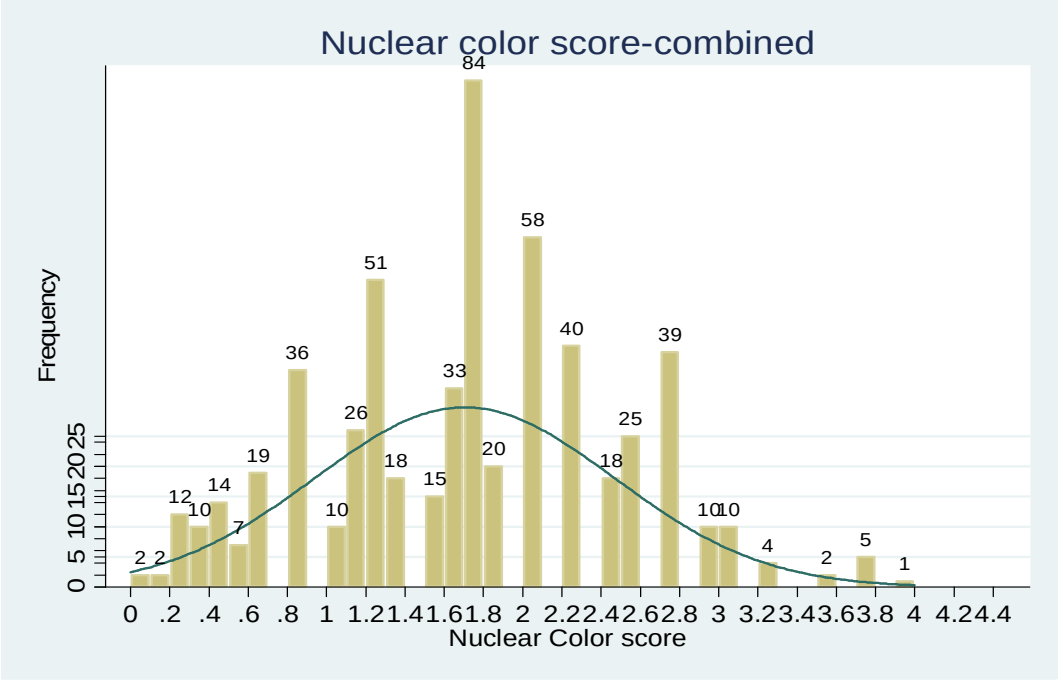
Scorer 1 Vs. Scorer2 Bland Altman Graph- Right Eye	**Scorer 1 Vs. Scorer 2 Bland Altman Graph- Left Eye**
	
Bland Altman regression graph- right eye	Bland Altman regression graph- left eye
	
Limits of agreement (Reference Range for difference): -1.132 to 1.143 Mean difference: 0.006 (CI -0.088 to 0.100) Range : 0.200 to 3.650 Pitman's Test of difference in variance: r = 0.071, n = 143, p = 0.401	Limits of agreement (Reference Range for difference): -0.834 to 1.104 Mean difference: 0.135 (CI 0.055 to 0.216) Range : 0.200 to 3.800 Pitman's Test of difference in variance: r = 0.056, n = 142, p = 0.505

Table 3.0.24 LOC III- All nuclear color scores (n=571)

Moments	Mean NC
Mean	1.70
Standard Deviation	0.76
Variance	0.58
Median	1.7
Smallest value	0
Largest value	3.9
1% percentile	0.2
25% percentile	1.2
75% percentile	2.2
99% percentile	3.7
Skewness	0.046
Kurtosis	2.67
Skewness/Kurtosis tests for normality	
Pr (Skewness)	0.644
Pr (Kurtosis)	0.073
Adjsted Chi-square (2)	3.43
Pr Chi-square	0.1801

Figure 3.0.6 Frequencies and distribution of nuclear color (NC) scores (n=571)



3.3.19 Cortical opacity (C)

3.3.19.1 Summary statistics of cortical opacity scores

Figures 3.0.7 and 3.0.8 summarizes the frequencies and distribution of cortical opacity scores for right and the left eyes from scorers 1 and 2, and table 3.0.25 summarizes the cortical opacity scores for both eyes provided by scorers 1 and 2. The intra correlation of scores between the right and left eyes provided by scorers 1 and 2 were similar (correlation: 0.69 vs. 0.67). Similarly the inter correlations of scores on the same eye provided by the two scorers were also similar (correlation: 0.76 vs. 0.77). The mean difference of scores was slightly higher on the right (0.169) than the left eyes (0.122). The BAP showed that 4.90% and 6.99% of values on right and the left eyes were outside the limits of agreement. The difference between the scores compared to mean did not show the increasing trend with increasing level of opacity. In terms of variability, the variance of scores was higher for scorer 1 than 2 (Pitman's p <0.05). Table 3.0.27 has the descriptive statistics of combined scores- four of each of the lens opacity grades for each subject (2 eyes, 2 graders). The mean score was 0.95 with standard deviation (SD) of 0.85. The distribution statistics of all scores (table 3.0.27) suggested that cortical opacity scores are not normally distributed (skewness (p=0.00) and kurtosis (p=0.00)). About 99 (17%) scores were zero.

Table 3.0.25 LOC III- Cortical opacity scores from scorers 1 and 2 (n=143)

Moments	**Scorer 1 Right Eye**	**Scorer 1 Left Eye**	**Scorer 2 Right Eye**	**Scorer 2 Left Eye**
Mean	1.01	1.02	0.85	0.90
Standard	0.93	0.94	0.79	0.74
Deviation	0.86	0.88	0.62	0.54
Variance	0.9	1.0	0.7	0.8
Median	0	0	0	0
Smallest value	3.8	3.9	3.2	3.1
Largest value	0	0	0	0
1% percentile	0	0.2	0.2	0.3
25% percentile	1.7	1.6	1.3	1.7
75% percentile	3.1	3.4	3.2	3.1
99% percentile	0.65	0.79	1.05	1.04
Skewness	2.61	2.99	3.56	3.69
Kurtosis				
Correlation (r^2)	0.69		0.67	
t test (p value)	-0.08 (0.94)		-0.98 (0.33)	

Figure 3.0.7 Frequencies and distribution of cortical opacity scores for right and left eyes from scorer 1

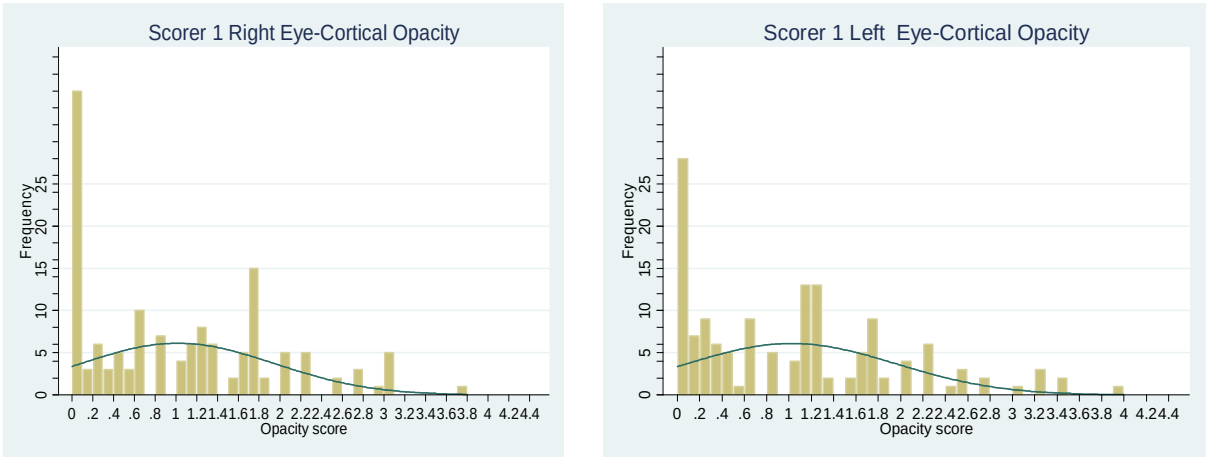


Figure 3.0.8 Frequencies and distribution of cortical opacity scores for right and left eyes from scorer 2

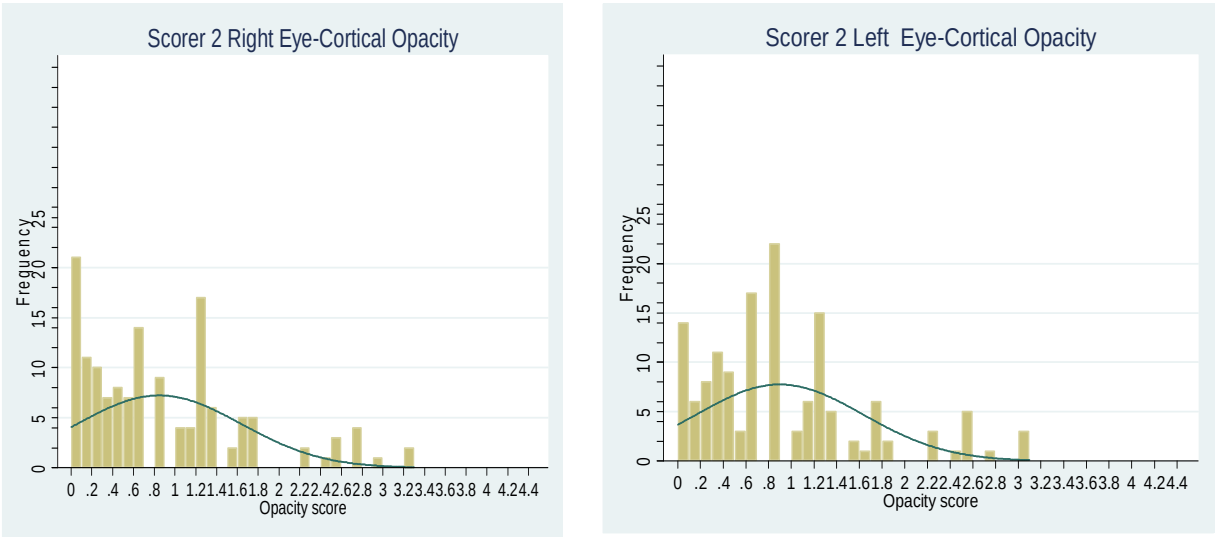


Table 3.0.26 Comparison of cortical opacity scores for right and left eyes (BAP and regression)

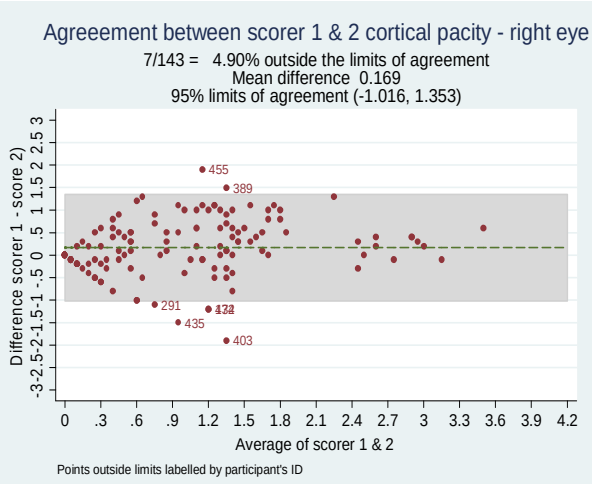
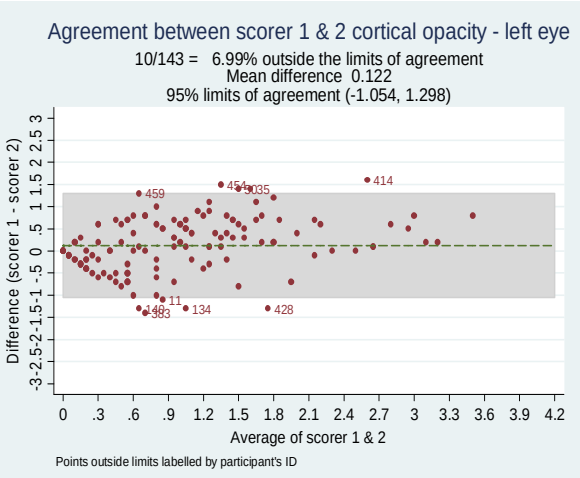
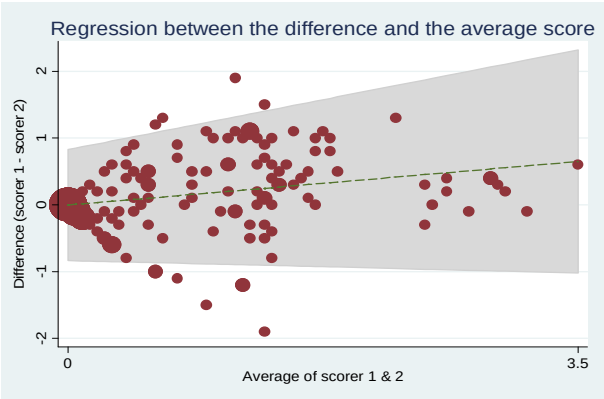
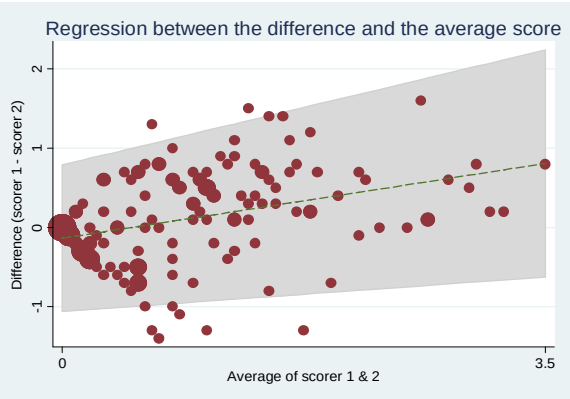
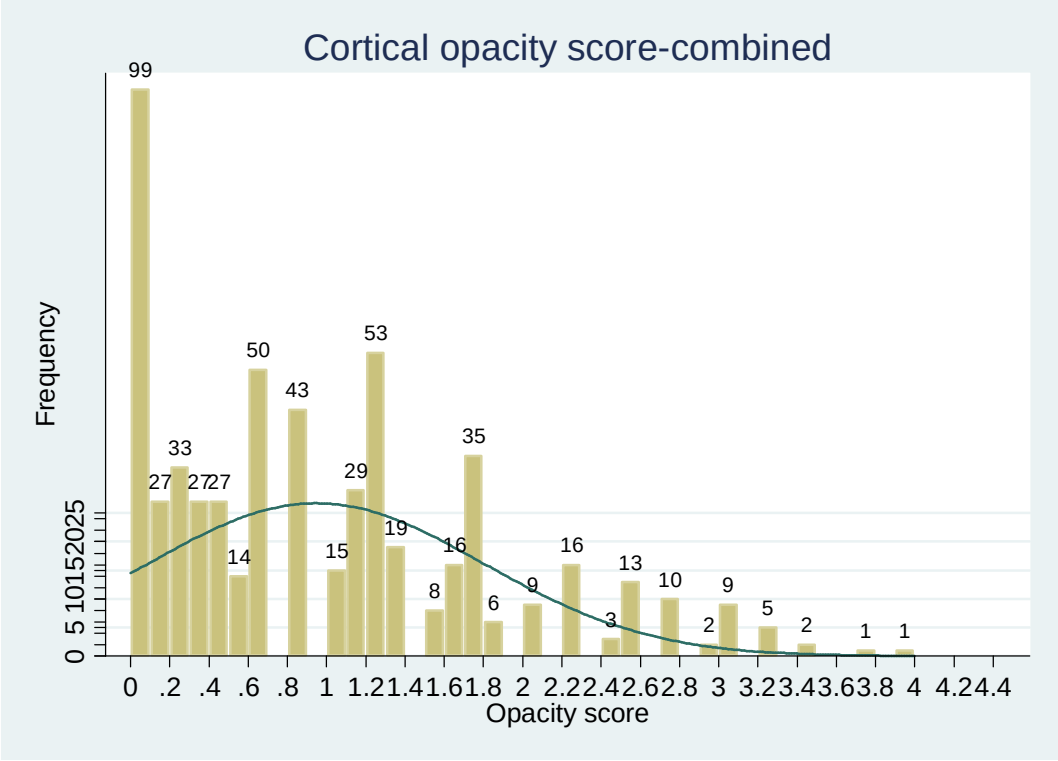
Scorer 1 Vs. Scorer 2 Regression Graph-Right Eye	**Scorer 1 Vs. Scorer 2 Regression Graph-Left Eye**
	
Bland Altman regression graph- right eye	Bland Altman regression graph- left eye
	
Limits of agreement (Reference Range for difference): -1.040 to 1.378 Mean difference: 0.169 (CI 0.069 to 0.268) Range : 0.000 to 3.500 Pitman's Test of difference in variance: r = 0.249, n = 143, p = 0.003	Limits of agreement (Reference Range for difference): -1.078 to 1.322 Mean difference: 0.122 (CI 0.023 to 0.222) Range : 0.000 to 3.500 Pitman's Test of difference in variance: r = 0.353, n = 143, p = 0.000

Table 3.0.27 Table 25 LOC III- All cortical opacity scores (n=571)

Moments	Mean cortical opacity
Mean	0.95
Standard Deviation	0.85
Variance	0.73
Median	0.80
Smallest value	0
Largest value	3.9
1% percentile	0.0
25% percentile	0.2
75% percentile	1.4
99% percentile	3.2
Skewness	0.89
Kurtosis	3.20
Skewness/Kurtosis tests for normality	
Pr (Skewness)	0.000
Pr (Kurtosis)	0.000
Adjsted Chi-square (2)	47.43
Pr Chi-square	0.000

Figure 3.0.9 Frequencies and distribution of cortical opacity (C) scores (n=571)



3.3.20 Posterior sub-capsular opacity (P)

3.3.20.1 Summary statistics of posterior sub-capsular opacity (P) scores

Figures 3.0.10 and 3.0.11 summarize the frequencies and distribution of posterior sub-capsular (P) scores for right and the left eyes from scorers 1 and 2. And table 3.0.28 summarizes the posterior sub-capsular opacity scores for both eyes provided by scores 1 and 2. The scorers' intra correlation of scores between right and the left eyes were markedly different (correlation: 0.57 vs. 0.73). Similarly the inter correlations of scores between right and the left eyes on the same participants provided by scorers were 0.67 and 0.50. The mean differences of scores were comparable between two scores. The BAP showed 6.29% and 6.99 % of values on the right and left eyes outside the limits of agreement. In terms of variability, the variance of scores was higher for scorer 1 than 2 (Pitman's p <0.05). The combined mean opacity score of posterior sub-capsular in the study participants was 0.22 with standard deviation (SD) of 0.46. Table 3.0.30 provides summary statistics of the combined score. The distribution statistics of all scores (table 3.0.30) - four of each of the lens opacity grades for each subject (2 eyes, 2 graders) suggests that posterior sub-capsular opacity scores are not normally distributed (skewness (p=0.00) and kurtosis (p=0.00)). See figure 3.0.12. About 291 (51%) scores were zero.

Table 3.0.28 LOC III- Posterior sub-capsular opacity (P) scores from scorers 1 and 2 (n=143)

Moments	**Scorer 1 Right Eye**	**Scorer 1 Left Eye**	**Scorer 2 Right Eye**	**Scorer 2 Left Eye**
Mean	0.17	0.22	0.21	0.27
Standard	0.48	0.55	0.36	0.42
Deviation	0.23	0.30	0.13	0.17
Variance	0	0	0.1	0.1
Median	0	0	0	0
Smallest value	3.7	3.5	2.9	2.7
Largest value	0	0	0	0
1% percentile	0	0	0	0
25% percentile	0.1	0.1	0.3	0.3
75% percentile	2.4	2.6	1.8	2.0
99% percentile	4.55	3.50	4.10	2.93
Skewness	27.18	16.09	26.74	13.59
Kurtosis				
Correlation (r^2)	0.57		0.73	
t test (p value)	-1.1384 (0.26)		-2.261 (0.03)	

Figure 3.0.10 Frequencies and distribution of posterior sub-capsular (P) scores for right and left eyes from scorer 1

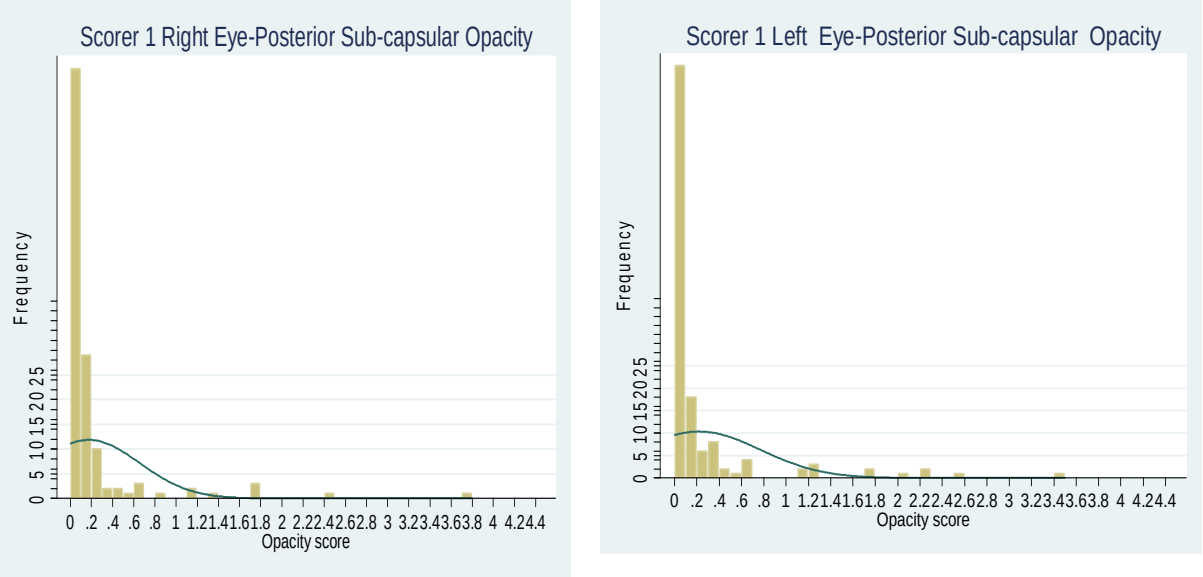


Figure 3.0.11 Frequencies and distribution of posterior sub-capsular (P) scores for right and left eyes from scorer 2

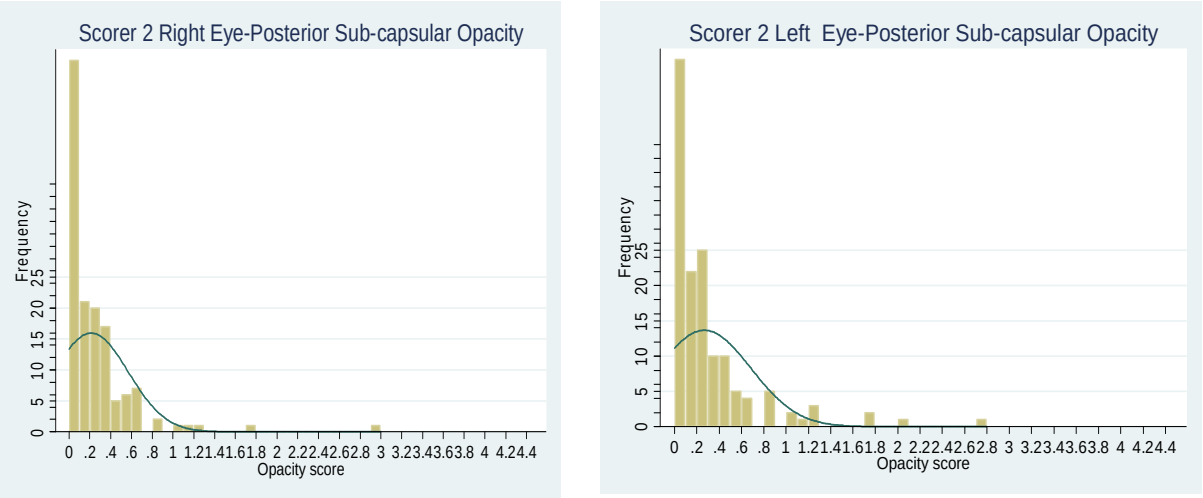


Table 3.0.29 Comparison of posterior sub-capsular (P) for right and left eyes (BAP and regression)

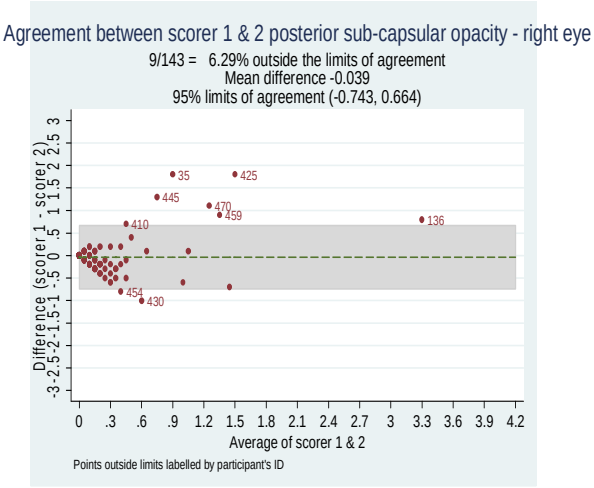
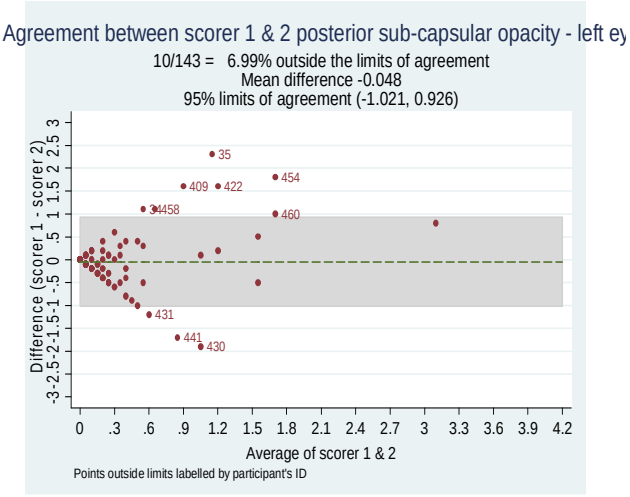
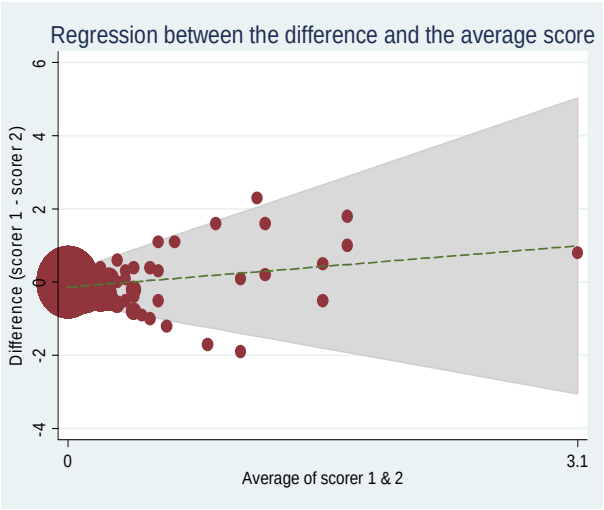
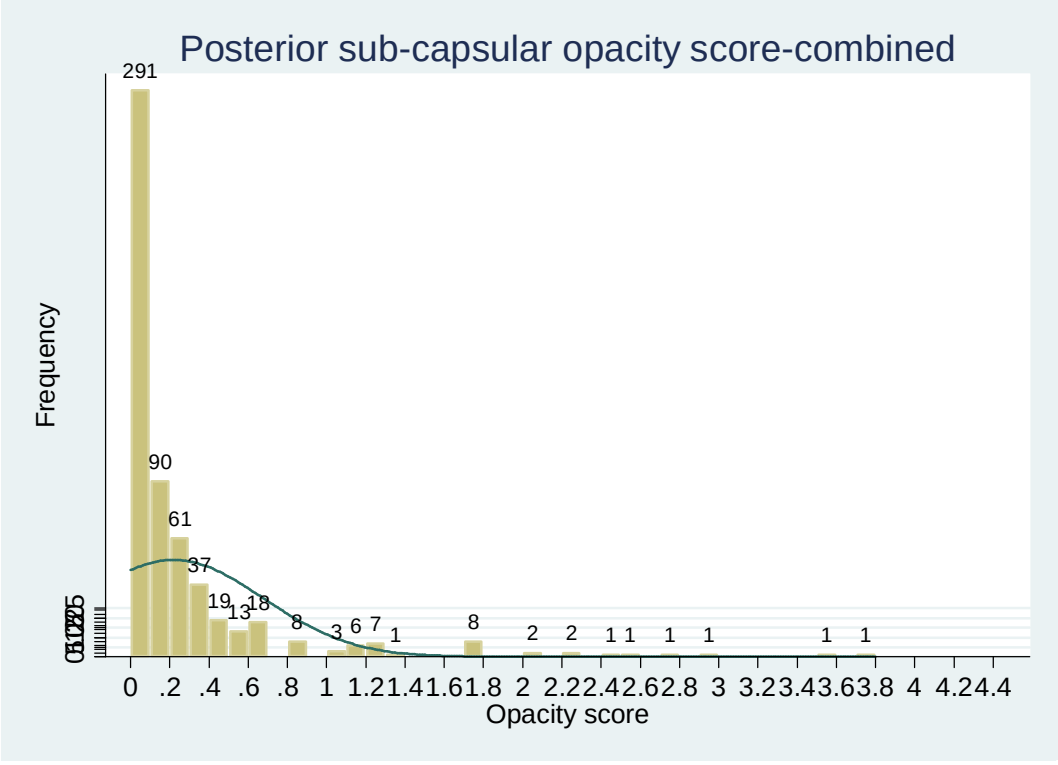
Scorer 1 Vs. Scorer2 Bland Altman Graph- Right Eye	**Scorer 1 Vs. Scorer 2 Bland Altman Graph- Left Eye**
	
Bland Altman regression graph- right eye	Bland Altman regression graph- left eye
	
Limits of agreement (Reference range for difference): -0.757 to 0.679 Mean difference: -0.039 (CI -0.099 to 0.020) Range : 0.000 to 3.300 Pitman's Test of difference in variance: r = 0.374, n = 143, p = 0.000	Limits of agreement (Reference range for difference): -1.041 to 0.946 Mean difference: -0.048 (CI -0.130 to 0.035) Range : 0.000 to 3.100 Pitman's Test of difference in variance: r = 0.308, n = 143, p = 0.000

Table 3.0.30 LOC III- All posterior sub-capsular (P) scores (n=571)

Moments	Mean cortical opacity
Mean	0.22
Standard Deviation	0.46
Variance	0.21
Median	0.00
Smallest value	0
Largest value	3.7
1% percentile	0.0
25% percentile	0.0
75% percentile	0.2
99% percentile	2.4
Skewness	3.85
Kurtosis	21.13
Skewness/Kurtosis tests for normality	
Pr (Skewness)	0.000
Pr (Kurtosis)	0.000
Adjsted Chi-square (2)	.
Pr Chi-square	0.000

Figure 3.0.12 Frequencies and distribution of posterior sub-capsular (P) scores (n=571)

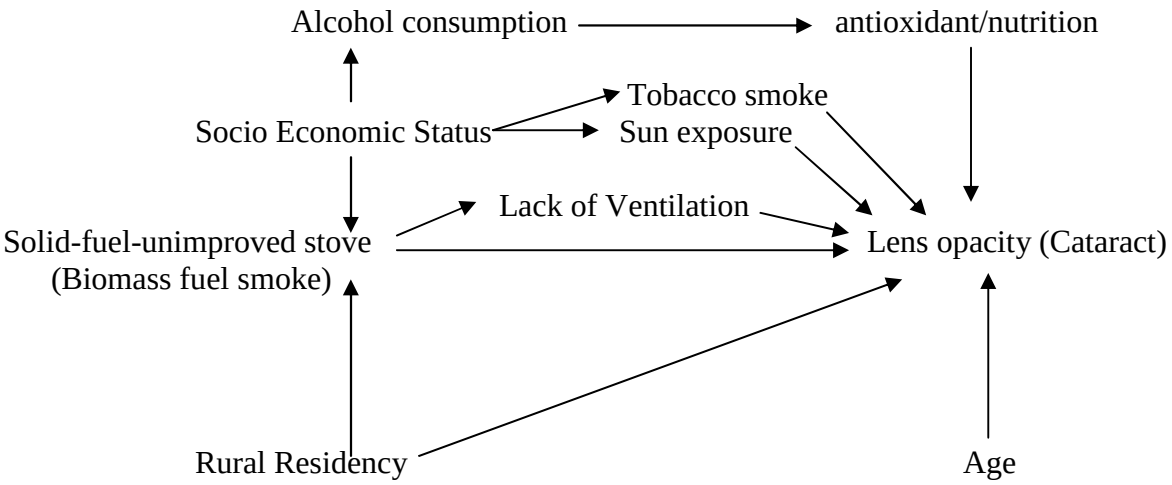


3.3.21 Opacity risk factor modeling

3.3.21.1 Potential confounders' selection criteria for the multivariate model

Summarization of causal links via graphs or diagrams has long been used as an informal aid in epidemiological studies to identify potential confounders [72]. Results of epidemiological studies show that besides biomass-fuel smoke, tobacco smoking, sunlight exposure, alcohol consumption, lack of antioxidants and dietary factors, age, rural residency, occupation could be risk factors for lens opacity or cataracts. Figure I provide a simple graph of possible confounders in the study of association between biomass fuel smoke exposure and risk of lens opacity. It is unlikely that it shows all the possible associations between potential risk factors.

Figure I Factors associated with the risk of formation of cataract



In the case of an association between biomass fuel smoke and lens opacity, socio-economic status (SES) including literacy and area of residency, occupation, exposure to sunlight, lack of antioxidants or nutrition and tobacco smoking (directly or through SES) could confound the relationship and may need to be considered. For example, SES could influence the use of cleaner burning fuel, such as liquefied petroleum gas (LPG) or biogas, compared with biomass fuel and also the tobacco consumption. Area of residency could play an important role because, compared with urban residents; rural residents may not have easy access to cleaner burning fuels. Rural residents on the other hand may have easy access to unprocessed biomass-based fuels and thus may use these fuels more frequently. But biogas is an exception, because this is more commonly used in rural areas than in urban areas of developing countries. Occupation could play an important role in the pathogenesis of cataract, if a person's work is mostly outdoors or on farm land, leading to less smoke and more ultraviolet light exposure. Thus, occupation could potentially confound the relationship between biomass fuel smoke exposure and risk of lens opacity or cataracts. Another important risk factor for cataract formation is lack of antioxidants, which can make people susceptible to oxidative damage. Lack of antioxidants could also be indirectly or directly related to SES of people. Alcohol consumption, on the other hand, may not play a direct role in the pathogenesis of cataract but it could suppress the antioxidant capabilities

of people, provoking oxidative damage. Age and tobacco smoke can independently increase the risk of cataract through duration of exposure and through suppression of antioxidant capabilities. Thus, these exposures were considered as potential covariates to be controlled in the risk models for the association between exposures to biomass fuel smoke and lens opacity. However, before running a risk model, the prevalence of lens opacity and the distribution of mean opacity across confounders/covariates were examined to further determine covariates that significantly affected the mean opacity scores.

3.3.21.2 Prevalence of lens opacity by exposure variables

Tables 3.0.31 to 3.0.37 summarizes the prevalence of lens opacity by age, area of residency, current main fuel stove, tobacco smoking and alcohol consumption habit, dietary practice and work in the sunlight for more than one hour every day. The prevalence of other covariates including other sources of indoor air pollution (use of mosquito coil and incense) and socio-economic indicators are presented in Annex 3.6 (tables B.0.1 and B.0.2). The numbers in tables represents four of each of the lens opacity grades for each participant (2 eyes, 2 graders).

Table 3.0.31 Prevalence of lens opacity by age group in years (% in age group and row total %)

Scores	**Age group in years**					**χ^2 (p value)**
	20-29	>29 - <=39	>39 - <=49	>49 - <=59	>59	
Nuclear Opacity						
0	2 (4.55%)	2 (1.43%)	1 (0.57%)	0 (0.00%)	0 (0.00%)	246.13
>0-≤0.9	26 (59.09%)	27 (19.29%)	24 (13.64%)	12 (8.82%)	0 (0.00%)	(0.00)
>0.9- ≤1.9	16 (36.36%)	97 (69.29%)	131 (74.43%)	63 (46.32%)	20 (26.32%)	
>1.9 - ≤2.9	0 (0.00%)	14 (10.00%)	19 (10.80%)	41 (30.15%)	45 (59.21%)	
>2.9	0 (0.00%)	0 (0.00%)	1 (0.57%)	20 (14.71%)	11 (14.47%)	
Total	44	140	176	136	76	
Nuclear color						
0	1 (2.27%)	0 (0.00%)	1 (0.57%)	0 (0.00%)	0 (0.00%)	400.88
>0-≤0.9	35 (79.55%)	43 (30.71%)	15 (8.52%)	7 (5.15%)	0 (0.00%)	(0.00)
>0.9- ≤1.9	8 (18.18%)	91 (65.00%)	115 (65.34%)	41 (30.15%)	2 (2.63%)	
>1.9 - ≤2.9	0 (0.00%)	6 (4.29%)	44 (25.00%)	73 (53.68%)	57 (75.00%)	
>2.9	0 (0.00%)	0 (0.00%)	1 (0.57%)	15 (11.03%)	17 (22.37%)	
Total	44	140	176	136	76	
Cortical						
0	10 (22.73%)	28 (20.00%)	38 (21.59%)	12 (8.82%)	11 (14.47%)	111.24
>0-≤0.9	28 (63.64%)	65 (46.43%)	69 (39.20%)	36 (26.47%)	23 (30.26%)	(0.00)
>0.9- ≤1.9	6 (13.64%)	46 (32.86%)	59 (33.52%)	52 (38.24%)	18 (23.68%)	
>1.9 - ≤2.9	0 (0.00%)	1 (0.71%)	10 (5.68%)	22 (16.18%)	18 (23.68%)	
>2.9	0 (0.00%)	0 (0.00%)	0 (0.00%)	14 (10.29%)	6 (7.89%)	
Total	44	140	176	136	76	
Posterior sub-capsular						
0	35 (79.55%)	74 (52.86%)	95 (53.98%)	56 (41.18%)	31 (40.79%)	55.68
>0-≤0.9	9 (20.45%)	63 (45.00%)	75 (42.61%)	65 (47.61%)	34 (44.74%)	(0.00)
>0.9- ≤1.9	0 (0.00%)	2 (1.43%)	6 (3.41%)	7 (5.15%)	10 (13.16%)	
>1.9 - ≤2.9	0 (0.00%)	1 (0.71%)	0 (0.00%)	5 (3.68%)	1 (1.32%)	
>2.9	0 (0.00%)	0 (0.00%)	0 (0.00%)	3 (2.21%)	0 (0.00%)	
Total	44	140	176	136	76	

Table 3.0.32 Prevalence of lens opacity by area of residency and level of literacy

Scores	**Area of residency**			**Literacy**		
	Rural	Urban & Peri-urban	χ^2 (p value)	Can read and write	Cannot read and write	χ^2 (p value)
Nuclear Opacity						
0	2 (2.50%)	3 (0.61%)	13.73 (0.01)	4 (1.39%)	1 (0.35%)	55.65
>0-≤0.9	20 (25.00%)	69 (14.02%)		61 (21.18%)	28 (9.86%)	(0.00)
>0.9- ≤1.9	42 (52.50%)	285 (57.93%)		178 (61.18%)	149 (52.46%)	
>1.9 - ≤2.9	16 (20.00%)	103 (20.93%)		45 (15.63%)	74 (26.06%)	
>2.9	0 (0.00%)	32 (6.50%)		0 (0.00%)	32 (11.27%)	
Total	80	492		288	284	
Nuclear color						
0	0 (0.00%)	2 (0.41%)	12.26 (0.02)	0 (0.00%)	2 (0.70%)	58.86
>0-≤0.9	20 (25.00%)	80 (16.26%)		71 (24.65%)	29 (10.21%)	(0.00)
>0.9- ≤1.9	42 (52.50%)	215 (43.70%)		150 (52.08%)	107 (37.68%)	
>1.9 - ≤2.9	18 (22.50%)	162 (32.93%)		61 (21.18%)	119 (41.90%)	
>2.9	0 (0.00%)	33 (6.71%)		6 (2.08%)	27 (9.51%)	
Total	80	492		288	284	
Cortical						
0	8 (10.00%)	91 (18.50%)	3.89 (0.42)	56 (19.44%)	43 (15.14%)	24.39
>0-≤0.9	33 (41.25%)	188 (38.21%)		122 (42.36%)	99 (34.86%)	(0.00)
>0.9- ≤1.9	27 (33.75%)	154 (31.30%)		93 (32.29%)	88 (30.99%)	
>1.9 - ≤2.9	8 (10.00%)	43 (8.74%)		14 (4.86%)	37 (13.03%)	
>2.9	4 (5.00%)	16 (3.25%)		3 (1.04%)	17 (5.99%)	
Total	80	492		288	284	
Posterior sub-capsular						
0	45 (56.25%)	246 (50.00%)	6.23 (0.18)	160 (55.56%)	131 (46.13%)	8.95
>0-≤0.9	35 (43.75%)	211 (42.89%)		116 (40.28%)	130 (45.77%)	(0.06)
>0.9- ≤1.9	0 (0.00%)	25 (5.08%)		10 (3.47%)	15 (5.28%)	
>1.9 - ≤2.9	0 (0.00%)	7 (1.42%)		2 (0.69)	5 (1.76%)	
>2.9	0 (0.00%)	3 (0.61%)		0 (0.00%)	3 (1.06%)	
Total	80	492		288	284	

Table 3.0.33 Prevalence of lens opacity by present cooking fuel stove

Scores	**Present cooking fuel stove**			**χ^2 (p value)**
	Biomass fuel stove	Kerosene fuel stove	Gaseous fuel stove	
Nuclear Opacity				
0	3 (1.32%)	0 (0.00%)	2 (0.62%)	13.05
>0-≤0.9	28 (12.28%)	3 (15.00%)	58 (17.90%)	(0.11)
>0.9- ≤1.9	133 (58.3%)	9 (45.00%)	185 (57.10%)	
>1.9 - ≤2.9	53 (23.25%)	4 (20.00%)	62 (19.14%)	
>2.9	11 (4.82%)	4 (20.00%)	17 (5.25%)	
Total	228	20	324	
Nuclear color				
0	2 (0.88%)	0 (0.00%)	0 (0.00%)	19.14
>0-≤0.9	48 (21.05%)	0 (0.00%)	52 (16.05%)	(0.01)
>0.9- ≤1.9	104 (45.61%)	6 (30.00%)	147 (45.37%)	
>1.9 - ≤2.9	66 (28.95%)	11 (55.00%)	103 (31.79%)	
>2.9	8 (3.51%)	3 (15.00%)	22 (6.79%)	
Total	228	20	324	
Cortical				
0	37 (16.23%)	5 (25.00%)	57 (17.59%)	5.22
>0-≤0.9	91 (39.91%)	9 (45.00%)	121 (37.35%)	(0.73)
>0.9- ≤1.9	77 (33.77%)	3 (15.00%)	101 (31.17%)	
>1.9 - ≤2.9	16 (7.02%)	2 (10.00%)	33 (10.19%)	
>2.9	7 (3.07%)	1 (5.00%)	12 (3.70%)	
Total	228	20	324	
Posterior sub-capsular				
0	123 (53.95%)	5 (25.00%)	163 (50.31%)	102.71
>0-≤0.9	97 (42.54%)	6 (30.00%)	142 (43.83%)	(0.00)
>0.9- ≤1.9	5 (2.19%)	4 (20.00%)	16 (4.94%)	
>1.9 - ≤2.9	3 (1.32%)	2 (10.00%)	3 (0.93%)	
>2.9	0 (0.00%)	3 (15.00%)	0 (0.00%)	
Total	228	20	324	

Table 3.0.34 Prevalence of lens opacity by ever smoked cigarette, *bidi* or *hukka* and alcohol consumption

Scores	**Ever smoked cigarette, *bidi* or *hukka*?**			**Alcohol consumption**		
	Never	Ever	χ^2 (p value)	Never	Ever	χ^2 (p value)
Nuclear Opacity						
0	4 (1.04%)	1 (0.53%)	23.46 (0.00)	5 (0.92%)	0 (0.00%)	0.89
>0-≤0.9	71 (18.49%)	18 (9.57%)		84 (15.44%)	5 (17.86%)	(0.93)
>0.9- ≤1.9	230 (59.90%)	97 (51.60%)		312 (57.35%)	15 (53.57%)	
>1.9 - ≤2.9	63 (16.41%)	56 (29.79%)		112 (20.59%)	7 (25.00%)	
>2.9	16 (4.17%)	16 (8.51%)		31 (5.70%)	1 (3.57%)	
Total	384	188		544	28	
Nuclear color						
0	0 (0.00%)	2 (1.06%)	49.96 (0.00)	2 (0.37%)	0 (0.00%)	7.62
>0-≤0.9	82 (21.35%)	18 (9.57%)		96 (17.65%)	4 (14.29%)	(0.11)
>0.9- ≤1.9	193 (50.26%)	64 (34.04%)		248 (45.59%)	9 (32.14%)	
>1.9 - ≤2.9	97 (25.26%)	83 (44.15%)		165 (30.33%)	15 (53.57%)	
>2.9	12 (3.13%)	21 (11.17%)		33 (6.07%)	0 (0.00%)	
Total	384	188		544	28	
Cortical						
0	70 (18.23%)	29 (15.43%)	30.67 (0.00)	95 (17.46%)	4 (14.29%)	8.03
>0-≤0.9	167 (43.49%)	54 (28.72%)		216 (39.71%)	5 (17.86%)	(0.09)
>0.9- ≤1.9	118 (30.73%)	63 (33.51%)		166 (30.51%)	15 (53.57%)	
>1.9 - ≤2.9	22 (5.73%)	29 (15.43%)		48 (8.82%)	3 (10.71%)	
>2.9	7 (1.82%)	13 (6.91%)		19 (3.49%)	1 (3.57%)	
Total	384	188		544	28	
Posterior sub-capsular						
0	195 (50.78%)	96 (51.06%)	11.73 (0.02)	280 (51.47%)	11 (39.29%)	7.99
>0-≤0.9	173 (45.05%)	73 (38.83%)		233 (42.83%)	13 (46.43%)	(0.09)
>0.9- ≤1.9	13 (3.39%)	12 (6.38%)		21 (3.86%)	4 (14.29%)	
>1.9 - ≤2.9	3 (0.78%)	4 (2.13%)		7 (1.29%)	0 (0.00%)	
>2.9	0 (0.00%)	3 (1.60%)		3 (0.55%)	0 (0.00%)	
Total	384	188		544	28	

Table 3.0.35 Prevalence of lens opacity by ever work outside in the sun >1 hour daily and land ownership

Scores	**Work outside in the sun >1 hour daily**			**Land ownership**		
	Yes	No	χ^2 (p value)	Yes	No	χ^2 (p value)
Nuclear Opacity						
0	5 (1.36%)	0 (0.00%)	10.53 (0.03)	5 (1.23%)	0 (0.00%)	9.74
>0-≤0.9	46 (12.50%)	43 (21.08%)		70 (17.16%)	19 (11.59%)	(0.05)
>0.9- ≤1.9	218 (59.24%)	109 (53.43%)		230 (56.37%)	97 (59.15%)	
>1.9 - ≤2.9	80 (21.74%)	39 (19.12%)		86 (21.08%)	33 (20.12%)	
>2.9	19 (5.16%)	13 (6.37%)		17 (4.17%)	15 (9.15%)	
Total	368	204		408	164	
Nuclear color						
0	2 (0.54%)	0 (0.00%)	6.81 (0.15)	1 (0.25%)	1 (0.61%)	1.95
>0-≤0.9	65 (17.66%)	35 (17.16%)		73 (17.89%)	27 (16.46%)	(0.75)
>0.9- ≤1.9	171 (46.47%)	86 (42.16%)		187 (45.83%)	70 (42.68%)	
>1.9 - ≤2.9	115 (31.25%)	65 (31.86%)		126 (30.88%)	54 (32.93%)	
>2.9	15 (4.08%)	18 (8.82%)		21 (5.15%)	12 (7.32%)	
Total	368	204		408	164	
Cortical						
0	66 (17.93%)	33 (16.18%)	1.83 (0.77)	61 (14.95%)	38 (23.17%)	6.80
>0-≤0.9	142 (38.59%)	79 (38.73%)		160 (39.22%)	61 (37.20%)	(0.15)
>0.9- ≤1.9	119 (32.34%)	62 (30.39%)		137 (33.58%)	44 (26.83%)	
>1.9 - ≤2.9	30 (8.15%)	21 (10.29%)		37 (9.07%)	14 (8.54%)	
>2.9	11 (2.99%)	9 (4.41%)		13 (3.19%)	7 (4.27%)	
Total	368	204		408	164	
Posterior sub-capsular						
0	188 (51.09%)	103 (50.49%)	2.07 (0.72)	216 (52.94%)	75 (45.73%)	12.26
>0-≤0.9	156 (42.39%)	90 (44.12%)		174 (42.65%)	72 (43.90%)	(0.02)
>0.9- ≤1.9	17 (4.62%)	8 (3.92%)		14 (3.43%)	11 (6.71%)	
>1.9 - ≤2.9	4 (1.09%)	3 (1.47%)		4 (0.98%)	3 (1.83%)	
>2.9	3 (0.82%)	0 (0.00%)		0 (0.00%)	3 (1.83%)	
Total	368	204		408	164	

Table 3.0.36 Prevalence of lens opacity by dietary habits

Scores	**Vegetarian vs. non-vegetarian**		**χ^2 (p value)**
	Vegetarian	Non-vegetarian	
Nuclear Opacity			
0	0 (0.00%)	5 (0.95%)	13.26 (0.01)
>0-≤0.9	2 (4.55%)	87 (16.48%)	
>0.9- ≤1.9	21 (47.73%)	306 (57.95%)	
>1.9 - ≤2.9	16 (36.36%)	103 (19.51%)	
>2.9	5 (11.36%)	27 (5.11%)	
Total	44	528	
Nuclear color			
0	0 (0.00%)	2 (0.38%)	24.63 (0.00)
>0-≤0.9	1 (2.27%)	99 (18.75%)	
>0.9- ≤1.9	12 (27.27%)	245 (46.40%)	
>1.9 - ≤2.9	25 (56.82%)	155 (29.36%)	
>2.9	6 (13.64%)	27 (5.11%)	
Total	44	528	
Cortical			
0	8 (18.18%)	91 (17.23%)	10.14 (0.04)
>0-≤0.9	13 (29.55%)	208 (39.39%)	
>0.9- ≤1.9	22 (50.00%)	159 (30.11%)	
>1.9 - ≤2.9	1 (2.27%)	50 (9.47%)	
>2.9	0 (0.00%)	20 (3.79%)	
Total	44	528	
Posterior sub-capsular			
0	20 (45.45%)	271 (51.33%)	3.38 (0.50)
>0-≤0.9	19 (43.18%)	227 (42.99%)	
>0.9- ≤1.9	4 (9.09%)	21 (3.98%)	
>1.9 - ≤2.9	1 (2.27%)	6 (1.14%)	
>2.9	0 (0.00%)	3 (0.57%)	
Total	44	528	

Table 3.0.37 Prevalence of lens opacity by annual family income in Nepali Rupees (NRs)

Scores	**Family annual income in NRs.**					**χ^2 (p value)**
	NRs. <=25,000	NRs. 25,000-50,000	NRs.50,000-100,000	NRs. >100,000	Refused to answer	
Nuclear Opacity						
0	4 (3.45%)	1 (0.52%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	44.62
>0-≤0.9	16 (13.79%)	29 (15.10%)	25 (16.03%)	18 (21.43%)	1 (4.17%)	(0.00)
>0.9- ≤1.9	54 (46.55%)	114 (59.38%)	95 (60.90%)	52 (61.90%)	12 (50.00%)	
>1.9 - ≤2.9	27 (23.28%)	40 (20.83%)	31 (19.87%)	10 (11.90%)	11 (45.83%)	
>2.9	15 (12.93%)	8 (4.17%)	5 (3.21%)	4 (4.76%)	0 (0.00%)	
Total	116	192	156	84	24	
Nuclear color						
0	0 (0.00%)	2 (1.04%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	12.22
>0-≤0.9	18 (15.52%)	37 (19.27%)	26 (16.67%)	15 (17.86%)	4 (16.67%)	(0.73)
>0.9- ≤1.9	47 (40.52%)	81 (42.19%)	81 (51.92%)	37 (44.05%)	11 (45.83%)	
>1.9 - ≤2.9	42 (36.21%)	61 (31.77%)	42 (26.92%)	26 (30.95%)	9 (37.50%)	
>2.9	9 (7.76%)	11 (5.73%)	7 (4.49%)	6 (7.14%)	0.00 (0.00%)	
Total	116	192	156	84	24	
Cortical						
0	14 (12.07%)	41 (21.35%)	29 (18.59%)	12 (14.29%)	3 (12.50%)	28.59
>0-≤0.9	43 (37.07%)	74 (38.54%)	58 (37.18%)	36 (42.86%)	10 (41.67%)	(0.03)
>0.9- ≤1.9	41 (35.34%)	54 (28.13%)	58 (37.18%)	21 (25.00%)	7 (29.17%)	
>1.9 - ≤2.9	11 (9.48%)	19 (9.90%)	10 (6.41%)	7 (8.33%)	4 (16.67%)	
>2.9	7 (6.03%)	4 (2.08%)	1 (0.64%)	8 (9.52%)	0.00 (0.00%)	
Total	116	192	156		24	
Posterior sub-capsular						
0	62 (53.45%)	88 (45.83%)	84 (53.85%)	42 (50.00%)	15 (62.50%)	21.61
>0-≤0.9	49 (43.24%)	91 (47.40%)	67 (42.95%)	31 (36.90%)	8 (33.33%)	(0.16)
>0.9- ≤1.9	4 (3.45%)	8 (4.17%)	3 (1.92%)	9 (10.71%)	1 (4.37%)	
>1.9 - ≤2.9	1 (0.86%)	2 (1.04%)	2 (1.28%)	2 (2.38%)	0 (0.00%)	
>2.9	0 (0.00%)	3 (1.56%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	
Total	116	192	156	84	24	

3.3.21.3 Analysis of Variance (ANOVA)

Table 3.0.38 presents the mean opacity scores by potential confounders for three types of opacities and nuclear color. Appendix 3.6 has ANOVA results (tables B.0.3-B.0.5) for other exposure and potential confounders (environmental tobacco smoke, frequencies of meat, fish and milk consumption) and sunlight protection.

Table 3.0.38 Analysis of variance (ANOVA and *p* values) between main exposure and potential confounders and lens opacity

Independent variables	**Dependent Variables : mean opacity score (standard deviation)**			
Exposure variables	Nuclear Opacity (NO)	Nuclear Color (NC)	Cortical Opacity (C)	Posterior sub-capsular Opacity (P)
Age in years				
20-29	0.75 (0.45) *	0.58 (0.38)*	0.48 (0.47)*	0.03 (0.06)*
>29 - ≤39	1.35 (0.53)	1.18 (0.46)	0.65 (0.56)	0.15 (0.30)
>39 - ≤49	1.43 (0.51)	1.64 (0.48)	0.76 (0.68)	0.15 (0.27)
>49 - ≤59	1.94 (0.80)	2.17 (0.63)	1.42 (0.98)	0.36 (0.69)
>59	2.39 (0.62)	2.65 (0.39)	1.33 (1.05)	1.33 (1.05)
Residence locality				
Rural	1. 39 (0.63) *	1.46 (0.67)*	0.92 (0.85)	0.12 (0.18)*
Urban and Peri-urban	1.64 (0.75)	1.74 (0.77)	1.09 (0.86)	0.23 (0.49)
Tobacco smoking				
Never	1.50 (0.72) *	1.55 (0.71)*	0.81 (0.75)*	0.18 (0.35)*
Ever	1.82 (0.75)	2.02 (0.77)	1.22 (0.97)	0.30 (0.61)
Pack years of smoking				
0	1.50 (0.72)*	1.55 (0.71)*	0.81 (0.75)*	0.18 (0.35)*
>0 & ≤9 pack-years	1.69 (0.73)	1.90 (0.74)	1.21 (0.89)	0.20 (0.40)
>9 pack-years	1.96 (0.75)	2.15 (0.78)	1.23 (1.05)	0.41 (0.76)

*F-statistics for one-way ANOVA significant at $p<0.05$

Table 3.0.38 contd. Analysis of variance (ANOVA and p values) between main exposure and potential confounders and lens opacity

Independent variables	**Dependent Variables : mean opacity score (standard deviation)**			
Present cooking fuel stove	Nuclear Opacity (NO)	Nuclear Color (NC)	Cortical Opacity (C)	Posterior sub-capsular Opacity (P)
Biomass fuel stove	1.63 (0.65) *	1.60 (0.72)*	0.92 (0.84)	0.16 (0.34)*
Kerosene fuel stove	2.06 (1.01)	2.27 (0.77)	0.80 (1.03)	0.96 (1.28)
Gaseous fuel stove	1.56 (0.77)	1.74 (0.78)	0.98 (0.85)	0.21 (0.40)
Heating fuel in the house				
Wood	1.58 (0.68) *	1.64 (0.69)*	0.94 (0.84)	0.21 (0.47)
No heating fuel used	1.65 (0.83)	1.79 (0.86)	0.95 (0.88)	0.23 (0.44)
Ventilation in the kitchen				
Fully ventilated kitchen	1.62 (0.71)	1.74 (0.72)*	0.93 (0.87)	0.21 (0.40)
Unventilated kitchen	1.57 (0.85)	1.56 (0.89)	0.99 (0.81)	0.26 (0.62)
Source of light				
Electricity & solar lamp	1.62 (0.74)*	1.72 (0.76)*	0.95 (0.86)	0.22 (0.46)
Kerosene lamp	1.08 (0.52)	0.61 (0.15)	0.86 (0.66)	0.18 (0.24)
Work outside in the sun >1 hour daily				
Yes	1.64 (0.70)	1.78 (0.84)	0.91 (0.83)	0.22 (0.49)
No	1.55 (0.82)	1.66 (0.72)	1.00 (0.89)	0.21 (0.40)
Nutritional status				
Vegetarian	2.02 (0.63) *	2.16 (0.52)*	0.93 (0.64)	0.34 (0.54)
Non-vegetarian	1.57 (0.74)	1.67 (0.77)	0.95 (0.87)	0.21 (0.45)
Alcohol consumption				
Never	1.61 (0.75)	1.69 (0.77)	0.93 (0.85)*	0.21 (0.46)
Ever	1.64 (0.60)	1.90 (0.65)	1.30 (0.86)	0.33 (0.47)
Burn mosquito coil indoors				
Yes	1.55 (0.73)	1.50 (0.78)*	0.74 (0.68)*	0.21 (0.51)
No	1.64 (0.75)	1.83 (0.73)	1.06 (0.92)	0.23 (0.43)

*F-statistics for one-way ANOVA significant at $p<0.05$

Table 3.0.38 contd. Analysis of variance (ANOVA and p values) between main exposure and potential confounders and lens opacity

Independent variables	**Dependent Variables : mean opacity score (standard deviation)**			
Exposure variables	Nuclear Opacity (NO)	Nuclear Color (NC)	Cortical Opacity (C)	Posterior sub-capsular Opacity (P)
Burn incense indoors				
Yes	1.58 (0.72)*	1.68 (0.75)*	0.91 (0.82)*	0.20 (0.43)*
No	1.89 (0.90)	1.90 (0.89)	1.27 (1.06)	0.45 (0.63)
Socio-economic variables				
Level of literacy				
Can read and write	1.39 (0.63) *	1.46 (0.68)*	0.78 (0.70)*	0.18 (0.35)*
Cannot read and write	1.84 (0.78)	1.93 (0.77)	1.12 (0.95)	0.30 (0.54)
Present occupation				
Farming on own land & Agriculture labor	1.69 (0.64) *	1.75 (0.71)*	0.97 (0.83)	0.20 (0.40)*
Non agriculture labor	1.48 (1.05)	1.23 (0.98)	0.36 (0.48)	0.51 (1.14)
Government services, commerce and business and teacher and student	1.25 (0.72)	1.42 (0.80)	1.04 (0.85)	0.19 (0.32)
Housewife	1.69 (0.75)	1.82 (0.73)	0.96 (0.89)	0.21 (0.40)
Family annual income in NRs				
Rs. <=25,000	1.73 (0.89)	1.79 (0.81)	1.09 (0.93)	0.19 (0.36)
Rs. 25,000-50,000	1.61 (0.68)	1.66 (0.79)	0.88 (0.83)	0.25 (0.54)
Rs.50,000-100,000	1.53 (0.68)	1.66 (0.67)	0.88 (0.73)	0.16 (0.35)
Rs. >100,000	1.52 (0.81)	1.80 (0.85)	1.01 (0.97)	0.32 (0.53)
Refused to answer	1.83 (0.50)	1.62 (0.56)	0.97 (0.88)	0.18 (0.39)
Land ownership				
Yes	1.56 (0.72) *	1.68 (0.74)	0.98 (0.84)	0.17 (0.34)*
No	1.74 (0.78)	1.77 (0.81)	0.87 (0.89)	0.33 (0.65)

*F-statistics for one-way ANOVA significant at $p<0.05$

3.3.21.4 Multivariate least square and ordered logistic regression

By including all 4 opacity scores- four of each of the lens opacity grades for each subject and using known confounders of cataracts (exposure to heating fuel smoke, tobacco smoking, dietary practice, alcohol consumption, exposure to sunlight, area of residency and literacy) and other covariates that affected the mean opacity scores for particular type of opacity in ANOVA test (table 3.0.38), a multivariate regression models (ordinary least square and ordered logistic regression) were constructed with the cluster option. In addition, the covariate-land ownership was included in all models as it is a proxy of socio-economic status and exposure to sun light but the covariate-main source of light was not included in the model as only two participants reported they burned kerosene wick lamp. Similarly years of use of biomass, and kerosene fuel stove from the age of active cooking till she stopped cooking (if participant had stopped cooking during the time of interview) were categorized into three 20-years bands and investigated the exposure response relationships. The exposure response relationship models were adjusted by all covariates adjusted in the main models. Results of least square regression, ordered logistic regression and exposure response relationship are presented in tables 3.0.39 – 3.0.46 by opacity types.

3.3.21.4.1 Regression models for nuclear opacity (NO) in women in Pokhara, Nepal

Table 3.0.39 Multivariate regression results for fuel use in relation to nuclear opacity (NO) in continuous and ordered scale in women in Pokhara, Nepal

Variable	**Adjusted linear regression with robust SE (95% CI)**	**Adjusted OR with ordered logistic regression and robust SE (95% CI)**
Present cooking fuel		
Gas	-	-
Biomass	0.164 (0.000 ;0.329)	1.759 (1.01;3.067)
Kerosene	0.138 (-0.615;0.891)	1.069 (0.103;11.064)
Heating fuel		
No heating fuel used or electricity	-	1.00
Biomass	0.005 (-0.015;0.024)	0.994 (0.931;1.061)
Pack-years of smoking		
0 pack-years	-	1.00
0 - ≤ 9 pack-years	-0.120 (-0.356;0.116)	0.842 (0.394;1.800)
>9 pack-years	0.032 (-0.296;0.360)	0.849 (0.321;2.248)
Burn incense indoors		
No	-	1.00
Yes	-0.001 (-0.401;0.398)	1.379 (0.335;5.666)
Literacy		
Can read and write	-	1.00
Cannot read and write	0.014 (-0.166;0.194)	1.178 (0.642;2.158)
Residence locality		
Urban	-	1.00
Rural	-0.218 (-0.440;0.003)	0.362 (0.158;0.825)

Table 3.0.39 contd. Multivariate regression results for fuel use in relation to nuclear opacity (NO) in continuous and ordered scale in women in Pokhara, Nepal

Variable	Adjusted linear regression with robust SE (95% CI)	Adjusted OR with ordered logistic regression and robust SE (95% CI)
Dietary practice		
Non vegetarian	-	1.00
Vegetarian	0.006 (-0.303;0.315)	0.962 (0.379;2.440)
Year- hours working in the sun	0.001 (-001;0.002)	1.002 (0.999;1.006)
Alcohol		
No	-	1.00
Yes	-0.390 (-0.812;0.032)	0.304 (0.053;1.732)
Main occupation		
Government service, teaching and student	-	1.00
Work in own farm and agriculture labor	0.105 (-0.117;0.324)	1.131 (0.544;2.351)
Non agriculture labor	0.212 (-0.247;0.671)	1.420 (0.332;6.079)
Housewife	0.022 (-0.203;0.247)	0.814 (0.369;1.798)
Land ownership		
Yes	-	1.00
No	0.220 (0.022;0.419)	2.159 (1.107;4.211)
Age in years	0.039 (0.030;0.048)	1.141 (1.106;1.177)

Table 3.0.40 Exposure response relationship based on duration of exposure to biomass and kerosene fuel stove and NO

Exposure to Biomass and kerosene fuel stove	OR (95% CI) for biomass fuel stove	OR (95% CI) for kerosene fuel stove
0 (never)	1.00	1.00
1-19 years	1.964 (0.755;5.107)	1.156 (0.417;3.207)
20-39 years	2.856 (0.924;8.833)	1.036 (0.206;5.218)
>40 years	5.894 (1.310;26.514)	-

Adjusted for Age, pack-years of tobacco smoking (ref: 0 pack-years); wood as a heating fuel (ref: no heating fuel used); burn incense everyday (ref: do not burn incense everyday) ; ever consumed alcohol (ref: never consumed alcohol); cannot read and write (ref: can read and write); rural residency (ref: urban residency); vegetarian (ref: non-vegetarian); hours of work outside in the sun everyday and years of working; main occupation (ref: government service, teaching and student); no land ownership (ref: own land).

3.3.21.4.2 Regression models for nuclear color (NC) in women in Pokhara, Nepal

Table 3.0.41 Multivariate regression results for fuel use in relation to nuclear color (NC) in continuous and ordered scale in women in Pokhara, Nepal

Variable	**Adjusted linear regression with robust SE (95% CI)**	**Adjusted OR with ordered logistic regression and robust SE (95% CI)**
Present cooking fuel		
Gas	-	1.00
Biomass	0.050 (-0.084;0.184)	1.040 (0.592;1.828)
Kerosene	0.319 (-0.082;0.719)	3.727 (0.614;22.619)
Heating fuel		
No heating fuel used or electricity	-	1.00
Biomass	0.005 (-0.008;0.018)	1.008 (0.952;1.068)
Pack-years of smoking		
0 pack-years	-	1.00
0 - ≤ 9 pack-years	-0.049 (-0.227;0.129)	0.961 (0.480;1.927)
>9 pack-years	0.105 (-0.145;0.355)	1.098 (0.397;3.039)
Burn incense indoors		
No	-	1.00
Yes	0.194 (0.121;0.510)	1.725 (0.462;6.444)
Burn mosquito coil indoors		
No	-	1.00
Yes	-0.086 (-0.229;0.058)	0.601 (0.329;1.099)
Ventilation		
Fully ventilated kitchen	-	1.00
Unventilated kitchen	-0.097 (-0.279;0.085)	0.790 (0.364;1.714)
Literacy		
Can read and write	-	1.00
Cannot read and write	-0.018 (-0.147;0.112)	1.076 (0.615;1.879)
Residence locality		
Urban	-	1.00
Rural	-0.236 (-0.435;0.037)	0.371 (0.158;0.870)
Dietary practice		
Non vegetarian	-	1.00
Vegetarian	-0.089 (-0.315;0.137)	1.267 (0.444;3.614)
Per year- hours working in the sun	-0.000 (-0.001;0.001)	0.999 (0.994;1.005)
Alcohol		
No	-	1.00
Yes	-0.240 (-0.634;0.153)	0.492 (0.086;2.791)

Table 3.0.41 contd. Multivariate regression results for fuel use in relation to nuclear color (NC) in continuous and ordered scale in women in Pokhara, Nepal

Variable	Adjusted linear regression with robust SE (95% CI)	Adjusted OR with ordered logistic regression and robust SE (95% CI)
Main occupation		
Government service, teaching and student	-	1.00
Work in own farm and agriculture labor	-0.075 (-0.292;0.142)	0.778 (0.324;1.864)
Non agriculture labor	-0.025 (-0.343;0.292)	0.890 (0.214;3.783)
Housewife	-0.144 (-0.347;0.059)	0.523 (0.227;1.205)
Land ownership		
Yes	-	1.00
No	0.096(-0.074;0.267)	1.347 (0.668;2.713)
Age in years	0.053 (0.045;0.060)	1.201 (1.164;1.240)

Table 3.0.42 Exposure response relationship based on duration of exposure to biomass and kerosene fuel stove and NC

Exposure to Biomass and kerosene fuel stove	OR (95% CI) for biomass fuel stove	OR (95% CI) for kerosene fuel stove
0 (never)	1.00	1.00
1-19 years	1.439 (0.546;3.797)	1.336 (0.533;3.353)
20-39 years	1.366 (0.455;4.105)	2.312 (0.570;9.376)
>40 years	2.041 (0.3812;10.903)	-

* Adjusted for Age, pack-years of tobacco smoking (ref: 0 pack-years); wood as a heating fuel (ref: no heating fuel used); burn incense everyday (ref: do not burn incense everyday) ; unventilated kitchen (ref: fully ventilated kitchen); burn mosquito coil (ref: do not burn mosquito coil); ever consumed alcohol (ref: never consumed alcohol); cannot read and write (ref: can read and write) rural residency (ref: urban residency); vegetarian (ref: non-vegetarian); hours of work outside in the sun everyday and years of working; main occupation (ref: government service, teaching and student); no land ownership (ref: own land).

3.3.21.4.3 Regression model for cortical opacity in women in Pokhara, Nepal

Table 3.0.43 Multivariate regression results for fuel use in relation to cortical opacity (C) in continuous and ordered scale in women in Pokhara, Nepal

Variable	**Adjusted linear regression with robust SE (95% CI)**	**Adjusted OR with ordered logistic regression and robust SE (95% CI)**
Present cooking fuel		
Gas	-	1.00
Biomass	-0.103 (-0.409;0.203)	0.776 (0.366;1.647)
Kerosene	-0.522 (-1.104;0.060)	0.339 (0.088;1.306)
Heating fuel		
No heating fuel used or electricity	-	1.00
Biomass	0.007 (-0.016;0.029)	1.006 (0.953;1.062)
Pack-years of smoking		
0 pack-years	-	1.00
0 - ≤9 pack-years	0.181 (-0.176;0.538)	1.513 (0.677;3.383)
>9 pack-years	0.132 (-0.341;0.606)	1.332 (0.434;4.087)
Burn incense indoors		
No	-	1.00
Yes	-0.302(-0.746;0.142)	0.549 (0.202;1.496)
Burn mosquito coil indoors		
No	-	1.00
Yes	-0.144 (-0.382;0.095)	0.848 (0.477;1.506)
Alcohol		
No	-	1.00
Yes	0.047 (-0.474;0.569)	0.944 (0.270;3.296)
Literacy		
Can read and write	-	1.00
Cannot read and write	-0.018 (-0.267;0.230)	0.970 (0.538;1.748)
Dietary practice		
Non vegetarian	-	1.00
Vegetarian	-0.237 (-0.654;0.179)	0.644 (0.233;1.780)
Per year- hours working in the sun	0.002 (-0.000;0.004)	1.004 (1.00;1.010)
Land ownership		
Yes	-	1.00
No	-0.028 (-0.289;0.233)	0.869 (0.469;1.610)
Age in years	0.023 (0.008;0.037)	1.044

Table 3.0.44 Exposure response relationship based on duration of exposure to biomass and kerosene fuel stove and cortical opacity

Exposure to Biomass and kerosene fuel stove	**OR (95% CI) for biomass fuel stove**	**OR (95% CI) for kerosene fuel stove**
0 (never)	1.00	1.00
1-19 years	1.139 (0.468;2.773)	0.536 (0.249;1.152)
20-39 years	0.960 (0.351;2.629)	0.921 (0.214;3.968)
>40 years	1.118 (0.239;5.208)	-

*Adjusted for Age, pack-years of tobacco smoking (ref: 0 pack-years); wood as a heating fuel (ref: no heating fuel used); burn incense everyday (ref: do not burn incense everyday) ; ever consumed alcohol (ref: never consumed alcohol); cannot read and write (ref: can read and write) rural residency (ref: urban residency); vegetarian (ref: non-vegetarian); hours of work outside in the sun everyday and years of working; no land ownership (ref: own land).

3.3.21.4.4 Regression model for posterior sub-capsular opacity (P) in women in Pokhara, Nepal

Table 3.0.45 Multivariate regression results for fuel use in relation to posterior sub-capsular opacity (P) in continuous and ordered scale in women in Pokhara, Nepal

Variable	**Adjusted linear regression with robust SE (95% CI)**	**Adjusted OR with ordered logistic regression and robust SE (95% CI)**
Present cooking fuel		
Gas	-	1.00
Biomass	-0.070 (-0.163;0.022)	0.781 (0.497;1.228)
Kerosene	0.549 (-0.398;1.496)	3.194 (0.254;40.216)
Heating fuel		
No heating fuel used or electricity	-	1.00
Biomass	-0.008 (-0.026;0.010)	0.980 (0.924;1.039)
Pack-years of smoking		
0 pack-years	-	1.00
0 - ≤ 9 pack-years	0.015 (-0.138;0.168)	0.641 (0.353;1.164)
>9 pack-years	0.105 (-0.141;0.351)	0.818 (0.361;1.858)
Burn incense indoors		
No	-	1.00
Yes	-0.058(-0.377;0.262)	0.470 (0.185;1.196)
Alcohol		
No	-	1.00
Yes	-0.126 (-0.399;0.147)	0.882 (0.288;2.698)
Literacy		
Can read and write	-	1.00
Cannot read and write	-0.038 (-0.177;0.101)	1.050 (0.628;1.758)

Table 3.0.45 contd. Multivariate regression results for fuel use in relation to posterior sub-capsular opacity (P) in continuous and ordered scale in women in Pokhara, Nepal

Variable	Adjusted linear regression with robust SE (95% CI)	Adjusted OR with ordered logistic regression and robust SE (95% CI)
Locality		
Urban	-	1.00
Rural	-0.069 (-0.175;0.037)	0.722 (0.368;1.417)
Dietary practice		
Non vegetarian	-	1.00
Vegetarian	0.113 (-0120;0.345)	1.060 (0.408;2.754)
Per year- hours working in the sun	0.000 (-0.001;0.001)	1.002 (1.00;1.010)
Land ownership		
Yes	-	1.00
No	0.091 (-0.033;0.216)	1.429 (0.880;2.321)
Main occupation		
Government service, teaching and student	-	1.00
Work in own farm and agriculture labor	-0.043 (-0.185;0.099)	0.825 (0.419;1.622)
Non agriculture labor	0.277 (-0.273;0.827)	0.838 (0.135;5.199)
Housewife	-0.079 (-0.195;0.036)	0.658 (0.372;1.164)
Age in years	0.009 (0.003;0.014)	1.039 (1.018;1.062)

Table 3.0.46 Exposure response relationship based on duration of exposure to biomass and kerosene fuel stove and posterior sub-capsular opacity

Exposure to Biomass and kerosene fuel stove	OR (95% CI) for biomass fuel stove	OR (95% CI) for kerosene fuel stove
0 (never)	1.00	1.00
1-19 years	2.813 (1.286;6.153)	2.426 (0.968;6.078)
20-39 years	2.260 (0.864;5.591)	0.930 (0.373;2.323)
>40 years	2.116 (0.594;7.536)	-

*Adjusted for Age, pack-years of tobacco smoking (ref: 0 pack-years); wood as a heating fuel (ref: no heating fuel used),;burn incense everyday (ref: do not burn incense everyday) ; ever consumed alcohol (ref: never consumed alcohol); cannot read and write (ref: can read and write) rural residency (ref: urban residency); vegetarian (ref: non-vegetarian); hours of work outside in the sun everyday and years of working; main occupation (ref: government service, teaching and student); no land ownership (ref: own land).

3.4. Discussion

The hypothesis of this study was that smokes from biomass fuel used in indoor stoves without flues increases the severity of lens opacity in women compared with cooking using gaseous fuels. And the main aim of this study was to identify cataracts or opacities at the pre-clinical stage in women that is mostly associated with the use of biomass fuel for cooking in the households. Lens opacity scores were graded with LOCS III classification system. Two graders provided scores for the right and the left eyes. Thus, there were four scores for each person. The opacity scores ranged from 0 to 4.2. The correlations of between scorer's scores for the same eye were better for the left than the right eye. The mean opacities for the right and left eyes were similar (student t test *p* value >0.05) for nuclear and cortical opacity but were different for nuclear color and posterior sub-capsular opacity. Except for nuclear color, the variances of opacity scores for nuclear, cortical and posterior sub-capsular markedly varied between two scorers. The minimum and maximum values that were outside the limits of agreement between scorers were 4.20% and 6.99% for right and the left eyes. Considering the difference in the variance of scores and some observers bias between scorers, all scores were combined for the statistical analysis. As between observations of opacity scores on the same individuals were highly correlated, the intra subject correlation was adjusted by putting a cluster option in the statistical estimate and in all risk models. The cluster option gave the robust standard error and unbiased 95% confidence intervals of the mean scores. For each type of opacity, two separate regression analyses- ordinary least square regression with untransformed but continuous scores, and ordered logistic regression by grouping opacity scores into five bins (ordinal categories) as 0 '0', 1 '0-<=0.9', 2 '>0.9- <=1.9', 3 '>1.9 - <=2.9' & 4 '>2.9' were constructed. Using continuous and ordered scale scores, the effects of exposure to cooking and heating fuel on the lens opacity were examined over the study participants. The opacity scores in five bins were also used to estimate the prevalence of cataracts in the study population. For example, participants were characterized as having age-related cataracts if any type of opacity had scores equal to or greater than 2. The grade 2 cutoff of opacity as cataracts has been adopted by some major population based cataract studies[65, 66].

Nuclear cataracts were the most prevalent type followed by cortical and posterior-sub-capsular. The overall prevalence of scores of 2.0 or greater for nuclear cataracts, nuclear color, cortical and posterior sub-capsular cataracts was 26.4%, 37.24%, 12.42% and 1.47%, respectively. The prevalence of cataracts monotonically increased by age (p=0.00). About 28% of participants who had biomass fuel stove had nuclear cataracts, whereas 40% of participants who had kerosene fuel stove had nuclear cataracts and 24% who had gas fuel stove had nuclear cataracts. The difference of mean nuclear opacity by fuel stove category was statistically significant.

In the ordinary regression analysis; after adjustment for age, pack-years of tobacco smoking, use of wood as a heating fuel, practice of incense burning indoors, literacy, area of residency, hours of work every day and years of work outside in the sun, alcohol consumption, dietary practice, main occupation and land ownership; the current use of biomass fuel increased the mean nuclear opacity by 0.164 (95% CI: 0.000; 0.329) compared with gas fuel stove. In the case of nuclear color of the lens, compared with the current use of gas fuel stove, the current use of biomass fuel stove increased the mean nuclear color by 0.050 (95% CI: -0.084 ; 0.184). For cortical opacity,

compared with the current use of gas fuel stove, the current use of biomass fuel decreased the mean cortical opacity by -0.103 (95% CI: -0.409 ; 0.203) and for posterior sub-capsular opacity, compared with the current use of gas fuel stove, the current use of biomass fuel decreased the mean opacity by -0.070 (95% CI: -0.163 ; 0.022).

In the case of current use of kerosene fuel stove, compared with the use of gas fuel stove, the mean nuclear opacity increased by 0.138 (95% CI: -0.615 ; 0.891). Whereas the current use of kerosene fuel stove increased the mean nuclear color by 0.319 (95% CI: -0.082 ; 0.719). For cortical opacity, compared with the current use of gas fuel stove, the current use of kerosene fuel stove decreased the mean cortical opacity by -0.522 (95% CI: -1.104 ; 0.060) and for posterior sub-capsular opacity the current use of kerosene fuel stove increased the mean opacity by 0.549 (95% CI: -0.398 ; 1.496).

Ordered logistic regression analysis of nuclear opacity grades after adjustment of potential confounders showed evidence of use of biomass fuel compared with gas fuel increasing the odds of nuclear opacity by 1.759 (95% CI: 1.010 ; 3.067). For nuclear color, cortical and posterior sub-capsular opacity, the odds ratios were 1.040 (95% CI: 0.592 ; 1.828), 0.776 (95% CI: 0.366 ; 1.647) and 0.781 (95% CI: 0.497 ; 1.228), respectively.

Similarly the odds of nuclear opacity from the current use of kerosene fuel stove compared with the gas fuel stove were 1.069 (95% CI: 0.103; 11.064). For nuclear color, cortical and posterior sub-capsular opacity the odds ratios were 3.727 (95% CI: 0.614 ; 22.619), 0.339 (95% CI: 0.088 ; 1.306) and 3.194 (95% CI: 0.254 ; 40.216), respectively.

The results of ordinary least square regression and ordered logistic regression above suggest indoor exposure to smoke from biomass fuel combustion increases the severity of nuclear opacity in women. Apart from use of biomass fuel stove, age and not having a land also showed a positive association with nuclear opacity. The risks of nuclear opacity increased from use of kerosene fuel stove but it was not statistically significant. No significant association was found between use of biomass fuel or kerosene and change in nuclear color and cortical and posterior sub-capsular opacity.

However, before concluding that the linkage is causal, it is important to consider alternative explanations, particularly the possibility that the study results might be a result of selection bias, information bias, or confounding in the study design or analysis. As with all epidemiological studies, the selection bias in recruitment of participants' is a potential concern. In this study, the possibility of recruiting only cooperative participants were avoided by recruiting only first five patients presented at the outpatient department (OPD) between 0009-1000 am, throughout the study period. There were no refusals to participate by selected participants. In comparison to my previous cataract study with case-control design, the cross-sectional design of the present study provided an internal control for selection bias as it did not exclude participants with refractive errors. In my previous cataract study, patients with refractive error were used as the control. Although there do not exist any studies showing an association between use of solid fuel for cooking and refractive error, there exist (inconclusive) evidence of the association of refractive error with education (indicator of higher socio-economic status) [52-57] [58-60]. Thus there was a possibility that people with refractive error may have different life styles or exposure pattern

and low odds of exposure to solid fuel smoke than cases[73]. In the present study, the level of literacy was adjusted in all models. In addition, essentially the 86% of participants in the study were from the urban area and for majority of participants', the study center is a primary referral for eye care. Therefore, I assume that selection bias in this study is unlikely.

Information bias may take the form of outcome misclassification or exposure misclassification. Since lens opacities were confirmed by evaluating the photographs by two scorers and in the case of nuclear opacity only about 4% of scores were outside the limits of agreement, thus, I consider that disease misclassification is unlikely. Exposure data were all obtained by questionnaire and graders were not aware of the participant's exposure status. Participants in this study were not aware of their lens opacity status. Thus, it is less likely that some participants may have remembered or over reported present and past exposure more than the others. Also in this study the questions asked were about common exposures, which all participants come across on a day-to-day basis. I expect that any such differential recall bias would thus be minimal. In particular, all of the participants know very well the types of stoves and fuel they have used, and there is no prevailing belief that indoor smoke exposure from unimproved stoves is harmful to eye. However, there is likely to be some degree of non-differential exposure misclassification. This is likely to affect some variables more than others. Ventilation status, for example, may be more substantively misclassified than, say, use of incense, because there is more variation in ventilation arrangements and it is hard to encapsulate these in a series of simple questions. However, I had verified the high level of accuracy of reporting of two key exposure variables, stove type and ventilation in the kitchen from validity study conducted in the homes of 28 study participants of another study conducted at the same time and in the same area [74]. Considering this and that there is no prevailing belief that indoor smoke exposure increases the risk of cataracts, I believe exposure misclassification is likely to be minimal. One possible limitation, however, is that I only asked about the main cooking fuel used. This might have led to some misclassification of exposure status as people may also use secondary fuel.

The third main area of potential bias is confounding. In one of the large case-control study, which suggested that indoor cooking smoke may be associated with cataract [32], authors reported that it was likely that the association found between solid fuel use and cataract formation was confounded by other factors related to socio-economic status. The present study collected a comprehensive range of data on potential confounding factors; particularly those associated with socio- economic status. In the univariate analysis (ANOVA), the mean opacity scores did not vary by annual family income, however, the mean opacity score varied by level of education and land ownership. I adjusted these two socio-economic variables in the models. Adjustment with these variables did not eliminate the key associations found with the nuclear opacity. Although I cannot totally rule out the possibility of an unknown confounding factor causing the associations found, it seems unlikely.

After eliminating the likelihood of bias being responsible for a potentially causal association, there are several other considerations that may be considered in inferring likely causality (Hill 1965). These include consistency with the findings of other studies, the existence of an exposure-response relationship, and biological plausibility. There have been six other studies that have suggested an association between exposure to biomass fuel stove and cataract or blindness [32, 38-41, 50]. I am not aware of any studies that have investigated the relationship

between cataract and indoor smoke exposure and found no evidence of an association. However, except study of Mohan et al [32], other studies on indoor air pollution and cataracts does not provide separate exposure odds ratio for the three main anatomical types of cataracts: nuclear, cortical and posterior sub-capsular. In the present study, exposure odds ratios were evaluated for three anatomical types and change in nuclear color by the use of biomass and kerosene fuel stove. Among different types of cataracts, the exposure to biomass fuel stove was mainly found to be associated with the nuclear opacity/cataract. This finding is consistent with the findings of Mohan et al [32], where use of cow dung and biomass-based fuels was found to be associated with increased risk of nuclear, cortical and mixed cataract, but not the posterior sub-capsular cataracts. In the present study the association of cortical opacity with use of biomass fuel was not found. Providing additional confirmation of the relationship between use of biomass fuel stove and nuclear opacity was a statistically significant exposure-response trend based on years of exposed to biomass fuel stove increasing the risk of nuclear opacity. None of the earlier studies had investigated such an exposure-response relationship by types of opacity.

A causal relationship between exposure to indoor smoke and cataract is biologically plausible. There is evidence that smoke can induce oxidative stress and deplete plasma ascorbate, carotenoids and glutathione, which provide antioxidant protection against cataract formation [43, 48, 75]. Tobacco smoke and bio-fuel smoke have many similarities[42, 43]. Several studies have indicated that tobacco smoking and fuel smoke condensate enhance the formation of super-oxide radicals, which decrease the formation of antioxidants and increase the risk of cataract [43, 76-79]. Studies have shown a possible association of cataract with exposure to naphthalene[80-82] and formaldehyde. Biomass fuel combustion emits naphthalene [44, 46, 83, 84] and formaldehyde [42, 45, 85]. In the indoor air pollution study conducted in the 28 households in study area (chapter I) , the naphthalene concentrations were found higher from biomass fuel and kerosene fuel stoves compared with gaseous fuel stove. However, assuming that the association found in this study reflects a true causal relationship, it is still unclear which route of exposure, inhalation or direct eye contact, leads to the pathogenic process of cataract formation.

In conclusion, this study confirms that use of biomass fuel stove is associated with an increased risk of nuclear opacity. Bias including potential confounding, is not likely to explain these associations, which are biologically plausible and consistent with the results of other epidemiological studies. However, a much larger population-based cross-sectional study is needed to confirm this finding. Irrespective of the evidence for associations between biomass-fueled stoves and nuclear opacity, it is clear that biomass fuel stove without flues produce substantial indoor air pollution with naphthalene and fine particulate matter, known to cause harm to health including eyes. Therefore, replacement of these stoves with cleaner alternatives is justified. One, at least partially effective, remedial measure would be to replace unflued stoves with chimney stoves, which vent cooking smoke directly to the exterior of the house. However, these improved stoves require continuing maintenance if they are to be useful in improving indoor air quality. Ideally, electric stoves or low-emissions biomass stoves, such as semi-gasifier stoves, or those with cleaner burning fuels (biogas or LPG) would be used. The public health benefits of earlier diagnosis of problem of refractive error and lens opacity, and widespread stove improvement, particularly addition of flues and promotion of semi-gasifier or gaseous stove, could be immense.

3.5 References

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3.6 Appendix Prevalence of lens opacity by exposure categories

Table B.0.1 Prevalence of lens opacity by main heating fuel and ventilation in the kitchen

Scores	Heating fuel used in the house			Ventilation status in the kitchen		
	Wood	No heating fuel used	χ^2 (p value)	Fully ventilated	Unventilated	χ^2 (p value)
Nuclear Opacity						
0	3 (0.87%)	2 (0.88%)	3.17 (0.53)	2 (0.44%)	3 (2.50%)	13.94
>0-≤0.9	51 (14.83%)	38 (16.67%)		62 (13.72%)	27 (22.50%)	(0.01)
>0.9- ≤1.9	203 (59.01%)	124 (54.39%)		267 (59.07%)	60 (50.00%)	
>1.9 - ≤2.9	72 (20.93%)	47 (20.61%)		99 (21.90%)	20 (16.67%)	
>2.9	15 (4.36%)	17 (7.46%)		22 (4.87%)	10 (8.33%)	
Total	344	228		452	120	
Nuclear color						
0	2 (0.58%)	0 (0.00%)	12.36 (0.02)	1 (0.22%)	1 (1.01%)	12.05
>0-≤0.9	58 (16.86%)	42 (18.42%)		67 (14.82%)	33 (27.50%)	(0.02)
>0.9- ≤1.9	172 (50.00%)	85 (37.28%)		208 (46.02%)	49 (40.83%)	
>1.9 - ≤2.9	97 (28.20%)	83 (36.40%)		153 (33.85%)	27 (22.50%)	
>2.9	15 (4.36%)	18 (7.89%)		23 (5.09%)	10 (8.33%)	
Total	344	228		452	120	
Cortical						
0	53 (15.41%)	46 (20.18%)	3.31 (0.51)	83 (18.36%)	16 (13.33%)	5.72
>0-≤0.9	139 (40.41%)	82 (35.96%)		177 (39.16%)	44 (36.67%)	(0.22)
>0.9- ≤1.9	112 (32.56%)	69 (30.26%)		134 (29.65%)	47 (39.17%)	
>1.9 - ≤2.9	28 (8.14%)	23 (10.09%)		40 (8.85%)	11 (9.17%)	
>2.9	12 (3.49%)	8 (3.51%)		18 (3.98%)	2 (1.67%)	
Total	344	228		452	120	

Table B.0.1 contd. Prevalence of lens opacity by main heating fuel and ventilation in the kitchen

Scores	Heating fuel used in the house			Ventilation status in the kitchen		
	Wood	No heating fuel used	χ^2 (p value)	Fully ventilated	Unventilated	χ^2 (p value)
Posterior sub-capsular						
0	174 (50.58%)	117 (51.32%)	5.03 (0.29)	229 (50.66%)	62 (51.67%)	11.95 (0.02)
>0-≤0.9	152 (44.19%)	94 (41.23%)		196 (43.36%)	50 (41.67%)	
>0.9- ≤1.9	11 (3.20%)	14 (6.14%)		21 (4.65%)	4 (3.33%)	
>1.9 - ≤2.9	4 (1.16%)	3 (1.32%)		6 (1.33%)	1 (0.83%)	
>2.9	3 (0.87%)	0 (0.00%)		0 (0.00%)	3 (2.50%)	
Total	344	228		452	120	

Table B.0.2 Prevalence of lens opacity by main source of light in the house

Scores	**Main source of light in the house**		**χ^2 (p value)**
	Electricity	Kerosene lamp	
Nuclear Opacity			
0	5 (0.89%)	0 (0.00%)	22.05 (0.00)
>0-≤0.9	83 (14.72%)	6 (75.00%)	
>0.9- ≤1.9	326 (57.80%)	1 (12.50%)	
>1.9 - ≤2.9	118 (20.92%)	1 (12.50%)	
>2.9	32 (5.67%)	0 (0.00%)	
Total	564	8	
Nuclear color			
0	2 (0.41%)	0 (0.00%)	38.30 (0.00)
>0-≤0.9	92 (16.31%)	8 (100.00%)	
>0.9- ≤1.9	257 (45.57%)	0 (0.00%)	
>1.9 - ≤2.9	180 (31.91%)	0 (0.00%)	
>2.9	33 (5.85%)	0 (0.00%)	
Total	564	8	
Cortical			
0	97 (17.20%)	2 (25.00%)	1.38 (0.85)
>0-≤0.9	218 (38.65%)	3 (37.50%)	
>0.9- ≤1.9	178 (31.56%)	3 (37.50%)	
>1.9 - ≤2.9	51 (9.04%)	0 (0.00%)	
>2.9	20 (3.55%)	0 (0.00%)	
Total	564	8	
Posterior sub-capsular			
0	287 (50.89%)	4 (50.00%)	0.59 (0.96)
>0-≤0.9	242 (42.91%)	4 (50.00%)	
>0.9- ≤1.9	25 (4.43%)	0 (0.00%)	
>1.9 - ≤2.9	7 (1.24%)	0 (0.00%)	
>2.9	3 (0.53%)	0 (0.00%)	
Total	564	8	

Table B.0.3 Prevalence of lens opacity by incense and mosquito burning indoors

Scores	**Burn incense indoors**			**Burn mosquito coil indoors**		
	Yes	No	χ^2 (p value)	Yes	No	χ^2 (p value)
Nuclear Opacity						
0	5 (0.96%)	0 (0.00)	17.24 (0.00)	2 (0.94)	3 (0.83)	7.50
>0-≤0.9	79 (15.19%)	10 (19.23%)		32 (15.09%)	57 (15.83)	(0.11)
>0.9- ≤1.9	305 (58.65%)	22 (42.21%)		134 (63.21)	193 (53.61)	
>1.9 - ≤2.9	108 (20.77%)	11 (21.15%)		32 (15.09)	87 (24.17)	
>2.9	23 (4.42%)	9 (17.31%)		12 (5.66)	20 (5.56)	
Total	520	52		212	360	
Nuclear color						
0	2 (0.38%)	0 (0.00)	8.03 (0.09)	1 (0.47%)	1 (0.281%)	34.02
>0-≤0.9	92 (17.69%)	8 (15.38%)		53 (25.00%)	47 (13.06%)	(0.00)
>0.9- ≤1.9	239 (45.96%)	18 (34.62%)		109 (51.42%)	148 (41.11%)	
>1.9 - ≤2.9	161 (30.96%)	19 (36.54%)		38 (17.92%)	142 (39.44%)	
>2.9	26 (5.00%)	7 (13.46%)		11 (5.19%)	22 (6.11%)	
Total	520	52		212	360	
Cortical						
0	90 (17.31%)	9 (17.31%)	10.36 (0.04)	40 (18.87%)	59 (16.39%)	19.12
>0-≤0.9	208 (40.00%)	13 (25.00%)		94 (44.34%)	127 (35.28%)	(0.00)
>0.9- ≤1.9	163 (31.35%)	18 (34.62%)		66 (31.13%)	115 (31.94%)	
>1.9 - ≤2.9	44 (8.46%)	7 (13.46%)		12 (5.66%)	39 (10.38%)	
>2.9	15 (2.88%)	5 (9.62%)		0 (0.00%)	20 (5.56%)	
Total	520	52		212	360	

Table B.0.3 contd. Prevalence of lens opacity by incense and mosquito burning indoors

Scores	**Burn incense indoors**			**Burn mosquito coil indoors**		
	Yes	No	χ^2 (p value)	Yes	No	χ^2 (p value)
Posterior sub-capsular						
0	277 (53.27%)	14 (26.92%)	25.30 (0.00)	114 (53.77%)	177 (49.17%)	7.21
>0-≤0.9	217 (41.73%)	29 (55.77%)		86 (40.57%)	160 (44.44%)	(0.13)
>0.9- ≤1.9	19 (3.65%)	6 (11.54%)		7 (3.30%)	18 (5.00%)	
>1.9 - ≤2.9	4 (0.77%)	3 (5.77%)		2 (0.94%)	5 (1.39%)	
>2.9	3 (0.58%)	0 (0.00%)		3 (1.42%)	0 (0.00%)	
Total	520	52		212	360	

Table B.0.4 Analysis of Variance (ANOVA and *p* values) between main exposure and potential confounders and lens opacity

Independent variables	**Dependent Variables : mean opacity score (standard deviation)**			
Exposure variables	Nuclear Opacity (NO)	Nuclear Color (NC)	Cortical Opacity (C)	Posterior sub-capsular Opacity (P)
Smoking filtered vs. non-filtered cigarette				
Do not smoke	1.50 (0.72)*	1.55 (0.71)*	0.81 (0.75)*	0.18 (0.35)*
Smoke unfiltered cigarette	1.68 (0.82)	1.69 (0.92)	1.30 (0.93)	0.47 (0.91)
Smoke filtered cigarette	1.64 (0.66)	1.93 (0.69)	1.16 (0.95)	0.20 (0.33)
Smoke both filtered and unfiltered cigarette	2.05 (0.72)	2.30 (0.60)	1.24 (1.02)	0.26 (0.50)
Number of family member smoke in the house				
No family member smoke	1.66 (0.77)*	1.84 (0.75)*	1.08 (0.88)*	0.24 (0.44)*
One family member smoke	1.44 (0.60)	1.35 (0.67)	0.54 (0.57)	0.10 (0.18)
>2 family members smoke	1.73 (0.94)	1.74 (0.82)	1.26 (0.98)	0.56 (1.05)

Table B.0.5 Analysis of Variance (ANOVA and *p* values) between main exposure and potential confounders and lens opacity

Independent variables	**Dependent Variables : mean opacity score (standard deviation)**			
Exposure variables	Nuclear Opacity (NO)	Nuclear Color (NC)	Cortical Opacity (C)	Posterior sub-capsular Opacity (P)
Frequency of meat & fish consumption				
Vegetarian	1.94 (0.66)*	2.14 (0.51)*	0.89 (0.64)	0.31 (0.53)*
Once per week	1.52 (0.81)	1.62 (0.83)	0.89 (0.79)	0.25 (0.54)
Once per month	1.65 (0.65)	1.72 (0.68)	1.03 (0.96)	0.16 (0.29)
Frequency of egg consumption				
Never	1.19 (0.50)*	1.38 (0.72)*	1.04 (0.53)*	0.22 (0.28)
Once per week	1.41 (0.71)	1.47 (0.80)	0.99 (0.93)	0.20 (0.38)
Once per month	1.60 (0.65)	1.81 (0.67)	1.20 (0.85)	0.18(0.36)
Daily	0.93 (0.44)	-	-	-
Rarely	1.78 (0.76)	1.84 (0.73)	0.85 (0.80)	0.24 (0.53)
Frequency of milk consumption				
Yes	1.58 (0.78)	1.64 (0.80)	0.93 (0.85)	0.20 (0.38)
No	1.63 (0.71)	1.75 (0.67)	0.83 (0.78)	0.25 (0.64)
Sometimes	1.67 (0.67)	1.80 (0.74)	1.07 (0.90)	0.23 (0.44)
Measures of UV protection (veil, sunglasses or hat used)				
No	1.62 (0.83)	0.20 (0.40)	0.95 (0.84)	0.22 (0.49)
Yes	1.63 (0.69)	0.24 (0.49)	0.99 (0.87)	0.21 (0.40)

CHAPTER 4

Tuberculosis and Indoor Biomass and Kerosene Use in Nepal: A Case-Control Study

Tuberculosis and indoor biomass and kerosene use in Nepal: A case-control study

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4.0 Chapter summary

Background: In Nepal, tuberculosis (TB) is a major problem. Worldwide, six previous epidemiologic studies have investigated whether indoor cooking with biomass fuel such as wood or agricultural wastes is associated with TB with inconsistent results.
Objectives: Using detailed information on potential confounders, we investigated the associations between TB and the use of biomass and kerosene fuels.
Methods: A hospital-based case-control study was conducted in Pokhara, Nepal. Cases (*n* = 125) were women, 20-65 years old, with a confirmed diagnosis of TB. Age-matched controls (*n* = 250) were female patients without TB. Detailed exposure histories were collected with a standardized questionnaire.
Results: Compared with using a clean-burning fuel stove (liquefied petroleum gas, biogas), the adjusted odds ratio (OR) for using a biomass-fuel stove was 1.21 [95% confidence interval (CI), 0.48-3.05], whereas use of a kerosene-fuel stove had an OR of 3.36 (95% CI, 1.01-11.22). The OR for use of biomass fuel for heating was 3.45 (95% CI, 1.44-8.27) and for use of kerosene lamps for lighting was 9.43 (95% CI, 1.45-61.32).
Conclusions: This study provides evidence that the use of indoor biomass fuel, particularly as a source of heating, is associated with TB in women. It also provides the first evidence that using kerosene stoves and wick lamps is associated with TB. These associations require confirmation in other studies. If using kerosene lamps is a risk factor for TB, it would provide strong justification for promoting clean lighting sources, such as solar lamps.
Key words: biomass fuel, cooking-fuel smoke, heating, indoor air pollution, kerosene lighting, kerosene stove, smoking, women. *Environ Health Perspect* 118:558-564 (2010). doi:10.1289/ ehp.0901032 [Online 17 December 2009]

4.1 Introduction

Tuberculosis (TB) is a major infectious disease that causes illness and death worldwide[1]. In 2006, there were about 9.2 million new TB cases and 1.7 million TB-related deaths[2]. Most new cases and deaths occurred in Asia and Africa. In Nepal, a South Asian country, TB is a major public health problem[3], with an overall annual incidence of all forms of TB estimated at 176 per 100,000 persons[4].

A range of social, environmental, and behavioral factors influence exposure and susceptibility to *Mycobacterium tuberculosis* infection. Identifying TB risk factors and minimizing exposure to them could reduce the TB burden in Nepal and other developing countries. Active tobacco smoking, for example, has been shown to be a risk factor for TB, presumably by damaging immune and other protective mechanisms, allowing TB infection to prosper [5-7]. The composition of tobacco smoke has many similarities to that of indoor cooking smoke from biomass fuel [8-10], exposure to which is common in the developing world, including Nepal. Therefore, an association of TB with indoor cooking smoke is plausible. Six previous epidemiologic studies have investigated whether an association exists between TB and exposure to cooking-fuel smoke [11-16]. Although four of these studies found some evidence of an association, all the studies had limitations. The first study to find an association between exposure to cooking-fuel smoke and TB presented limited data on potential confounding factors, and the risk model was adjusted only for age, which left open the possibility of confounding by socioeconomic factors or smoking [12]. Mishra et al. (1999)[14] also reported evidence of an association; however, they used data from the 1992-1993 Indian National Family Survey, which was based on self- reported TB status. This leaves the possibility of outcome misclassification. A third study found an association between cooking smoke exposure and TB but included no validation of key components of the questionnaire [15]. In a study conducted in Malawi, Crampin et al. (2004)[11] found no association between cooking smoke exposure and TB, but the study participants varied little in the type of fuel they used, and the risk model was adjusted only for age, sex, area of residence, and HIV status, leaving open the possibility of confounding by other socioeconomic factors or smoking. The fifth study, conducted in South India by Shetty et al. (2006) [16], also found no association of cooking-fuel smoke with TB, but they did find an association between TB and not having a separate kitchen. The sixth study was conducted by Kolappan and Subramani (2009) in Chennai, India; they found a marginal association between biomass fuel and pulmonary TB in their study population [adjusted OR = 1.7; 95% confidence interval (CI), 1.0-2.9]. The study participants in this study were primarily men (87%) but because women do most of the cooking, they are more likely to be exposed to smoke from cooking fuel [13].

We conducted a TB case-control study in the Pokhara municipality of Nepal where cooking with biomass fuels in unvented indoor stoves is a common practice. Our main objectives were to confirm results of earlier studies using clinically confirmed TB cases and to investigate possible confounding of the relationship using a validated questionnaire and exposure assessment in the kitchens of a subset of participants' houses.

4.2 Methods

Subjects' approvals were obtained from the institutional review boards at the University of California-Berkeley, and at the Nepal Health Research Council.

The study was conducted at the Regional Tuberculosis Center (RTC) and the Manipal Teaching Hospital (MTH), Manipal College of Medical Sciences, in Pokhara. The RTC and MTH are the two major health centers [directly observed treatment short-course (DOTS) clinics] that specialize in diagnosing TB and caring for people who live in Kaski (Pokhara) and seven adjoining hill districts: Syangja, Parbat, Tanahu, Lamjung, Myagdi, Baglung , and Gorkha, which

are in the midwestern development region of Nepal. All subjects were recruited and interviewed between July 2005 and April 2007. The climate of the region is temperate but can be cool at times. For example, in Pokhara city (latitude 28.2° N), which is 827 m above sea level (Central Bureau of Statistics 2009), the mean temperature and mean daily minimum temperatures in January 2006 (the coldest month of the year) were 14.3°C and 7.2°C, respectively (Department of Hydrology and Meteorology 2006/2007)[17]. Other, more elevated parts of the region can be colder.

4.2.1 Recruitment procedure for cases and controls

Cases were all female patients, 20-65 years old, who visited TB clinics in RTC (90.4%) and MTH (9.6%) and who had been newly diagnosed with active pulmonary TB by chest X-ray and positive active sputum smears (two sputum specimens positive for acid-fast bacilli by microscopy), which are routinely conducted at the hospital using methods recommended by the WHO[18]. Women who were pregnant, who were on chemotherapy for cancer, who had HIV/ AIDS or diabetes, or who had a history of TB were excluded from the study.

Controls were recruited from outpatient and inpatient departments (dental, 1.6%; ear, nose, and throat, 1.6%; ophthalmology, 25.6%; general medicine, 56%; obstetrics and gynecology, 7.2%; orthopedics, 2.4%; skin, 1.6%; surgery, 3.2%; and psychiatry, 0.8%) at the MTH, in the same months when cases were identified. For each case, the control subjects were the first eligible female patients without pulmonary TB, matched to cases on age (5-year frequency bands), who presented at MTH between 0900 and 1000 hours after case enrollment. Controls were excluded from the study for the same reasons as for the cases. Control subjects were interviewed only after medical screening confirmed that they did not have TB. Confirmation procedures included a chest X-ray and an on-the-spot sputum examination. The ratio of cases to controls was 1:2.

After obtaining an informed oral consent to participate, all cases and controls were interviewed face-to-face by trained interviewers shortly after diagnosis while they were still at the hospital. The three interviewers were unavoidably aware of the case or control status of the interviewees but were not aware of the main exposure of interest or hypothesis of the study. All interviewers interviewed both cases and controls.

The questionnaire collected data on education level, area of residence (urban, peri-urban, and rural), history of use of cooking fuels and stoves that included present and previous (including in parents' houses, before marriage) cooking fuels and stoves, present kitchen type and location, kitchen ventilation, house type, participant's smoking history and smoking status of family members, alcohol consumption, vitamin supplement consumption, use of mosquito coils and incense, household crowding, vehicle ownership, and annual income level.

4.2.2 Statistical analysis

Liquefied petroleum gas (LPG) and biogas were designated "gaseous- fuel stoves" (GFS), which was used as the reference category for most analyses compared with kerosene-fuel stoves (KFS) and biomass-fuel stoves (BFS). Very few participants (two cases and four controls) reported burning biomass in stoves with flues or chimneys venting to the outside, and no one reported using an

electric cooker. For this reason, no separate category was created for vented BFS, and these subjects were included in the BFS category.

We examined the extent of agreement of responses on the exposure information (current stove/fuel type and ventilation) obtained during face-to-face interviews at the hospital with data obtained from actual inspection of these features in the houses of the first 28 study participants (13 cases and 15 controls). The effect of misclassification was calculated in terms of sensitivity and specificity.

We combined information on kitchen location and windows in the kitchen to create a composite dichotomous variable for ventilation. "Fully and partially ventilated kitchens" included open-air kitchens, separate kitchens outside the house, and partitioned kitchens with windows inside the house. This was used as the reference category for ventilation. Unventilated kitchens included partitioned and non-partitioned kitchens without windows inside the house. We were unable to clearly interpret questionnaire data on closing doors in a way that could be used to characterize ventilation.

To calculate the number of pack-years of smoking, we combined the information on the average number of tobacco products (cigarettes or *bidis*) smoked every day multiplied by the duration of smoking in years divided by 20, assuming that a pack of cigarette contains twenty cigarettes/*bidis*. One participant who reported she smoked a *hukka* (water pipe) was excluded from this analysis.

We calculated crude odds ratios (ORs) between exposure and outcome. We decided a priori to include all statistically significant ($p \leq 0.05$) variables in the model, as well as any other recognized risk factors for TB. Then we applied a stepwise backward elimination model, with a variable selection criterion of $p = 0.2$, to all the variables to identify any others that should be included in the final model. Using the selected covariates, we constructed a multivariate unconditional logistic regression model for risk of TB. We calculated adjusted female population-attributable fractions and associated CIs using the aflogit command in Stata (version 10; Stata Corp LLC, College Station, TX, USA) statistical software. This procedure assumes that the proportion of controls exposed is a good estimate of the proportion exposed in the target population.

4.3 Results

Four potential interviewees (all cases) did not meet the inclusion criteria: two were diabetic and two were HIV positive. During recruitment, one potential control was found to have pulmonary TB and was transferred to the case group. Except for one control, all potential interviewees agreed to participate in this study. In total, we recruited and interviewed 125 cases and 250 controls. Cases were more likely to be referred by a health care professional (30.4%) than were controls (7.2%). This might reasonably be expected because TB causes serious illness, but many of the controls would have had much less severe conditions.

Table 4.0.1 lists descriptive data for the cases and controls, with unadjusted ORs and CIs. With the exception of the income variable, few data were missing. Confirming the success of the matching process, distributions of cases and controls were similar in terms of age. Most cases and

controls (72.0% of cases, 94.4% of controls) were from the Kaski district. Cases were more likely than controls to be Buddhist, to live in urban and peri-urban areas, to reside in poorer quality houses (*kuccha*), to be illiterate, to have non-partitioned and unventilated kitchens indoors, and to use kerosene wick lamps as their main source of light. Cases were also more likely than controls to regularly consume alcohol, to be tobacco smokers, to have more smokers in the family than controls, and to have not always lived in their present house. We think that, to some extent, the latter variable probably captures the likelihood of previously having used other cooking fuels. Except for three cases, none of the participants who had smoked reported that they had ever quit smoking for 6 months or more. Therefore, we classified smokers as ever-smokers and never-smokers. The median smoking experience for both cases and controls was 8 pack- years (SD = 13.37 pack-years). More cases than controls had had household members with TB. Moreover, cases were more likely to be using BFS or KFS than were controls ($p = 0.004$). The distribution of cooking fuel used by the study participants was biomass from wood or crop residues (44.3%), LPG (42.7%), kerosene (11.2%), and biogas (1.9%).

We created a heating fuel variable that treated participants who reported either using electricity (1 case, 3 controls) or using no heating fuel (38 cases, 137 controls) as the reference category, and the remaining subjects, who mainly used wood (84 cases, 107 controls), as the biomass fuel category. The biomass group included a few women who used coal (one control) and kerosene (one case, one control) for heating.

We verified stove-fuel types and ventilation characteristics in the houses of 28 participants. All 18 participants who had reported their main cookstove as being a biomass stove were found to be correct, as were the five reporting use of a LPG stove. One of the four participants who had reported using kerosene stove, however, was found to be using an LPG stove. On that basis, the accuracy (true reports ÷ total reports) of stove reporting was 96%. In the inspection of ventilation characteristics, one participant who had reported not having a window in her kitchen was found to have a temporary outside kitchen with a window- sized opening. Two participants who reported having a window in the kitchen actually did not have a window. Based on these data, the accuracy for reporting ventilation was 89%.

As shown in Table 4.0.1, the unadjusted exposure ORs for cooking in BFS and KFS were 1.98 (95% CI, 1.24-3.17) and 2.54 (95% CI, 1.26-5.12), respectively. Use of kerosene lamps had an unadjusted OR of 10.35 (95% CI, 3.42-31.3), and use of biomass fuel for heating had an OR of 2.81 (95% CI, 1.78-4.42). Compared with cooking in a fully ventilated or partially ventilated kitchen, cooking in an unventilated kitchen was associated with a doubling of the risk of TB (OR = 2.02; 95% CI, 1.31-3.13).

The univariate analysis showed statistically significant ($p \leq 0.05$) associations of TB with use of mainly biomass, coal, and kerosene as a source of heating fuel, urban/rural locality of residence, residence outside the Kaski district, religion, literacy, construction type of present house, not always having lived in the present house, ventilation, use of a kerosene lamp, tobacco smoking, one or more smokers in the family, alcohol consumption, vitamin consumption, and having had a family member with TB. Although not selected by the stepwise algorithm, in the multivariate model we also included annual family income in Nepali rupees as an additional indicator of socioeconomic status, and age, because it was a matching variable. Table 4.0.2 shows the results

of the main logistic regression model. Compared with use of GFS, use of a biomass- fueled stove for cooking showed a slight positive relationship, but the CI was so wide that this provides little evidence of an association with TB. Kerosene cooking-fuel use, however, was associated with TB. Also particularly strongly associated with TB in the model were use of biomass as a heating fuel (OR = 3.45; 95% CI, 1.44-8.27) and kerosene lamps as the main source of lighting in the house (OR = 9.43; 95% CI, 1.45-61.3).

We investigated possible effect modification of the biomass fuel variables by other exposures. However, investigation was limited because of small numbers of participants in many of the exposure categories, leading to very unstable estimates. Covariates with sufficient numbers in separate categories permitting some useful examination of effect modification were ventilation, literacy, and house construction. We found evidence of effect modification of the effects of heating fuel by ventilation status: participants who lived in houses with unventilated kitchens were at much higher risk (adjusted OR = 26.0; 95% CI, 4.24-159) than were those who lived in houses with ventilated kitchens (adjusted OR = 7.07; 95% CI, 1.48-33.9). Corresponding estimates for biomass cooking fuel were much more equivocal, with the adjusted ORs for ventilated and unventilated kitchens being 0.80 (95% CI, 0.19-3.37) and 0.47 (95% CI, 0.08-2.94), respectively. For illiterate and literate participants, adjusted ORs for heating fuel use were 5.12 (95% CI, 0.96-27.4) and 2.93 (95% CI, 0.87-9.91), respectively. We found no evidence of effect modification of literacy status on biomass cooking-fuel effects. Finally, participants who lived in *kuccha* construction houses (bamboo and mud, with thatched roofs) appeared to be at higher risk from both biomass cooking and heating fuels than were participants who lived in *pucca* or semi-*pucca* construction houses (brick and cement or brick and mud). For heating fuel, the adjusted ORs were 11.9 (1.38-102) and 2.73 (0.88-8.41) for *kuccha* and *pucca*/semi-*pucca* houses, respectively. The corresponding values for biomass cooking-fuel use were 4.07 (95% CI, 0.43-38.8) and 0.73 (0.22-2.40), respectively. With the possible exception of the modification by ventilation of the effects of biomass cooking fuel, these effects might generally be considered to be in the predictable direction—higher ORs associated with less ventilation and more-deprived socioeconomic circumstances.

Table 4.0.1 Characteristics of TB cases and controls, Pokhara, Nepal

Characteristic	Cases (%)	Controls (%)	Univariate OR (95% CI)
All participants	125 (100)	250 (100)	-
Age (years)			
20-29	54 (43.2)	108 (43.2)	-
30-39	26 (20.8)	52 (20.8)	-
40-49	22 (17.6)	44 (17.6)	-
50-59	3 (2.40)	6 (2.40)	-
≥60	20 (16.0)	40 (16.0)	-
Mean±SD	35±13	35±13	-
Residence in Kaski district			
Yes	90 (72.0)	236 (94.4)	1.00
No	35 (28.0)	14 (5.60)	6.56 (3.37-12.8)
Area of residence			
Urban/peri-urban	87 (69.6)	212 (84.8)	1.00
Rural	38 (30.4)	38 (15.2)	2.44 (1.46-4.08)
Education			
Literate	61 (48.8)	154 (61.6)	1.00
Illiterate	64 (51.2)	96 (38.4)	1.68 (1.09-2.60)
Religion			
Hindu	89 (71.2)	236 (94.4)	1.00
Buddhist	31 (24.8)	9 (3.60)	9.13 (4.18-19.9)
Christian	4 (3.00)	5 (2.00)	2.65 (0.75-9.38)
Muslim	1 (0.01)	0 (0.00)	
Occupation			
Government services and commerce	16 (12.8)	32 (12.8)	1.00
Farming	41 (32.8)	57 (22.8)	1.44 (0.70-2.96)
Nonagricultural labor	9 (7.20)	47 (18.8)	0.97 (0.49-1.94)
Teacher and student	11 (8.80)	15 (6.00)	1.47 (0.55-3.92)
Housewife	48 (38.4)	99 (39.6)	0.97 (0.49-1.94)
Present house construction			
Pucca or semi-*pucca* [c]	66 (53.0)	171 (68.0)	1.00
Kuccha house [d]	59 (47.0)	79 (32.0)	1.93 (1.25-3.00)
Always lived in the present house			
Yes	38 (30.4)	111 (44.4)	1.00
No	87 (69.6)	139 (55.6)	1.83 (1.16-2.88)
Crowding			
≤3 people per room	104 (83.2)	206 (82.4)	1.00
>3 people per room	21 (16.8)	44 (17.6)	0.95 (0.53-1.67)
Age started cooking			
>13 years	74 (59.2)	129 (51.6)	1.00
≤13 years	51 (40.8)	121 (48.4)	0.73 (0.48-1.13)

a No missing data, except as indicated. ***b*** Tanahu, Syangja, Baglung, Parbat, Myagdi, and Lamjung districts. ***c*** *Pucca* house made with brick and cement; semi-*pucca* house made with brick and mud. ***d*** *Kuccha* house made ith bamboo and mud (with thatched roof).

Table 4.0.1 contd. Characteristics of TB cases and controls, Pokhara, Nepal

Characteristic	Cases (%)	Controls (%)	Univariate OR (95% CI)
Current fuel and stove use			
Gas (GFS)	41 (32.8)	126 (50.4)	1.00
Kerosene (KFS)	19 (15.20)	23 (9.20)	2.54 (1.26-5.12)
Biomass (BFS)	65 (52.0)	101 (40.4)	1.98 (1.24-3.17)
Main heating fuel use in the house			
Electricity	1 (0.8)	3 (1.20)	-
No heating fuel	38 (30.4)	137 (54.8)	-
Combined	39 (31.2)	140 (56.0)	1.00
Wood	85 (68.0)	109 (43.6)	-
Coal	0 (0.00)	1 (0.40)	-
Kerosene	1 (0.8)	0 (0.00)	-
Combined	86 (68.8)	110 (44.0)	2.81 (1.78-4.42)
Kitchen location			
Open air kitchen and separate kitchen outside	18 (14.4)	37 (14.8)	1.00
Partitioned kitchen inside house	45 (36.0)	134 (53.6)	0.69 (0.36-1.33)
Non partitioned kitchen inside house	62 (49.6)	79 (31.6)	1.61 (0.84-3.10)
Windows in the kitchen			
Yes	117 (95.1)	231 (92.8)	1.00
No	6 (4.90)	18 (7.20)	0.66 (0.25-1.70)
Missing	2	1	
Overall ventilation in the kitchen			
Fully ventilated	59 (47.2)	161 (64.4)	1.00
Unventilated	66 (52.8)	89 (35.6)	2.02 (1.31-3.13)
Source of light in the house			
Electricity	107 (85.6)	246 (98.4)	1.00
Kerosene lamp	18 (14.4)	4 (1.6)	10.35 (3.42-31.3)
Smoking status			
Never smoked	83 (66.4)	200 (80.0)	1.00
Ever smoked	42 (33.6)	50 (20.0)	2.02 (1.25-3.28)
Pack-years of smoking			
0	84 (66.2)	200 (80.0)	1.00
≤8	16 (12.8)	31 (12.4)	1.23 (0.64-2.37)
>8	25 (20.0)	19 (7.60)	3.13 (1.64-5.99)
Smokers in the family			
None	58 (46.4)	165 (66.0)	1.00
One	48 (38.4)	72 (28.8)	1.90 (1.18-3.04)
Two or more	19 (15.2)	13 (5.20)	4.16 (1.93-8.95)
Burn mosquito coils indoors			
No	76 (60.8)	136 (54.8)	1.00
Yes	49 (39.2)	113 (45.2)	0.78 (0.50-1.21)
Missing	0	1	

Table 4.0.1 contd. Characteristics of TB cases and controls, Pokhara, Nepal

Characteristic	Cases (%)	Controls (%)	Univariate OR (95% CI)
Burn incense indoors			
No	28 (22.4)	46 (18.4)	1.00
Yes	97 (77.6)	204 (81.6)	0.78 (0.46-1.32)
Alcohol consumption			
No	106 (85.5)	238 (95.6)	1.00
Yes	18 (14.5)	11 (4.40)	3.67 (1.68-8.05)
Missing	1	1	
Taking vitamin supplements			
No	120 (97.56)	214 (85.94)	1.00
Yes	3 (2.44)	35 (14.06)	0.15 (0.05-0.51)
Missing	2	1	
Household member had TB			
No	77 (61.6)	227 (90.8)	1.00
Yes	48 (38.4)	23 (9.20)	6.15 (3.51-10.8)
Annual income (Nepalese rupees)			
≤25,000	26 (23.9)	72 (30.3)	1.00
25,000-50,000	58 (53.2)	90 (37.8)	1.78 (1.02-3.13)
>50,0000 to ≤100,000	16 (14.7)	51 (21.4)	0.87 (0.42-1.79)
>100,000	9 (8.20)	25 (10.5)	0.99 (0.41-2.42)
Missing	16	12	
Land ownership			
No	32 (25.6)	83 (33.3)	1.00
Yes	93 (74.4)	166 (66.7)	1.47 (0.91-2.38)
Missing	-	1	
Personal transportation			
Yes	15 (12.0)	47 (18.8)	1.00
No	110 (88.0)	203 (81.2)	1.70 (0.91-3.17)

4.3.1 Exposure response

We investigated whether associations with TB varied according to duration of cooking with BFS or KFS (Table 4.0.3). We categorized the total durations of cooking on BFS and KFS by cases and controls into bands. The adjusted exposure ORs were 1.17 (95% CI, 0.32-4.32), 0.64 (95% CI, 0.18-2.20), and 0.47 (95% CI, 0.11-2.02) for use of a BFS for less than 5 years, 5-10 years, and >10 years, respectively. For KFS, the unadjusted ORs were 4.96 (95% CI, 1.44-17.1) and 4.60 (95% CI, 1.34-15.7) for less than and more than 5 years of use, respectively, relative to no KFS use. Because we did not collect duration data for either heating fuel use or household lighting, we could not carry out comparable analyses for these variables.

As one measure of the potential public health implication of the association, we estimate that the population-attributable fractions of TB from exposure to BFS, KFS, biomass fuel heating, and kerosene lamps in our target population were 9% (95% CI, -42% to 41%), 12% (0.2 to 22%), 47% (22 to 64%), and 13% (4 to 22%), respectively.

Table 4.0.2 Multivariate logistic regression model for fuel use in relation to TB in women in Pokhara, Nepal (log likelihood = -118.73, R 2 = 0.44).

Variable	OR (95% CI) [a]
Fuel stove	
GFS	1.00
BFS	1.21 (0.48-3.05)
KFS	3.36 (1.01-11.22)
Heating fuel	
No heating fuel use or electricity	1.00
Biomass, coal, or kerosene	3.45 (1.44-8.27)
Main light source in the house	
Electricity	1.00
Kerosene lamp	9.43 (1.45-61.32)

[a] Adjusted for age, religion, income, residence locality, residence district, literacy, type of present house construction, always lived in the present house, pack-years of smoking, number of family members who smoked indoors, alcohol consumption, taking vitamin supplements, family history of TB, and ventilation in the kitchen

Table 4.0.3 Exposure-response relationships based on duration of cooking with BFS and KFS

Exposure to fuel stove	Cases (%)	Controls (%)	OR (95% CI)	
			Adjusted [a]	Unadjusted
Exposure to BFS (Years)				
0	26 (20.8)	43 (17.2)	1.00	1.00
>0 to ≤5	20 (16.0)	28 (11.2)	1.17 (0.32-4.32)	1.18 (0.55-2.52)
>5 to ≤10	18 (14.4)	51 (20.4)	0.64 (0.18-2.20)	0.58 (0.28-1.22)
>10	61 (48.8)	128 (51.2)	0.47 (0.11-2.02)	0.79 (0.44-1.40)
Exposure to KFS (years)				
0	86 (68.8)	209 (83.6)	1.00	1.00
>0 to ≤5	12 (9.6)	14 (5.60)	4.96 (1.44-17.1)	2.09 (0.93-4.73)
>5 to ≤10	27 (21.6)	27 (10.8)	4.60 (1.34-15.7)	2.54 (1.39-4.64)

[a] Adjusted for duration of use of BFS and KFS, GFS, biomass heating fuel, ventilation, use of kerosene lamp, pack-years of smoking, number of family members smoking indoors, religion, residence district, locality, literacy, present house construction, always lived in the present house, alcohol consumption, family members had TB in the past, taking vitamin supplements, income and age.

4.4 Discussion

The results of this study suggest that indoor exposure to smoke from biomass fuel combustion is a risk factor for TB. The association, however, appears to be mainly with use of biomass for heating, rather than cooking. The study also strongly suggests that exposure to smoke from kerosene fuel combustion, either in stoves or in lamps, is a risk factor for TB.

Religion, income, residence outside Kaski district, vitamin consumption, a family history of TB, and not always having lived in the present house also showed statistically significant associations with TB (Table 4.0.1). Pack-years of smoking (> 8 pack-years) showed an association with TB (p = 0.06), which did not change appreciably after adjustment. Smoking is now an established risk factor for TB [5, 19-22]. The very elevated relative risk estimate for Buddhists relative to Hindus is striking. We considered the possibility that this may have been because some Buddhists who live around Pokhara are Tibetan and reside in refugee camps. Crowded conditions in those camps could facilitate TB transmission. However, only 8 of 40 Buddhists in the study (six cases, two controls) were Tibetan refugees—an insufficient number to explain the finding. Other studies have also shown differences in TB rates between racial and religious groups, including Tibetan Buddhists [14, 23-26].

Before concluding that statistical associations are causal, it is important to consider alternative explanations, particularly whether study results might be a result of selection bias, information bias, or confounding in the study design, data collection, or analysis. As with all case-control studies, selection bias in the recruitment of controls is a potential concern. In this study, a systematic procedure for recruitment of all controls from inpatient and outpatient departments of MTH was used, and only one potential control refused to participate. Because most cases were recruited from the RTC, and all controls from MTH, the catchment areas for MTH and RTC might have been different. RTC patients came from a broader area, because it is a referral center for the western development region of Nepal. A higher proportion of cases (28%) than controls (6%) were from five districts other than Kaski. The Kaski district includes Pokhara city, and in general, Kaski residents are more likely to live in urban areas and to be wealthier. This could simply mean that living outside of Kaski is associated with higher exposure to TB risk factors but, alternatively, could indicate some selection bias. We adjusted for area of residence (Kaski or other districts) in the final model, but this would not necessarily have eliminated such a bias.

Another possible source of selection bias arises because we did not exclude some other, non-TB respiratory disease cases from the control group. Unfortunately, control diagnoses were not collected at the time of the study and proved impossible to obtain in retrospect, because of the limited period for which the hospital retains patient records. Because absence of TB was confirmed in controls by X-rays, we can, however, be confident that no chronic obstructive pulmonary disease or pneumonia cases were among our controls. It is possible that inclusion of respiratory disease cases among the controls could have produced a bias toward the null, if risk factors for those cases were similar to risk factors for TB.

Information bias may take the form of outcome misclassification or exposure mis- classification. Because all cases were newly diagnosed with active pulmonary TB on the basis of evidence from clinical tests, and controls were also confirmed by chest X-ray and on-the-spot sputum smear testing as not having active pulmonary TB, we consider that disease misclassification is unlikely to have occurred. We obtained all the exposure data by questionnaire. Case-control studies are often considered susceptible to recall bias, in that cases may be more likely than controls to remember past exposures. Because questions asked in this study were about common exposures, however, which both cases and controls experience on a day-to-day basis, we expect recall to have been accurate and any differential recall to have been minimal. We verified the high level of accuracy of reporting of two key exposure variables (stove type and ventilation) by visiting the homes of 28

study participants. Considering this and that there is no prevailing belief that indoor smoke exposure from biomass-burning stoves or kerosene-burning stoves or lamps is related to TB occurrence, we believe exposure mis- classification is likely to be minimal. One possible limitation, however, is that we only asked about the main cooking fuel used. This might have led to some misclassification of exposure status.

The third main area of potential bias is confounding. We collected data on a much more comprehensive range of exposures than did previous studies and investigated their potential to confound the associations with fuel use. Although confounding was present, adjustment with these variables did not eliminate the key associations. There may, of course, be some residual confounding due to misspecification of the variables, and there is no way to rule out the possibility of unknown confounding factors causing the associations found. One possibility is malnutrition, for which we obtained no data and which a known risk factor for TB. However, family income, for which we did obtain data and which is an excellent indicator of a family's ability to feed itself, was taken into account.

A notable finding in our study was the association with biomass used as a heating fuel. This was unexpected because the study design focused on cooking-fuel use. Hence, the study population was limited to women, who generally do the cooking in Nepal. Although we collected data on history of stove and cooking- fuel use, we did not collect a comparable level of data for heating fuels and so are unable to examine heating-fuel use for evidence of an exposure-response relationship.

In hindsight, the findings with biomass as a heating and a cooking fuel make sense. Women may light a cooking fire, set the pot atop it, and leave the room, returning only periodically while cooking takes place. On the other hand, use of heating fuel involves minimization of ventilation and deliberate exposure, as the family sits around the fire. In tropical India and Africa, where several of the other TB and biomass studies have been carried out, use of heating fuel is less common than in the mid-hills of Nepal, where night- time and winter temperatures are lower.

Our study also found the OR for TB to be high among both kerosene stove and lamp users, particularly the latter. Kerosene cooking fuel and kerosene lamp users were for the most part mutually exclusive groups. Only one of the 22 kerosene lamp users in the study used a kerosene stove. Kerosene stove users were more likely to use electricity for lighting. With one exception, as far as we are aware, no previous studies have examined a relationship between kerosene and TB[15]. This one study, carried out in Mexico, obtained crude ORs for use of kerosene-burning stoves of 1.9 (95% CI, 0.8-4.5) for active TB and 4.4 (95% CI, 1.7-11.5) for past TB; no adjusted estimates were presented. We have been unable to find any studies where the relationship between kerosene lighting and TB has been investigated or even incidentally reported.

The question arises as to why kerosene as a cooking fuel could be a TB risk factor but not biomass cooking fuel. This could have something to do with the nature of the emissions. Biomass burning produces very obvious smoke, which may irritate the eyes and respiratory tract, encouraging avoidance behavior. Kerosene, on the other hand, has the appearance of burning more cleanly, even if it does produce substantial amounts of fine particulate matter and vapor-phase chemicals, and may not encourage the same avoidance behavior as biomass smoke. Cooks may

be more likely to remain in the room while cooking with kerosene fuel. There are also likely to be differences in the toxic effects of the pollutant mixtures from the two fuels.

Kerosene is one of the main sources of cooking fuel in urban areas and lighting fuel in rural areas of developing countries, including Nepal. Therefore, if kerosene burning can be confirmed as a TB risk factor in other studies, the public health implications would be substantial. In rural areas not connected with electric power, kerosene wick lamps are burned at least 4-5 hr every day. Commonly, these lamps are homemade devices that are highly energy inefficient, with low luminosity. Simple wick kerosene lamps emit substantial amounts of smoke and particles[27]. A study conducted in rural Malawi has shown a higher loading of particulates in alveolar macrophages in men from exposure to kerosene in lamps compared with candles, hurricane lamps, and electric lamps[28]. Other emissions from kerosene combustion include carbon monoxide, carbon dioxide, sulfur dioxide, nitrogen dioxide, formaldehyde, and various VOCs (volatile organic carbons)[29]. An indoor air pollution study conducted in Bangladesh slums has shown significantly higher concentrations of benzene, toluene, xylene, hexane, and total VOCs emitted from kerosene stoves than from wood burning stoves [30].

The use of kerosene fuel is associated with harmful effects that have been documented in a few studies. These effects include impairment of ventilatory function and a rise in blood carboxyhemoglobin in women exposed to kerosene fuel smoke[31], and a higher incidence of acute lower respiratory infection in children in homes using KFS and BFS[32].

A causal relationship between exposure to biomass fuel smoke and TB is biologically plausible. The smoke could affect either risk of infection or risk of disease in infected people, or both, as has been shown to be the case with tobacco smoking[5]. Without knowledge of the time of infection, however, the present study cannot distinguish between the two possibilities. Inhalation of respirable particles and chemicals found in smoke from these sources generates an inflammatory response and impairs the normal clearance of secretions on the tracheobronchial mucosal surface, and may allow TB bacteria to escape the first level of host defenses, which prevent bacilli from reaching the alveoli[33]. Smoke also impairs the function of pulmonary alveolar macrophages, an important early defense mechanism against bacteria[34]. Alveolar macrophages isolated from the lungs of smokers have reduced phagocytic ability compared with macrophages from nonsmokers and secrete a lower level of pro-inflammatory cytokines[35]. Exposure to wood smoke in rabbits has been shown to negatively affect antibacterial properties of alveolar macrophages, such as their ability to phagocytize bacteria [36].

4.5 Conclusion

Our study provides evidence that the use of biomass fuel for household heating is a risk factor for TB, but little evidence that the use of biomass as a cooking fuel is a risk factor in this population. The association is biologically plausible and consistent with the results of some other epidemiologic studies. Nonetheless, there is the possibility of a selection bias arising from differences in the sources of cases and controls. The study also strongly suggests that kerosene fuel burning, particularly for lighting, is a risk factor for TB. That kerosene lamp burning was more strongly associated with TB than kerosene stove use may be because lamps are likely to be kept burning for longer periods than are stoves, which are used only during the period of cooking, and

the lamps may be kept closer to people during the evening, increasing the effective intake fraction. In addition, most of the kerosene lamps were wick lamps (21 of 22), whereas most (33 of 42) of the stoves were pressurized (pumped), which produce fewer emissions per unit fuel. Because these kerosene findings are apparently unique, more studies in different settings are needed to confirm them. Should the association with kerosene lamp use be confirmed, replacement of the kerosene lamps with solar lamps or other clean lighting systems would be a solution. Considering the strong associations of both religion and district of residence in this study, in any future case-control study examining this issue in Nepal, consideration should be given to matching on these factors.

Irrespective of the evidence for associations between indoor biomass use and TB, it is clear that such use produces substantial indoor air pollution with health-damaging chemicals and particulate matter. One, at least partially effective, remedial measure is to replace unflued stoves with chimney stoves. Such stoves, however, require continuing maintenance to maintain good indoor air quality, and because they usually just exhaust emissions to the near outdoors but not reduce them, even well-operating chimney stoves can only partly reduce total exposures [37, 38]. Ideally, electric stoves or low-emission biomass stoves, such as semi- gasifier stoves, or those with cleaner burning fuels (biogas or LPG) would be used. It is more difficult to generalize about kerosene stoves and lamps, because emissions vary greatly by type of device and fuel quality, which is not uniform[10]. Pressurized kerosene stoves and lamps using good-quality fuel may have low particulate emissions if properly maintained, but inexpensive wick lamps can be dirty, particularly with low-quality fuel. Their replacement with cleaner burning devices may also be justified.

4.6 Reference

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CHAPTER 5

Conclusion

Conclusion

5.1 Dissertation summary

It is estimated that about half of the world's households cook daily with biomass fuel, most using unvented stoves, with women, infants and young children experiencing the highest levels of exposure. In addition to biomass fuel, many poor households in developing countries also use kerosene for cooking and or lighting. Burning of biomass in unimproved devices and burning of kerosene, particularly in inexpensive devices like wick stoves and wick lamps, emits fine particulates (PM2.5) and many organic chemicals. Although some health impacts from use of biomass fuel are well documented, the possible health impacts of use of kerosene for cooking and lighting are not. The major objectives of this dissertation were to document levels of fine particulates, nicotine and naphthalene in kitchens from three main cookstoves (unimproved biomass, kerosene and liquefied petroleum gas) and to investigate the associations between tuberculosis (TB) and pre-clinical damage of lens (lens opacity) from use of biomass and kerosene cookstoves. These issues were separately explored in chapters 2, 3 and 4 of this dissertation.

In chapter 2, concentrations of PM2.5 and naphthalene from three main cookstoves--biomass, kerosene and LPG were measured. In addition, the extent of agreement of responses on the exposure information (stove types and ventilation in the kitchen) obtained during face-to-face interviews at the hospital were compared with data obtained from actual inspection of these features at the houses of a subset participants. The accuracy (true reports÷total reports) of stove reporting was 96% and the accuracy of ventilation reporting was 89%. The results suggested that the questionnaire used during face-to-face interviews at the hospital was adequate and reliable for collecting main exposure information (stove type and ventilation) from the study participants in Pokhara, Nepal.

Newly designed passive naphthalene badges were deployed in the field to measure naphthalene concentrations indoors. Weekly average naphthalene concentrations from biomass, kerosene and LPG cookstoves were 3.20 $\mu g/m^3$ (SD: 3.42), 3.26 $\mu g/m^3$ (SD: 2.22) and 0.79 $\mu g/m^3$ (SD: 0.53), respectively. The maximum weekly naphthalene concentration was found in a kitchen that used a biomass cookstove (15.76 $\mu g/m^3$). The difference in weekly average naphthalene concentrations between kerosene and biomass stoves was not statistically significant (p=0.23). Housing characteristics and presence of other exposure covariates in the kitchen did not influence the mean naphthalene concentrations. For example, better ventilation in the kitchen did not reduce the naphthalene concentration. Having smokers in the house and use of incense also did not increase naphthalene concentrations. However, duration of use of non-electric lamps and stoves were good predictors of naphthalene concentration in the multivariate regression. The naphthalene to PM2.5 ratio was 0.004, 0.02 and 0.01 for biomass, kerosene and LPG stoves, respectively. The results of the naphthalene study suggest that passive naphthalene badges can be reliably deployed in the field in future.

A statistically significant difference in PM2.5 concentrations was found between biomass, kerosene and LPG cookstoves. Results indicated significantly higher PM2.5 from biomass followed by kerosene and LPG cookstoves. Weekly mean PM2.5 concentrations in the kitchens

from three stoves were, 78, 16 and 8 times higher than the WHO annual air quality guidelines value (10 µg/m^3).

The UCB particulate matter monitor documented the highest measured 15 minutes PM2.5 concentration (75 mg/m^3) on a weekday from a biomass stove. Within the biomass stove group, for PM2.5, there was a 495 exposure-fold range (ratio of 97.5th and 2.5th percentiles of the log-normally distributed concentrations). Corresponding exposure-fold ranges within the kerosene and LPG stove groups were 14 and 6, respectively. Relatively high intra household variability of PM2.5 concentrations was observed in the biomass and kerosene stove groups. For LPG stoves, the total variance of PM2.5 explained by within-household variability was similar (45%) to that of between-household variability (55%). In the biomass stove group, housing characteristics such as kitchen location, house construction, use of secondary stoves and non electric lamps increased the between-household variance of PM2.5, whereas covariates like windows opened all the time, burned incense indoors and observer assigned ventilation status decreased the between-household variance of PM2.5.

On the basis of acceptability of temporal variability of 20% (within-household variability of PM2.5 level), the estimated variance components of between- and within-households indicated that averaging times of 48 hours are needed to reliably characterize between-household differences in PM2.5 levels in Pokhara. Since high intra-class correlation (ICC) indicates lower temporal variance relative to total variance, and suggests increasing the duration of measurements is no longer necessary; 82% of the variability in 48-hours average PM2.5 concentrations was explained by between-household differences, while only 11% of the 1 hour concentration variability was explained by between-household factors. The ICC values were similar (~0.82) for moving averages of 60 hours and more and approached 0.90 on seventh days.

In addition to PM2.5 and naphthalene, higher mean nicotine concentrations were found in the bedroom (0.29 µg/ m^3) than in the kitchen (0.20 µg/ m^3) and in houses reporting smoking family members compared with those reporting no smoking family members (0.63 vs. 0.11 µg /m^3 in the kitchen; and 0.65 vs. 0.06 µg /m^3 in the bedroom). A higher maximum nicotine concentration was found in the kitchen than the bedroom (1.87 µg/ m^3 vs. 1.53 µg/ m^3). However, the difference in mean concentrations between bedrooms and kitchens was not statistically significant (p=0.33). The mean concentration of nicotine found in this study was lower than the mean concentration that is generally observed in the homes of smokers (1-3 µg /m^3) and work places where smoking is allowed (2.14 µg/ m^3) in the US [1, 2]. However, the median concentration of nicotine was higher than the median nicotine concentration documented in the homes with smokers in Asia (0.15 vs. 0.09 µg/ m^3) and lower than that of median nicotine concentrations documented in the homes with smokers in Nepal (~0.4 µg/ m^3) [3].

In chapter 3, using a cross-sectional study design, the association between use of cooking and heating fuels on the risk of pre-clinical damage to the lens was investigated. Women between 20-65 years were recruited from the Ophthalmology and outpatient departments at Manipal Teaching Hospital (MTH), Pokhara, Nepal. After recruitment and obtaining oral consent, all participants' visual acuities were measured, followed by measurements of intra ocular pressure, refraction and photographs of the lens. Altogether six photographs of the cross-sectional view and the retro-illumination view were collected from each of the 144 participants. The changing

patterns of lens and opacity scores were graded by reference to the LOCS III classification system. Two graders provided scores for the right and the left eyes. Thus, there were four scores for each participant. The opacity scores ranged from 0 to 4.2 (on a scale of 0 to 6.0). In the study population, nuclear cataracts were the most prevalent type, followed by cortical and posterior-sub-capsular cataracts. In the linear regression analysis, after adjustment for age, pack-years of tobacco smoking, use of wood as a heating fuel, practice of incense burned indoors, main light source in the house, education, area of residency, ventilation, hours of work every day and years of work in the sun (years-hours), alcohol consumption, dietary practice, main occupation and land ownership; the current use of biomass cookstoves increased mean nuclear opacity score by 0.164 (0.000 ;0.329) compared with use of gas cookstoves. The current use of kerosene cookstoves, compared with the use of gas cookstoves increased the mean nuclear opacity score by 0.138 (-0.615; 0.891), but it was not statistically significant. Ordered logistic regression analysis of nuclear opacity grades after adjustment for thirteen confounders showed use of biomass fuel compared with gas increased the odds of nuclear opacity by 1.759 (1.01; 3.067).

This study provides evidence that the use of biomass cookstoves is associated with an increased risk of nuclear opacity but not cortical or posterior sub-capsular opacity. Supporting a relationship between use of biomass stoves and nuclear opacity was a statistically significant exposure-response trend showing years of exposure to biomass fuel stoves associated with increasing risk of nuclear opacity. Bias including potential confounding, is not likely to explain these associations, which are biologically plausible and consistent with the results of epidemiological studies showing an association with clinically diagnosed cataracts.

In chapter 4, using a case-control study design, the associations of tuberculosis (TB) in women with cooking, heating and lighting fuels was investigated. The main objectives of this study were to confirm results of earlier studies using clinically confirmed TB cases and to investigate possible confounding of the relationship using a standardized questionnaire and confirm self-reported exposure assessment in the kitchens of a subset of participants' houses. Cases in this study were all female patients, 20–65 years old, who visited TB clinics in the Regional Tuberculosis Center (RTC) and Manipal Teaching Hospital (MTH) and who had been newly diagnosed with active pulmonary TB by chest X-ray and positive active sputum smears (two sputum specimens positive for acid-fast bacilli by microscopy), which are routinely conducted at the hospital using methods recommended by the WHO[4]. Controls were women between 20-65 years attending MTH who did not have TB. Women who were pregnant, who were on chemotherapy for cancer, who had HIV/AIDS or diabetes, or who had a history of TB were excluded from the study.

Controls were recruited from outpatient and inpatient departments (dental, 1.6%; ear, nose, and throat, 1.6%; ophthalmology, 25.6%; general medicine, 56%; obstetrics and gynecology, 7.2%; orthopedics, 2.4%; skin, 1.6%; surgery, 3.2%; and psychiatry, 0.8%) at the MTH in the same months when cases were identified. For each case, the control subjects were the first eligible female patients without pulmonary TB, matched to cases on age (5-year frequency bands), who presented at MTH between 0900 and 1000 hours after case enrollment. Control subjects were interviewed only after medical screening confirmed that they did not have TB. Confirmation procedures included a chest X-ray and an on-the-spot sputum examination. The ratio of cases to controls was 1:2 and there were 125 cases and 250 controls.

Most cases and controls (72.0% of cases, 94.4% of controls) were from the Kaski district. Cases were more likely than controls to be Buddhist, to live in urban and peri-urban areas, to reside in poorer quality houses (kuccha), to be illiterate, to have non-partitioned and unventilated kitchens indoors, and to use kerosene wick lamps as their main source of light. Cases were also more likely than controls to regularly consume alcohol, to be tobacco smokers, to have more smokers in the family than controls, and to have not always lived in their present house. Except for three cases, none of the participants who had smoked reported that they had ever quit smoking for 6 months or more. More cases than controls had had household members with TB. More cases (14.4%) used kerosene wick lamps as their source of light than controls (1.6%) and more cases (69%) used wood, kerosene and coal as a source of heating fuel than controls (44%). Moreover, cases were more likely to be using a biomass cookstove or kerosene cookstove than were controls ($p = 0.004$). The distribution of cooking fuel used by the study participants was biomass from wood or crop residues (44.3%), LPG (42.7%), kerosene (11.2%), and biogas (1.9%).

In the multivariate logistic regression model, after controlling for age, religion, income, residence locality, residence district, literacy, type of present house construction, always living in the present house, pack-years of smoking, number of family members who smoked indoors, alcohol consumption, taking vitamin supplements, family history of TB, and ventilation in the kitchen, use of a biomass cookstove compared with a gas cookstove showed a slight positive relationship (OR=1.21; 95% CI, 0.48-3.05) but the confidence interval was so wide that this provides little evidence of an association with TB. Kerosene cooking, however, was associated with TB (OR=3.36; 95% CI, 1.01-11.22). Also particularly strongly associated with TB in the model were use of biomass as a heating fuel (OR = 3.45; 95% CI, 1.44–8.27) and use of a kerosene lamp (OR = 9.43; 95% CI, 1.45–61.32). As one measure of the potential public health implication of the association, the population-attributable fractions of TB from exposure to biomass and kerosene cookstoves and biomass heating fuel, and kerosene lamps in the target population were 9% (95% CI, –42% to 41%), 12% (0.2 to 22%), 47% (22 to 64%), and 13% (4 to 22%), respectively.

5.2 Future research directions

5.2.1 Examination of severity of lens opacity by cumulative exposure to naphthalene

Both in vivo and in vitro animal studies have shown an association of cataracts with naphthalene [5-8]. The lens opacity study (chapter 3) showed an association between use of a biomass stove and nuclear opacity. The risk of opacity was also found higher among kerosene stove users but it was not statistically significant. The results in chapter 2 show that the mean concentrations of naphthalene from biomass and kerosene cookstoves are similar. However, as there were only three kerosene stove users in IAP monitoring study, the results for naphthalene needs further confirmation. If confirmed that kerosene emits higher naphthalene concentrations comparable to biomass cookstoves then this will provide some justification for reconsidering policy to replace kerosene as well as unimproved biomass devices with suitable alternatives like biogas or LPG stoves. Kerosene is heavily subsidized in many developing countries, a policy that would need to be re-examined if significant health impacts were found to be associated with it.

There have been no epidemiologic studies looking at the association between severity of lens opacity or cataracts with cumulative dose of naphthalene. Lack of affordable measurement tools have been one reason for lack of such studies. However, the passive naphthalene badges developed and deployed in this study suggest that these badges can detect and collect naphthalene in kitchens that use biomass or kerosene cookstoves. Thus, these badges can be deployed in the field in the future to collect mean concentration as well as variance components. Using the unbiased mean estimates, researchers can compare the degree of lens opacity with cumulative exposure to naphthalene.

5.2.2 Indoor air pollution and lens opacity

The evidence found in the current study that use of biomass fuel is associated with pre-clinical damage of lens or lens opacity is coherent. The results of chapter 3 of this dissertation suggest that present and past use of biomass cookstoves is associated with nuclear opacity. In the present study, the prevalence of nuclear opacity (opacity scores $\geq$2.0) was 26.4%, which is comparable to the prevalence of nuclear opacity in women in India[9]. Much larger population-based studies covering both rural and urban residents are needed to confirm the findings of this study. If the association is established, then the public health benefit could be large as nuclear opacity dominates the cataracts burden in developing countries. Recently some studies have indicated that severe heat stress or exposure to high ambient temperature also damage lens [10-12]. In future, studies should include investigation of the association of lens opacity with heat stress or exposure to higher temperatures.

5.2.3 Indoor air pollution and tuberculosis infection

Tuberculosis (TB) is a major infectious disease that causes illness and death worldwide[13]. In 2006, there were about 9.2 million new TB cases and 1.7 million TB-related deaths[14]. Worldwide, six previous epidemiologic studies have investigated whether indoor cooking with biomass fuels, such as wood or agricultural wastes, is associated with TB--with inconsistent results. The results of chapter 4 of this dissertation suggest that indoor biomass burning, particularly as a heating fuel, and use of kerosene, as a cooking or lighting fuel is associated with TB. The results of use of kerosene for cooking and lighting increasing the risk of TB in women are unique. However, as the present study had a case-control design where hospital controls were compared with the cases, the possibility of selection bias could not be completely ruled out. The implications will be profound if the association of TB with the use of kerosene for cooking and lighting is confirmed. As all of the previously conducted studies of IAP and TB have focused on active pulmonary TB disease, there have been no studies investigating the role of use of biomass or kerosene on the risk of TB infection. Such information would be useful for policy making, particularly in countries where the prevalence of TB infection is relatively low.

5.3 Concluding remarks

In conclusion the results of the three main chapters of this dissertation suggest that in Nepal cooks who use unvented biomass cookstoves experience very high mean and peak exposure to PM2.5 compared to cooking with kerosene and LPG stoves. By contrast, the cooks experience higher concentrations of naphthalene from both kerosene and biomass cookstoves compared with LPG cookstoves. Current and past use of biomass cookstoves is associated with an increase in the risk of nuclear opacity. Similarly use of biomass as a heating fuel and kerosene, either in stoves or in lamps, is a risk factor for TB. Irrespective of the evidence for associations between indoor biomass use and risk of lens opacity and TB, it is clear that such use produces substantial indoor air pollution with health-damaging chemicals and particulate matter. One, at least partially effective, remedial measure is to replace unflued stoves with chimney stoves. Such stoves, however, require continuing maintenance to maintain good indoor air quality, and because they usually just exhaust emissions to the near outdoors, but not reduce them, even well-operating chimney stoves can only partly reduce total exposures [15, 16]. Ideally, electric stoves or low-emission biomass stoves, such as semi-gasifier stoves, or those with cleaner burning fuels (biogas or LPG) would be used. It is more difficult to generalize about kerosene stoves and lamps, because emissions vary greatly by type of device and fuel quality, which is not uniform[17]. Pressurized kerosene stoves and lamps using good-quality fuel may have low particulate emissions if properly maintained, but inexpensive wick lamps can be dirty, particularly with low-quality fuel. Their replacement with solar lamps or cleaner burning devices may also be justified.

5.4 Reference

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